STUDIA FORESTALIA SUECICA

Nr 2 1963

Studies on the Germination in Seeds of Scots Pine

(*Pinus silvestris* L.)

with special reference to the light factor

Studier över frögroningen hos tall med särskild hänsyn till ljusfaktorn

by

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Ms received Jan 14th 1963
Ms mottagit 14 jan. 1963
Esselte, Sthlm 63

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Chapter I. General Introduction

In the broad research field concerning the relation between light and plants photosynthesis occupies a central position. This is connected with its fundamental importance for the conversion of radiant energy to chemical energy required for the maintenance of life in all organisms. However, in addition to photosynthesis light influences many other processes in plant life as growth, development and differentiation. Such processes dependent on light but independent of photosynthesis are included under the designation photomorphogenesis (MOHR 1962). The light-influenced germination of seeds constitutes only a smaller part of these photomorphogenic processes.

The first time when the problem about an eventual effect of light on the seed germination was investigated was evidently 1788, when INGENHOUSZ studied this problem (quoted from LEHMANN and AICHELE 1931). According to JÖNSSON (1893) the first investigation in this field was carried out by MIESSE as early as 1775. However, the first investigation in which an evident influence of light on germination was found was made by CASPARY (1860) on seeds of *Bulliarda aquatica* DC. (quoted from CROCKER 1936). Since that time the literature in this field has become extensive, and it has been reviewed by a number of authors (LEHMANN and AICHELE l.c., CROCKER l.c., EVENARI 1956, TOOLE *et al.* 1956 a, KOLLER *et al.* 1962 and EVENARI in press). From these reviews it is evident that visible light affects both positively and negatively the germination of a large number of seeds from species belonging to different parts of the plant system.

Seeds of different coniferous tree species do not constitute any exception (cf. reviews in BALDWIN 1942 and JONES 1961). The first species where an effect of light on the germination has been established seems to be *Pinus nigra* Arnold, according to a paper by CIESLAR and LIEBENBERG (quoted from CIESLAR 1885). The first corresponding report on *Pinus silvestris* L. was published by ATTERBERG (1906) and HAACK (1906). After that time several authors repeatedly have shown stimulating effects of light on the germination of seeds from Scots pine (*P. silvestris* L.) as well as from other pine species. This will be further dealt with in the introduction to Chapter III (p. 20).

In the above papers the stimulating action of light was shown by irradiations *during* the germination process. According to NORDSTRÖM (1953 a and b) the germination of Scots pine seeds can be stimulated by irradia-

tion of the unimbibed seeds, *i.e.*, *before* the start of the germination process. With these results as a starting point the intention with the present work was to investigate the physiological background to the light-dependent germination in this species.

In Chapters III and IV results are presented from experiments, in which the physiological actions of the irradiations have been evaluated from the fraction of the irradiated seeds which after a definite time had protruding rootlets ("germinated" seeds). In Chapter III effects of irradiations with white light have been studied, in Chapter IV the spectral regions physiologically active were determined from studies on the action spectrum of the germination. In Chapters V and VI results are given from experiments on the effects of irradiations (both with white and coloured light) on the occurrence of embryo-mitosis and on the respiration of the seeds. In these experiments most of the observations have been restricted to these processes in the seeds before the start of the directly visible germination ("ungerminated" seeds). In this way the light-influenced respiration, occurrence of embryo-mitosis and final germination could be compared with each other in regard to their spectral dependence. A time sequence for the first appearances of the light responses in these processes also could be established. From this time sequence it seems possible to obtain information about the more primary steps in the physiology of the irradiated seeds, in which the light regulates the final germination responses.

The investigation was started in January of 1955 at the Department of Silviculture and from 1956, it was carried on at the Department of Forest Botany at the Royal College of Forestry in Stockholm, Sweden.

Chapter II. General Methods

A. Experimental Material

1. Origin. Data concerning the materials employed, which consisted of seeds of Scots pine (*Pinus silvestris* L.), are given in table 1. Seeds of the provenances Nos. 2—4 constituted the materials on which most of the experiments were done. Information concerning the experimental material is given in the texts to the separate tables and figures. In spite of the fact that seeds of different provenances were used for experiments in different parts of the investigation, no fundamental deviations between the responses in the materials could be found in comparable experiments (cf. the comparisons in figure 1 over the germination responses and in table 2 over the respiration responses).

2. Seed extraction. The cones have been collected after ordinary cuttings in normal stands of trees not specially selected for seed collection. Because the unimbibed seeds have been shown to be sensitive to light (NORDSTRÖM 1953 a and b) all cones with opened cone scales were discarded before the start of the extractions in order to obtain as uniform seed material as possible with regard to eventual effects of irradiations before the start of the experiments. For that reason all handlings of the seeds were done in safelight (see further about safelight p. 14). All extractions were performed in the laboratory. The cones were placed in darkness at +45° C for 16 hours in a kiln with ventilation, whereupon the seeds were separated from the opened cones.

3. Seed storage. Directly after the extraction the seeds were transferred to dark glass bottles, which were airtightly sealed with rubber stoppers, wrapped in aluminium foil and placed in a cold storage room at +3—4° C. Stored under such conditions the seeds retain their germinative capacities unchanged for years (HUSS 1953, cf. also figure 9). The moisture content of the seeds was about 5 per cent of the dry weight (cf. also table 7). Each bottle had a volume of 200 ml. The content of two such bottles is called a sub-collection. The seeds were stored under such conditions till the time when new seeds were needed. At that moment a sub-collection was treated in the following way.

4. Dewinging and cleaning. The seeds were rubbed in a satchel. Chaff and empty seeds were removed in a cleaner with a vertical air stream (cf. HUSS 1951), whereupon the dewinged and cleaned seeds from the sub-collection were ready for sampling.

Because mechanical treatments at the dewinging can influence the germinative capacity of the Scots pine seeds (HUSS 1950) and also influence their light dependence at the germination (NORDSTRÖM l.c.), control experiments were performed, in which the seeds individually dewinged and dewinged by the rubbing method were compared. The germinative capacities of these seeds were tested in continuous white light and in darkness. No differences could be found (cf. also figure 17 A and B), and consequently the rubbing method has been used for the dewinging throughout the investigation.

5. Sampling technique. In order to minimize the variation between individual samples from a seed collection a sampling technique described by TIRÉN (1948) was used (cf. also HUSS 1951). The sizes of the samples were 50 or 100 seeds depending on the experiments, in which the seeds were to be used (cf. the methods in corresponding chapters). With the use of an X-ray technique (cf. further in Chapter III) the distribution of seeds with differently developed embryos in replicates was studied. This study showed that the

Table 1. Provenances of the Scots pine seeds used in the investigation.

Provenance No.	Indication[1]	Locality	Parish	County	Altitude meter	Latitude	Date of collection
1		Krp. Stryckberget	Degerfors	Västerbotten	250	64° 11′	Feb. 1955
2		Ljusdal	Ljusdal	Gävleborg	230	61 30	Apr. 1956
3		Forsnäset	Ljusdal	Gävleborg	140	61 30	Apr. 1958
4		Kläppaåsen	Ljusdal	Gävleborg	160—190	61 30	Mar. 1960
5	A (9556)	Skatte by	Gällivare	Norrbotten	220	66 55	Oct. 1957
6	B (9575)	Bondersbyn	Töre	Norrbotten	45	65 55	» 1957
7	C (9523)	Mickelsträsk	Umeå	Västerbotten	190	64 10	» 1957
8	D (9520)	Åte	Själevad	Västernorrland	90	63 17	» 1957
9	E (9503)	Fällforsån	Umeå	Västerbotten	60	63 55	» 1957
10	F (9512)	Söderäng	Attmar	Västernorrland	100	62 20	» 1957
11	G (9519)	Utanede	Fors	Jämtland	160	62 57	» 1957
12	H (9517)	Bönsaberg	Vånhus	Kopparberg	250	61 12	» 1957
13	I (9509)	S. Viken	Sunne	Värmland	125	59 50	» 1957
14	J (9502)	Arvidstorp	Kalv	Älvsborg	137	57 15	» 1957
15	K (9505)	Gransjö	Algutsboda	Kronoberg	155	56 42	» 1957
16	L (9506)	Troxhult	Gullabo	Södra Kalmar	112	56 28	» 1957
17	M (9564)	Vombs Fure	Vomb	Malmöhus	40	55 40	» 1957
18	A (10348)	Ängesträsk	Råneå	Norrbotten	80	65 59	Nov. 1958
19	B (10347)	Degersel	Råneå	Norrbotten	70	65 59	» 1958
20	C (10318)	Tjälamark	Umeå	Västerbotten	60	63 55	» 1958
21	D (10319)	Åte	Själevad	Västernorrland	50	63 17	» 1958
22	E (10338)	Bölan	Enånger	Gävleborg	65	61 30	» 1958
23	F (10327)	Kofsta	Hedesunda	Gävleborg	60	60 25	» 1958
24	G (10321)	Väster-Ledinge	Skederid	Stockholm	40	59 41	» 1958
25	H (10328)	V. Arninge	Täby	Stockholm	30	59 30	» 1958
26	I (10304)	Alevede	Hogrän	Gotland	40	57 15	Oct. 1958
27	J (10362)	Halahult	Åryd	Blekinge	70	56 12	Nov. 1958
28	K (10350)	Vombs Fure	Vomb	Malmöhus	45	55 40	» 1958
29	c (10317)	Rödåliden	Umeå	Västerbotten	175	64 5	» 1958
30	d (10367)	Rusksele	Lycksele	Västerbotten	240	64 50	» 1958
31	e (10366)	Vormträsk	Lycksele	Västerbotten	290	64 54	» 1958
32	aa (10345)	Norrskedika	Börstil	Stockholm	15	60 15	» 1958
33	cc (10364)	Nyhammar	Grangärde	Kopparberg	180	60 40	» 1958
34	dd (10303)	Rågsveden	Äppelbo	Kopparberg	269	60 30	Oct. 1958
35	ee (10315)	Högheds fäbod	Orsa	Kopparberg	410	61 18	Nov. 1958
36	ff (10314)	Dråbergs fäbod	Älvdalen	Kopparberg	500	61 15	» 1958

[1] The numbers refer to the current series of cone samples at the Departement of Reforestation, the Royal College of Forestry, Sweden.

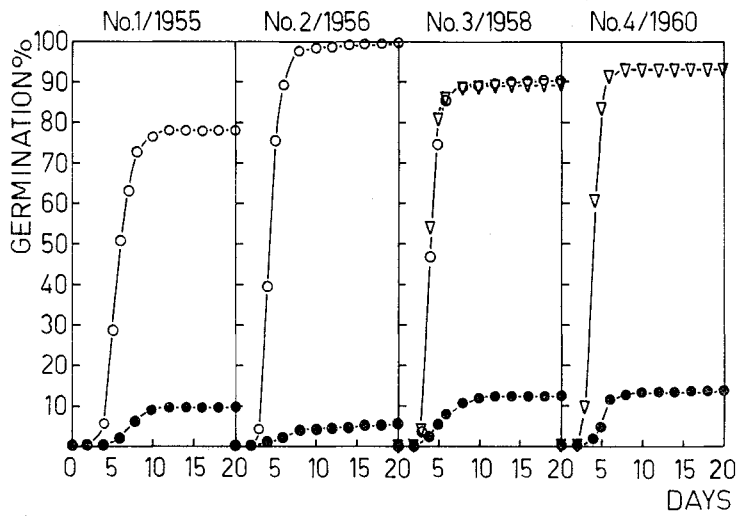

Figure 1. Germination responses of some seed provenances of Scots pine under continuous irradiation (empty circles, white fluorescent tubes TL/33, 1700 lux), in darkness after a standard red irradiation (inverted triangles, red fluorescent tubes TL/15, 350 $\mu W/cm^2$, 30 min., 6 hours of imbibition) and in darkness without any irradiation (filled circles). For details about the seed provenances, see table 1.

Table 2. Comparison of the respiration rates in Scots pine seeds of different provenances. Measurements after the seeds had been under germinating conditions for 48 hours.

Provenance No.[1]	Date of exper.	Table No.[2]	Gas	Respiration rate after irradiation with		
				Red	Red + far-red	Dark control
2	18/7-56	19	Q O_2	(44.6 ± 0.4)[3]	—	9.8 ± 0.3
			Q CO_2	(27.1 ± 0.4)	—	7.5 ± 0.3
3	5/11-59	20	Q O_2	47.4 ± 0.7	17.2 ± 0.6	—
			Q CO_2	28.5 ± 0.8	11.6 ± 0.8	—
3	11/12-59	25	Q O_2	48.9 ± 0.6	19.6 ± 1.1	13.5 ± 0.4
			Q CO_2	31.0 ± 0.8	14.2 ± 1.4	9.9 ± 0.3
3	21/3-60	23	Q O_2	—	—	15.1 ± 0.4
			Q CO_2	—	—	9.8 ± 0.4
4	12/12-60	26	Q O_2	43.1 ± 1.7	18.3 ± 0.8	13.1 ± 0.5
			Q CO_2	27.6 ± 2.2	14.3 ± 0.9	9.6 ± 0.7
4	20/12-60	22	Q O_2	33.4 ± 0.9	—	14.1 ± 0.4
			Q CO_2	22.5 ± 1.0	—	9.3 ± 0.1

[1] Cf. table 1.
[2] Number of the tables from which the figures are gathered.
[3] In continuous white light.

Table 3. Distribution of empty seeds in random samples of Scots pine seeds after cleaning. Every sample consisted of 4 × 100 seeds. The number of empty seeds was determined by cutting.

Sample No.	Number of empty seeds in replicates	Mean percentage of empty seeds	Chi square	P
1	6, 1, 6, 7 (20)	5.0	3.16 (3 d. f.)	$0.50>P>0.25$
2	5, 12, 5, 4 (26)	6.5	5.10 (3 d. f.)	$0.25>P>0.10$
3	3, 9, 4, 9 (25)	6.3	3.54 (3 d. f.)	$0.50>P>0.25$
4	9, 2, 3, 7 (21)	5.3	4.57 (3 d. f.)	$0.25>P>0.10$
1—4	(92)	5.8	1.20 (3 d. f.)	$0.90>P>0.75$

sampling technique gave unbiased samples from the collections. The variations between the proportions of seed with a defined embryo development in repeated samples from a collection satisfied tests of homogeneity (chi square tests after SNEDECOR 1957).

These results were also the basis for the method used in the determinations of empty seeds in the germination tests. Instead of cutting every seed without a protruding rootlet at the end of the germination experiments (cf. *e.g.* HUSS 1951) the mean number of empty seeds per sample was determined by direct cutting of 4×100 seeds. These samples were taken in the same way as the samples which were used for experiments. This determination was repeated every time seeds from a new sub-collection were used. An illustration of results with this method is given in table 3.

B. Method for the Germination Tests

All germination tests were done in Jacobsen apparatuses (for a general description, cf. BALDWIN 1942, HUSS 1951) if not otherwise stated. Also in the experiments in which embryo-mitosis and respiration were studied the seeds were kept in these apparatuses till time for the special investigations.

Each apparatus consisted of a water container ($65 \times 70 \times 16$ cm) and a perforated cover, both made of stainless plate. The containers were filled with tap water. The distance between the water level and the cover was kept at 8 cm. This was important for maintaining constant and optimal moisture conditions in the germination beds (SCHMIDT 1930, HUBER and MERKENSCHLAGER 1951, KAUSCH 1952). The germination beds, which received water from the container through wicks of filter paper, consisted of mats woven of cotton yarn covered with filter papers (cf. HUSS l.c.). Glass bell jars were placed over the germination beds. For experiments in continuous white light bell jars made of colourless glass (constant spectral transmission between 3600 and 10000 Å), for experiments in darkness similar bell jars made of black, nontransparent glass (Skruf) were used. With these two types of bell jars simultaneous experiments with continuous irradiation and darkness could be performed in the same apparatus, which was irradiated by a bank of white fluorescent tubes (cf. p. 16, see also SARVAS 1950). Control experiments with germination in darkness under such conditions and in darkness without any irradiations of the apparatus showed that the bell jars were lightproof.

Six apparatuses were used in two temperature-regulated rooms, three in each one. In experiments with continuous irradiation the temperature was controlled by a thermostatically regulated refrigatory system; in experiments without such an irradiation a

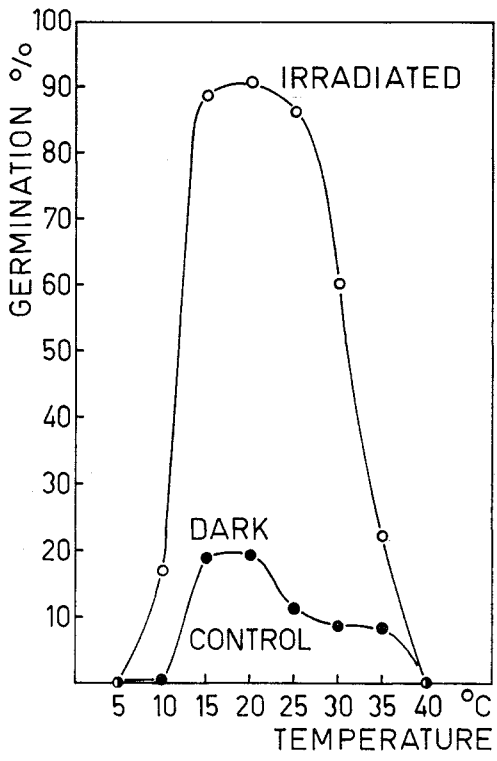

Figure 2. Effect of the temperature on germination in darkness of Scots pine seeds irradiated in unimbibed condition (white fluorescent tubes TL/33, 1700 lux, 4 hours). Dark control seeds unirradiated. Provenance No. 3.

thermostatically regulated heat-blower was used. The temperature of the germination beds was measured with thermometers thrust down into specially drilled holes through the top of a colourless and a dark bell jar. In experiments with continuous irradiation no difference in temperature between the germination beds under the respective type of bell jar could be found on measurement with this equipment.

As a standard temperature for the germination tests as well as for other experiments (measurements of the respiration, cf. Chapter VI) +25° C was selected. This was based on the results obtained by HAACK (1912), who found that this temperature was the most suitable one for the germination of Scots pine seeds. Also SCHMIDT (l.c.) stated that the same temperature was the optimal one for the germination of coniferous seeds in general. However, in this connection it may be pointed out that after the international rules for seed testing (ANONYMOUS 1959) the Scots pine seeds shall be tested at alternating temperatures (16 hours at +20° C, 8 hours at +30° C). Because alternating temperatures decrease the light dependence of the germination in Scots pine seeds (HAACK l.c., ROHMEDER 1951) as in many other light-requiring seeds (cf. *e.g.* reviews by EVENARI 1956 and in press) and thus is a problem *per se* which is not dealt with in this investigation, it was decided to perform all germination tests at a constant temperature (+25.0 ± 0.2° C).

Besides this effect of alternating temperatures it has also been shown in many cases (cf. EVENARI l.c.) that the light dependence of the germination can vary at different constant temperatures. For the Scots pine seeds HAACK (1906) found that the greatest difference between the germination in light and darkness appeared at +23° C. A similar

experiment was performed here, in which the germination tests were carried out in Petri dishes in darkness in thermostats with different temperatures (results in figure 2). Because there were no facilities for continuous irradiation of the seed samples in such an experiment, half of the seed samples were stimulated by irradiating the unimbibed seeds before the start of the germination tests. The results showed a maximal difference at +25° C in the following germination of all the seed samples in darkness. The evidence that this temperature was slightly supraoptimal could not be confirmed in the experiments with Jacobsen apparatuses, where nearly complete germination (100 per cent) could be obtained (cf. *e.g.* figures 9 and 10).

Concerning the length of the germination period this was extended to 30 days in preliminary investigations. This was in accordance with HUSS (1951) (cf. 21 days in ANONYMOUS l.c.). However, the results showed that practically no further germination was obtained with the actual seed materials, either in continuous irradiation, or in darkness, between the 20th and the 30th day of the germination period. For that reason the experiments were terminated after 20 days (cf. also the use of 40 days in special experiments, figures 18 and 19; see also the field experiment in figures 12 and 13). In most of the experiments the course of the germination has been followed day by day. The seeds with a rootlet length of 5 mm or longer have been registered each day and removed from the germination beds. In the last registrations of every experiment all seeds with signs of incipient germination (protruding rootlets) have been included regardless of their state of development. The registrations also included all germinated seeds irrespective of their appearance. The germination percentages were calculated on the number of full seeds (concerning empty seeds, cf. above p. 12). If not otherwise stated the results are given as the mean values from experiments on 4×100 seeds.

C. Radiation Sources and Filters

1. Safelight. As remarked earlier (see p. 9) all handling of the seeds was performed in safelight. In view of the fact that HAACK (1906) found in the germination of Scots pine seeds under continuous irradiation blue light had the slightest stimulating effect compared with light qualities of longer wavelengths and that in 1912 the same investigator reported that intermittent blue irradiation did not influence the germination of the same species, a blue safelight was considered to be the most suitable one at the start of the present investigation. For this purpose a blue incandescent lamp (Philips 25 W) was used. This was in accordance with NORDSTRÖM (1953 a and b). A spectrophotometric determination of the transmission curve for its lamp-glass (performed with a Beckman spectrophotometer Model B, also used in corresponding investigations below) showed a peak at 4000 Å (80 per cent transmittance), less than 1 per cent transmittance in the region 5750—6750 Å and then gradually increasing with longer wavelengths. The safelight from this source was used in the experiments, the results of which are presented in figures 1 (No. 1, 2), 9, 10, 14—19, 43 and tables 10, 11, 19 and 24.

However, the establishment of a red—far-red sensitive mechanism in the seeds (NYMAN 1957) made it expedient to use a safelight more suitable for the material. Such a one was adopted from WITHROW and PRICE (1957) and used in the rest of the experiments (green safelight, see figure 3). Comparative control experiments were done concerning eventual physiological effects (stimulation and inhibition of the germination) of the blue and green safelights employed. The preparation of the seeds was carried out in green safelight. After the start of the germination experiments (in darkness) the seeds

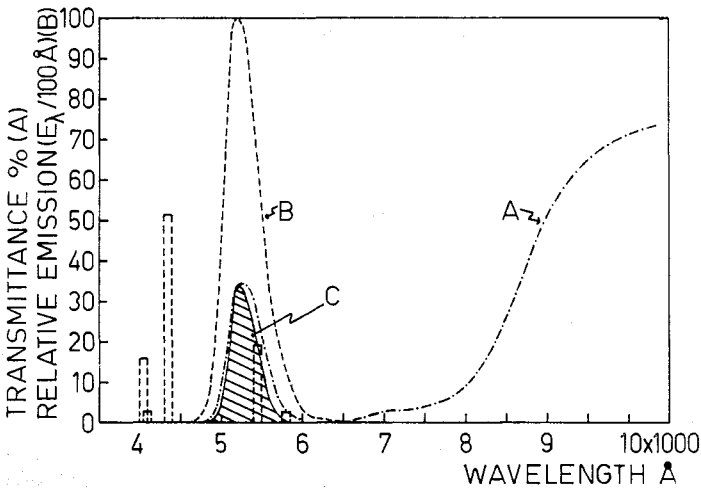

Figure 3. A: Spectral transmission of a green filter made after WITHROW and PRICE 1957. The transmission curve was determined with a Beckman spectrophotometer Model B. B: Relative spectral emission from a green fluorescent tube (Philips TL 10 W/17). After data from Svenska AB Philips. C: Spectral composition of the green safelight obtained by a combination of A and B.

were exposed daily to the blue or the green safelight under conditions corresponding to those observed in the daily registrations of the germination experiments. Control seeds were left unirradiated for the entire experimental period (for testing stimulatory effects) or given only a limited irradiation after 6 hours of imbibition for induction of complete germination (for testing inhibitory effects). The results given in table 4 show that both types of safelights did not influence the germination. Neither have any further indications been found suggesting effects of the safelights on the unimbibed seeds during the preparatory work before the start of the experiments (cf. *e.g.* the germination percentages of the dark control seeds and of the maximally far-red inhibited seeds in figures 23 and 24).

Table 4. Control experiments concerning the safelights used in the investigation. Provenance No. 3.

Irradiation	Safelight	Germination percentage (after 30 days)
White light (TL/33, continuous, 350 μW/cm²)	—	98 ± 1
White light (TL/33, 350 μW/cm² for 60 min. after 6 hours of imbibition)	—	94 ± 1
	Green	94 ± 1
	Blue	93 ± 1
—	—	11 ± 2
—	Green	7 ± 1
—	Blue	10 ± 2

Table 5. Relative spectral emission from white fluorescent tubes (Philips TL/33). After data from Svenska AB Philips.

Wavelength range Å	Colour	Relative spectral emission
3800—4200	Far violet	0.02
4200—4400	Violet	0.67
4400—4600	Blue	0.67
4600—5100	Blue-green	10.28
5100—5600	Green	71.98
5600—6100	Yellow	100.00
6100—6600	Light red	17.74
6600—7600	Dark red	0.42

2. White light. In accordance with SARVAS (1950) the germination experiments with continuous irradiation were done in Jacobsen apparatuses (cf. above p. 12), over which banks with white fluorescent tubes were placed. One bank with eight tubes (Philips 20 W/33) was placed 80 cm above each apparatus. The spectral composition of the light

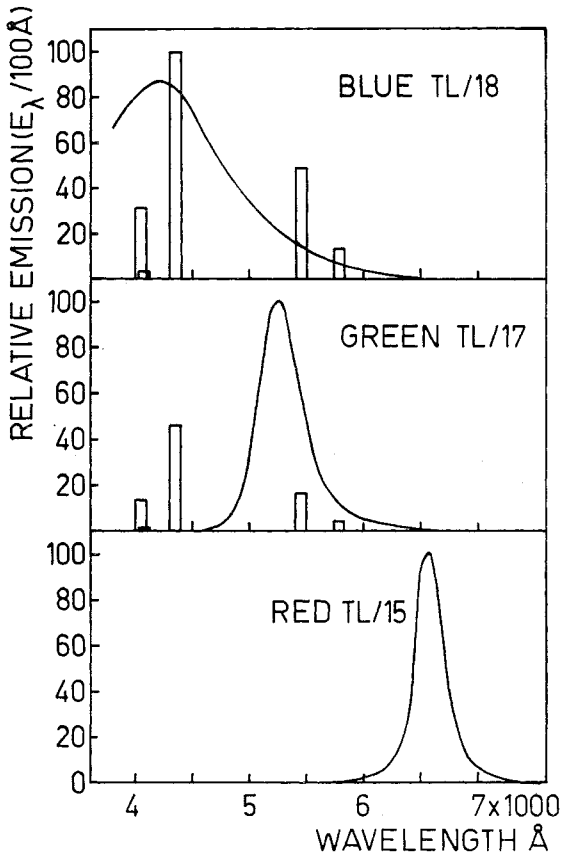

Figure 4. Relative spectral emission from blue, green and red fluorescent tubes (Philips TL/18, TL/17 and TL/15, respectively). After data from Svenska AB Philips.

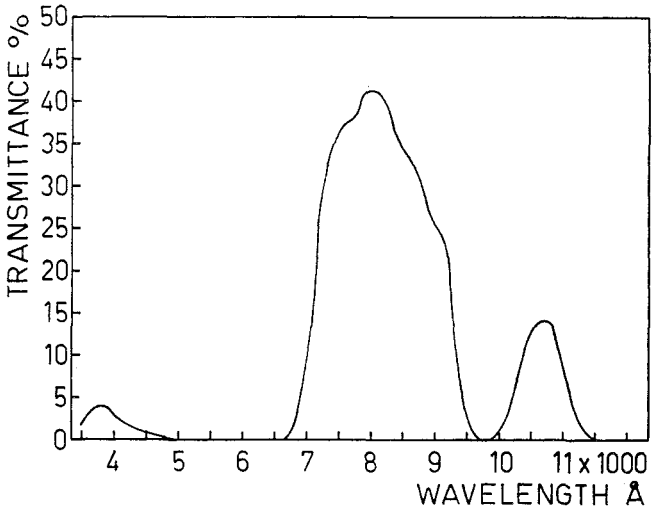

Figure 5. Spectral transmission of the compound filter (water—red-blue cellophane) used in the standard far-red irradiations.

from this source is given in table 5. The mean irradiance (measured under the colourless glass bell jars) was 1700 lux. Measurements at different places in the apparatuses showed a variation of the irradiance reaching ±200 lux, but these variations did not influence the germination responses. The same light source also was used in experiments in which only limited irradiations for induction of the germination were performed. In these cases the seeds were placed in a restricted area with uniform irradiance.

3. Standard red and far-red light. As an irradiation source in the standard red irradiations a bank with eight red fluorescent tubes (Philips TL 20 W/15) was used. The spectral composition of the light from this source is given in figure 4, "Red TL/15." The bank was placed 54 cm above a Jacobsen apparatus in a separate, thermostatically regulated room (+25° C). The approximate irradiance was 350 μW/cm^2 measured under the colourless glass bell jars, which covered the germination beds during the irradiations.

As a source for standard far-red irradiations (cf. *e.g.* Downs *et al.* 1957) a combination of an incandescent lamp (Osram 500 W), a water filter 10 cm deep (with a cooling coil of glass just below the surface of the water) and a filter composed of double layers of a red and a blue cellophane (18×20 cm) was used. From data on the spectral absorption of water in Withrow and Withrow (1956) and the measured transmission curve of the combined layers of double red and blue cellophane, the transmission curve of the water—red-blue cellophane filter was calculated. See figure 5. Through adjustment of the distance between the incandescent lamp and the water filter an approximate, total irradiance of 350 μW/cm^2 was obtained in the irradiation cabinet directly under the red-blue filter. Thirty-six per cent of this total radiation was found in the wavelength region 7000—8000 Å (integrated from the complete transmission curve, of which the part from 10000—12000 Å was calculated on the assumption that the transmission of the red-blue filter was constant in this region). In this cabinet four germination beds with seeds placed in open Petri dishes (7 cm diameter) were irradiated at the same time. The whole equipment was

placed in the same room as the equipment for the standard red irradiations (cf. above). Control measurements of the temperature in the irradiation cabinet never showed a greater increase of the temperature than 1—2° C during the irradiations.

4. Other coloured light. Besides the use of white and red fluorescent tubes (cf. above) also green and blue ones were employed in one of the experiments (see figure 22). A comparison of the spectral emission from all these types of coloured fluorescent tubes is given in figure 4.

For further studies on the effect of light in different, narrower wavelength regions (action spectra) an apparatus with interference filters has been used. The principal features of this were adopted from MOHR and SCHOSER (1959). In accordance with this a projector was used as the radiation source (Prado 500, Leitz, Wetzlar; objective Hektor f = 20 cm, 1 : 2.5; incandescent lamp Osram 588880 EasZ, 110 V, 500 W). This apparatus was connected with a variable transformer (Sundberg LA 8) for the regulation of the voltage to the projector lamp and another transformer (type Rstuu 500 VA, Leitz, Wetzlar) by means of which the input voltage on the variable transformer could be controlled and kept at 110 V. The projector was placed in a dark cabinet (68 × 54 × 40 cm) with a blower for ventilation. The objective was placed against a circular aperture (6 cm) in the wall of this cabinet. Through this aperture the light beam entered the upper part of a connected cabinet (33 × 107 × 40 cm). In this the light was reflected downwards with a plane, silver-surfaced mirror (10 × 10 cm, Jungner) on an object holder (60 cm below the centre of the mirror). The seeds to be irradiated were placed on the holder in the centre of the irradiated area (14 × 14 cm). If not otherwise stated 4 × 100 seeds were irradiated at the same time. In the irradiations the seeds were placed in a single layer on a square germination bed. The individual samples were separated by a frame (6.5 × 6.5 cm) of plexiglass bars with four separate compartments, each for 100 seeds. For further details in this connection, the reader is referred to the different experiments. The whole equipment was placed in a dark room without temperature regulation. All irradiations were performed at room temperature (+18— +22° C).

This equipment was combined with interference filters, which were placed in the slide-holder of the projector. The filters were second-order transmission interference filters of the Fabry-Pérot type, supplied by the manufacturer with supplementary filters for elimination of transmission peaks of other orders (5 × 5 cm, Filtraflex B-40, Balzers, Lichtenstein). For the wavelengths 4075, 5500, 6080, 7125 and 9575 Å corresponding filters made by Schott, Jena, were used. All the filters were tested in a spectrophotometer (Beckman Model B) in the wavelength region 3200—10200 Å. The transmission curves are given in figure 6. None of the investigated filters showed any further transmission peaks. When using the filters with transmission maxima at wavelengths less than 7750 Å a heat-absorbing filter (Schott KG 1, 5.5 mm, transmission curve in MOHR and SCHOSER l.c., figure 5) belonging to the projector was placed between the lamp and the interference filter.

In the experiments on the action spectra one and the same irradiance (100 $\mu W/cm^2$) was used at the different wavelengths. This irradiance could be produced with maximal voltage (110 V) in combination with the filter 4100 Å. At longer wavelengths the same irradiance was obtained by decreasing the applied voltage to the lamp (cf. WITHROW and WITHROW l.c.). In some experiments with red (6600 Å) and far-red (7300 Å) light also neutral filters (Kodak-Wratten ND 01-20) in combination with a varied voltage were used for adjustment of the irradiances.

5. Measurements of the irradiance. The measurements of the irradiance of white light were performed with a selenium photocell calibrated in lux (EEL, Evans Electro-

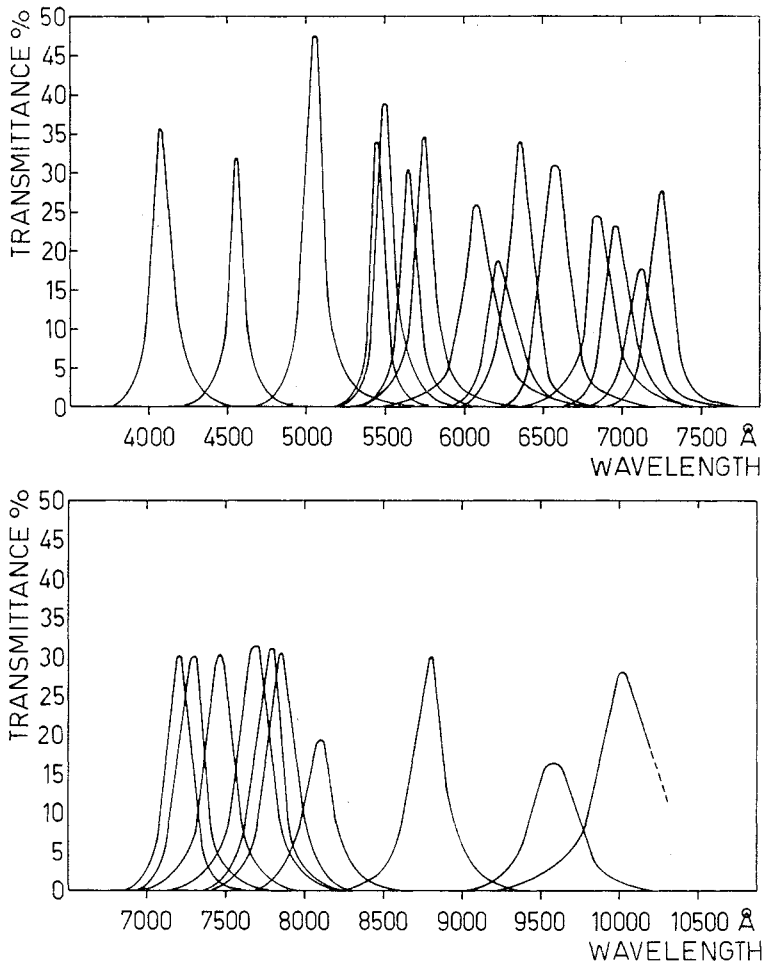

Figure 6. Spectral transmission of the interference filters used determined with a Beckman spectrophotometer Model B.

selenium Ltd, Harlow, Essex). For measurements of the irradiance of coloured light a compensated thermopile (E 20, Kipp & Zonen, Delft) connected with a galvanometer (A 70, Kipp & Zonen, Delft) was used. This equipment was calibrated by the manufacturer (1 erg/sec. corresponded 3.1×10^{-8} V) and gave a deflection of 1 scale division per 500 ergs/cm² sec. (50 μW/cm²) with a slit-width of 1 mm on the thermopile.

In the measurements with the apparatus with interference filters (cf. above) the holder for the germination bed was replaced with the thermopile. In order to get the same irradiance when using different filters through adjustments of the voltage to the projector lamp relative measurements also were made, in which the thermopile was placed nearer the mirror (10 cm). In this way greater deflections on the galvanometer were obtained, which permitted a more accurate choice of the proper voltages.

D. Statistical Methods

In the presentation of the experimental results in the figures the means are given. In the tables calculated standard errors also are given together with the means. In the cases where the means are expressed as percentages (from germination experiments), the standard errors were calculated from the formula $\sqrt{pq/n}$, in other cases from the formula $\sqrt{S(x - \bar{x})^2/n(n-1)}$. In estimations of the significances for differences between means (expressed as percentages from germination experiments) the chi square method or variance analysis after arcsin transformation was used, in other cases variance analysis without such a transformation (BONNIER and TEDIN 1957, SNEDECOR 1957). For further special, statistical methods, the reader is referred to the different experiments.

E. Other Methods

Concerning further special methods, see the method section in the different chapters.

Chapter III. Studies on the Germination — Effect of Irradiations with White Light

A. Introduction

As pointed out in the general introduction (cf. p. 7), also seeds from coniferous trees show a light-influenced germination. This has been only sparsely observed in general reviews of the light germination problem. In the review of forest tree seed by BALDWIN (1942) it is also remarked that in the most cases light is not necessary but often stimulates the germination rate. Actually there are several investigations on tree seeds, in which the essential importance of light for the germination of such seeds has been shown. An attempted survey of this literature, restricted to the genus *Pinus* is given in table 6 (for *P. silvestris* L., see in the text below). This survey shows that a light-stimulated germination is a widespread phenomenon in this genus.

In the first investigation on seeds of *Pinus silvestris* L. by HAACK (1906) it was shown that a limited irradiation (2.5 hours per day) with white light of low irradiance doubled the germination percentage compared with that in darkness. HAACK (1912) confirmed this and showed that a complete germination could be attained under continuous irradiation with only 10 lux

Table 6. A survey of *Pinus*-species with light-stimulated germination. For *P. silvestris* L., see the text.

Species	References
P. banksiana Lamb.	HEIT & ELIASON 1940[1], HEIT 1958
P. caribaea Morelet	NELSON 1940, WAKELEY 1951
P. contorta Dougl.	ALLEN 1941, PARROTT 1942
P. contorta var. *latifolia* Engelm.	HEIT 1958
P. densiflora Sieb. & Zucc.	HASEGAWA & FURUKAWA 1953, HASHIMOTO *et al.* 1954, IWAKAWA & KOTANI 1954, HASEGAWA & FURUKAWA 1955, ISIKAWA & ARAKI 1955 (q.f. TOOLE *et al.* 1962), FURUKAWA 1956, ASAKAWA 1956, 1959, GOO 1956
P. echinata Mill.	NELSON 1940, PARROTT 1942, WAKELEY 1951
P. elliottii Engelm.	JONES 1961
P. mugo Turra	PARROTT 1942, HEIT 1958
P. nigra Arnold	CIESLAR 1885, PITTAUER 1912, 1914, HEIT & ELIASON 1940, HEIT 1958
P. rigida Mill.	HEIT 1958
P. palustris Mill.	NELSON 1940, PARROTT 1942, WAKELEY 1951
P. ponderosa Laws.	HEIT & ELIASON 1940[1]
P. strobus L.	SCHWAPPACH 1906, HAACK 1906, PITTAUER 1912, ASAKAWA 1957, TOOLE *et al.* 1962
P. taeda L.	NELSON 1940, WAKELEY 1951, LANE 1957, TOOLE *et al.* 1958, JONES 1961, TOOLE *et al.* 1962
P. thunbergii Parl.	HEIT & ELIASON 1940, HASEGAWA & FURUKAWA 1953, HASHIMOTO *et al.* 1954, ISIKAWA & SHIMOGAWARA 1954, IWAKAWA & KOTANI 1954, HASEGAWA & FURUKAWA 1955, ISIKAWA & ARAKI 1955 (q.f. TOOLE *et al.* 1962), FURUKAWA 1956, GOO 1956, ASAKAWA 1956, 1959, HEIT 1958, ASAKAWA & INOKUMA 1961
P. virginiana Mill.	TOOLE *et al.* 1956 b, LANE 1957, TOOLE *et al.* 1961

[1] The authors have remarked that light probably affects the germination.

(from an incandescent lamp). Comparable results were obtained by ELIASON and HEIT (1940), who found that a continuous irradiation of such a low irradiance, which barely permitted an observation of the germination beds, induced nearly complete germination. Also SARVAS (1950) has made corresponding observations. On reduction of the irradiance from about 2000 lux (from fluorescent tubes) to about 40 lux (in daylight) the same germination responses were obtained, but the germination rate was somewhat less at the lower irradiance. Concerning the required irradiation time HAACK (1912) found that an irradiation of 8 hours per day with 10 lux gave the same germination responses as in continuous light. However, 2 and 4 hours' irradiation per day was not sufficient for full stimulation. An irradiation of only 5 minutes per day was without any effect. Also VAARTAJA (1956) found that continuous irradiation with white light from fluorescent tubes (about 120 lux) gave a greater germination than daily irradiations of 5 hours (supplemented by indirect sunlight, about 200—500 lux for 7 and 5

hours per day). On the other hand, Huss (1961) obtained the same germination responses with a daily irradiation of 1 hour as in complete daylight.

In addition to the above papers stimulating effects of indirect daylight on the germination of Scots pine seeds have been described also by Atterberg (1906), Pittauer (1912), Schmidt (1930), Heit and Eliason (1940), Parrott (1942), Stefansson (1950), Rohmeder (1951), Nordström (1953 a and b)and Bergman (1959). Heit (1958) obtained the same results with continuous, artificial light (also with an irradiation of 8 hours per day).

These stimulating actions of repeated or continuous irradiations have in all these cases resulted from irradiations performed on imbibed seeds. It has been generally accepted that seeds dependent on light for their germination react to irradiations only after the start of the imbibition (Evenari 1956). However, Nordström (l.c.) has reported that an irradiation of unimbibed Scots pine seeds with direct sunlight or with UV-light could increase their germinative capacity in the following germination in darkness, an effect also found by Huss (l.c.). Similar results from irradiation experiments with light from white fluorescent tubes have been preliminarily reported by the author (Nyman 1957) and will be further dealt with in the present investigation (cf. p. 30). Bergman (1957) found no stimulating effects of a preirradiation with sunlight on the following germination "in light" but remarked that in sowing experiments the irradiated seeds yielded more seedlings than the unirradiated ones. However, also Furukawa (1956) has found that an irradiation of unimbibed seeds of *Pinus thunbergii* Parl. and *P. densiflora* Sieb. & Zucc. could increase the following germination in darkness. From experiments with lettuce seeds results have been described (Evenari and Neumann 1953 b, Borthwick *et al.* 1954) indicating light effects on the unimbibed seeds (cf. also the remarks in Evenari in press). In connection with this it may be pointed out that Jensen (1941, 1942, 1945) could increase the length of life in a number of ornamental and vegetable seeds after an irradiation of the unimbibed seeds.

In many species it has been shown that light-stimulated seeds after a prolonged stay in imbibed condition in darkness no longer react to irradiations (development of skotodormancy, Evenari 1956, cf. also Evenari in press). This phenomenon was not found in Scots pine seeds (Eliason and Heit l.c., Sarvas l.c., Nordström l.c., Huss l.c.). However, Huss found that the age of the seeds influenced this phenomenon. The increased germination after a change of the germination conditions from darkness to light was less in older seeds.

Concerning the interaction of light and temperature this has been studied in some cases with Scots pine seeds. Haack (1906, 1912) found that light stimulated the germination at all investigated temperatures between

+ 15° C and + 33° C. Contrary to these experiments with constant temperatures ATTERBERG (l.c.) and ROHMEDER (l.c.) found an increased germination in darkness with alternating temperatures, but such temperature conditions could not completely substitute the stimulating effect of the light. HEIT (l.c.) obtained no effects in this respect. On the other hand, HEIT and ELIASON (l.c.) and ELIASON and HEIT (l.c.) have found that the Scots pine seeds, after subjecting the imbibed seeds to + 2—4° C for 20—25 days (stratification or pre-chilling), germinated to the same extent in darkness as in light. VAARTAJA (l.c.) found no effect on the light dependence in the same species after a treatment of a similar kind (+ 5° C for 10 days).

Also studies on the effect of storage have given different results. SCHMIDT (l.c.) found that seeds, which at the start of the experiment had a difference between the germination percentages in light and darkness amounting to 44 per cent, after storage for 4 months no longer showed any difference between these germination responses. Also NORDSTRÖM (l.c.) and HUSS (l.c.) have found a decreased light dependence with increased age of the seeds (from comparison between seeds of different provenances and different ages). Contrary to these results ELIASON and HEIT (l.c.) could not observe any increase of the germinative capacity in darkness of seeds which had been stored for 3 years.

Mechanical treatments have been found to increase the germination in darkness. Thus NORDSTRÖM (l.c.) found that the administration of repeated shocks to the unimbibed seeds for shorter periods stimulated the subsequent germination in darkness but for longer periods decreased the germinative capacity. In germination tests in light all the treatments caused a decrease of the germinative capacity, which was greater the longer the period of treatment (cf. also HUSS 1950). Other mechanical treatments as puncturing or removal of the seed coats have been preliminarily reported to affect the light dependence of the germination (NYMAN l.c. and 1961). This will be further dealt with below (p. 40). SCHMIDT (l.c.) has also mentioned the importance of an intact seed coat for the light dependence at the germination.

The seed origin as a significant factor for the light-dependent germination in Scots pine seeds has been studied in some cases. Thus SCHMIDT (l.c.) found that seeds collected from separate trees showed different responses to the light factor in their germination. The trees which produced the most light-dependent seeds also during the following two years produced seeds that showed the greatest differences between their germination responses in light and darkness. A corresponding result has been obtained by HEIT (l.c.) with seeds from one tree of *Pinus mugo* Turra. About the same light-darkness difference was found in the germination with seeds that were collected at four different times during a period of fourteen years. Also in

Scots pine seeds of diverse provenances SCHMIDT (l.c.) found different magnitudes of the light-darkness responses. These could be confirmed during the following years. ELIASON and HEIT (l.c.) found that Scots pine seeds from Europe were less dependent on light for germination than seeds of an American origin, a case also described by HEIT (l.c.) in a comparison between a greater number of seed provenances. For seeds of Scots pine from Sweden NORDSTRÖM (l.c.) has pointed out that seeds from the northern part of the country should be more dependent on the light factor for their germination and that this ought to be correlated with the degree of ripeness of the seeds (see further below). For a general review of this and connected problems, see also BALDWIN (1933, 1942).

From the above survey it is evident that the stimulating action of irradiations with artificial white light or indirect daylight is a well-established fact for seeds of Scots pine. This also seems to be valid for other pine species hitherto investigated. However, it may be noticed that there are some cases in which no effects have been found: *Pinus silvestris* L.—MAYER and von PESCH (1882) (q.f. ATTERBERG l.c.); SCHLICH (1904) (q.f. BALDWIN 1942); *P. ponderosa* Laws.—ALLEN (1941); *P. resinosa* Ait.—HEIT (1958); *P. strobus* L.—LANE (1957).

In the present chapter some experiments will be described in which the influence of some of the above-mentioned factors on the light-darkness responses have been further investigated.

The foregoing statements concerning the importance of the seed provenances gave rise to experiments, in which the germination responses in continuous white light and darkness were compared between seeds of different Swedish provenances. In connection with this the influence of the seed ripeness on the light dependence was investigated. From a number of authors (HAGEM 1917, HEIKENHEIMO 1921, OLDERTZ 1921, WIBECK 1920, 1928, 1929, KUJALA 1927, SIMAK and GUSTAFSSON 1954) it is known that the embryo in Scots pine seeds from regions with colder climate can be weakly developed and that the embryo development (embryo ratio: by WIBECK 1928 and OLDERTZ l.c. defined as the ratio between the length of embryo and endosperm) is correlated with the germinative capacities of the seeds. For that reason it seemed pertinent to investigate the existence of a connection between the morphological ripeness as it is expressed in the embryo ratio and the light dependence. With the use of an X-ray technique developed by SIMAK and GUSTAFSSON (1953 a and b) it was possible to study the embryo developments and the light reactions in the same individual seeds.

In connection with the provenance studies also the influence of irradiations on unimbibed seeds was investigated and compared in laboratory

and field tests. The last type of experiments is related to the discussion about the importance of the light factor for the germination of Scots pine seeds under natural conditions (HAACK 1906, WIKSTEN 1948, SARVAS l.c., ROHMEDER l.c., NORDSTRÖM l.c., BERGMAN 1957, RICHTAR 1959, HUSS l.c.).

Finally, the widespread occurrence of seeds with light-controlled germination, in which the light control is connected with the presence of an intact seed coat (EVENARI 1956 and in press, cf. also SCHMIDT l.c.), made it advisable to investigate further the same phenomenon in the Scots pine seeds.

B. Methods

The germination experiments described in this chapter were performed—if not otherwise stated—in the same manner as described in Chapter II (p. 12). A comparative field experiment has been carried out in a nursery (Ådalen, Bogesund, Stockholms län). See further the description of this experiment below (p. 30).

For all irradiation of the seeds, both for the germination experiments in continuous light as well as in experiments in which the seeds were irradiated before the start of the germination tests (in unimbibed condition), the same radiation source has been used (white, fluorescent tubes Philips TL 20 W/33, cf. also p. 16). The different irradiances were obtained with different distances between the radiation source and the seed material.

The method used for the X-ray investigations corresponded principally to the method which has been described by MÜLLER-OLSEN and SIMAK (1954). The X-ray apparatus employed (Schönander TEA-25) has been described by SIMAK and GUSTAFSSON (1953 a). Data for the exposures were: 10 mA, 10 kV, time 2 sec., focal distance 25 cm, film Ilford X-ray (Ilflex). From the X-ray photographs the seeds were grouped in different embryo-classes depending on the size of the embryo in relation to the size of the embryo-cavity. For definitions of the different embryo-classes, see page 38. These definitions are principally the same as in SIMAK and GUSTAFSSON (l.c.) and MÜLLER-OLSEN and SIMAK (l.c.) with the exception for class III, which here has been made broader. The mutual positions of the seeds on the X-ray photographs were maintained during the germination tests, which were carried out with 50 seeds per germination bed.

C. Results

1. *Effect of Irradiations on Imbibed Seeds*

a. Effect on seeds of different provenances. In order to investigate an eventual connection between seed provenance and light dependence for the germination seeds of different origins were simultaneously germinated in continuous white light and darkness. The origins of the seeds were chosen so as to represent as wide a distribution as possible in a north-south direction (with Swedish Scots pine seeds) and at the same time localities in a range of

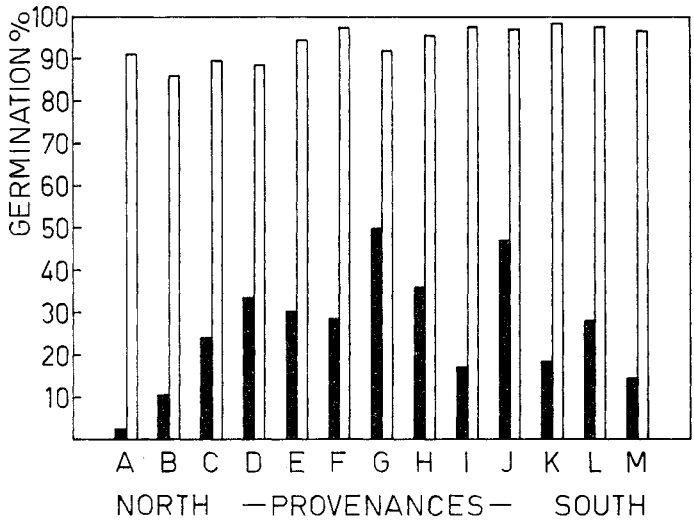

Figure 7. Germination responses of Scots pine seeds of different provenances under continuous irradiation (empty columns, white fluorescent tubes TL/33, 1700 lux) and darkness (filled columns). For details about the provenances A (9556)—M (9564), see table 1. Seeds collected in 1957.

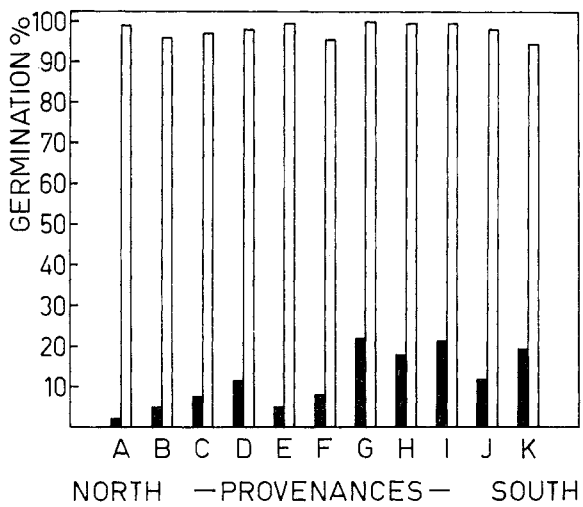

Figure 8. Germination responses of Scots pine seeds of different provenances under continuous irradiation (empty columns) and in darkness (filled columns). Irradiation as in figure 7. For details about the provenances A (10348)—K (10350), see table 1. Seeds collected in 1958.

altitudes as narrow as possible. The experiment was repeated the following year. The seeds from year 1957 came from localities situated between the northern latitude 66°55′—55°40′ in the range of altitude 40—250 m above sea level (cf. table 1, A(9559)—M(9564), provenance Nos. 5—17). Corresponding data for the seeds from year 1958 were: northern latitude 65°59′—55°40′, range of altitude 40—80 m above sea level (cf. table 1, A(10348)—K(10350), provenance Nos. 18—28). The results from the experiments with these seeds are given in figures 7 and 8, respectively.

In all the cases the stimulating action of the continuous irradiation with white light was evident. There were no certain trends indicating an increasing importance of the light factor for the germination of seeds from more northern localities. Of course, such a trend can be traced in the results in figure 8, but the material does not permit a definite conclusion on this point. However, it may be noticed that in both the experiments the lowest germination percentages in darkness have been found in the seeds from the most northern localities (A in figure 7 and A in figure 8).

Some experiments were also done with seeds from localities representing a wide altitude range in as narrow a latitude range as possible. Experiments with seeds from two ranges of northern latitude were performed (65°59′—64°5′ and 60°15′—61°15′, respectively). In the first case the altitudes were 70—290 m (provenance Nos. 18, 19, 29—31, see table 1), in the second one 15—500 m above sea level (provenance Nos. 23, 32—36, see table 1). In the first series the germination percentages in darkness varied between 1 and 5 per cent, in the second one between 1 and 26 per cent, where also a gradually decreasing germination in darkness was obtained with increasing altitude of the localities. No corresponding decrease of the germinative capacity in continuous light was found. Thus the inconsistent results in these last two experiments do not permit any definite conclusion concerning the influence of the altitude on the germination in darkness.

In the above experiments the degrees of light dependence have been evaluated only from the differences between the germination percentages in light and darkness. A further attempt to a comparative investigation of the sensitivity to an irradiation in seeds of different provenances will also be described below (see p. 30).

b. Effect of storage. In order to investigate the influence of storage on the light dependence of the germination the following experiments were undertaken.

The cleaned and sampled seeds were transferred to small glass tubes with rubber stoppers (100 seeds per tube) and stored under the same conditions as described in Chapter II. After different periods of time the germinative capacities in continuous white light and darkness were tested

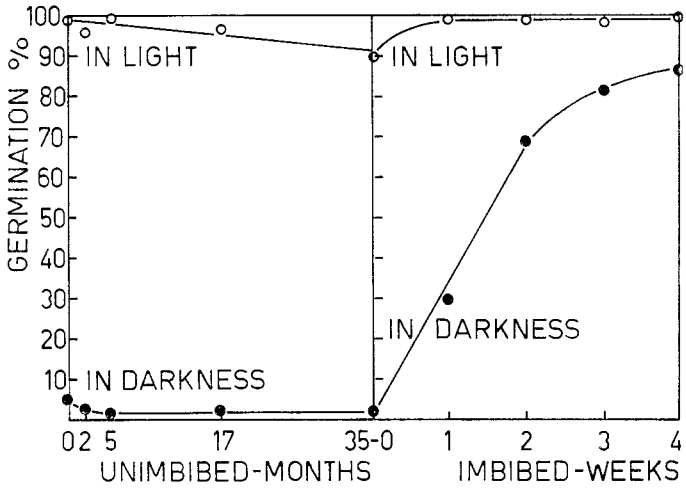

Figure 9. Germination responses of Scots pine seeds under continuous irradiation and darkness after storage in unimbibed and imbibed condition in darkness at + 3—4° C. Irradiation as in figure 7. Provenance No. 2.

with the usual method. The results, which are given in figure 9, showed that storage even as long as for about 3 years did not influence the germinative capacity in darkness and the corresponding one in light showed only a slight decrease. After the end of this experiment the rest of the seeds were transferred to Petri dishes with moist germination beds and stored there in darkness for a period of 1—4 weeks. After different times the germination beds were placed in a Jacobsen apparatus (at + 25° C) in light and darkness, respectively. The results showed that the germinative capacity in darkness rapidly increased during this treatment (stratification). See also corresponding results in figure 32.

These results showed that even a prolonged storage of the unimbibed seeds did not increase the ability of the seeds to germinate in darkness. Storage of imbibed seeds at the same temperature, however, rapidly caused a loss of the light dependence for the germination.

c. Effect of continuous and limited irradiation. From the review in the introduction to this chapter it is evident that the stimulating action of irradiations on the germination in Scots pine seeds (imbibed seeds) has been shown only in experiments in which the seeds have been irradiated, either continuously or during repeated periods of different duration. The possibility of irradiating for only one limited and shorter period and determining the optimal imbibition period required for the development of the greatest sensitivity to light does not seem to have been investigated, though this has been performed on seeds from many other species with light-controlled germination (cf. Evenari 1956 and in press).

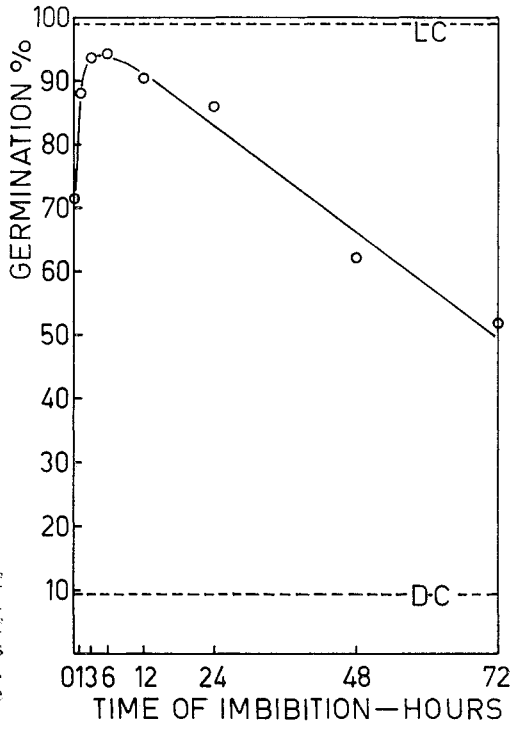

Figure 10. Germination responses of Scots pine seeds after an irradiation for 1 hour with different periods of imbibition. Controls under continuous irradiation (LC) and in darkness (DC). Irradiation as in figure 7. Provenance No. 3.

An experiment arranged to test this possibility was performed in such a way that all the seeds were given a fixed irradiation after different periods of imbibition. Before and after this irradiation the seeds were kept in darkness. Unirradiated (DC) and continuously irradiated (LC) seeds were used as controls in the experiment, the results of which are given in figure 10.

The results showed that the sensitivity had a maximum after around 6 hours of imbibition. With this combination of imbibition period and irradiation about the same germination response was obtained as with continuous irradiation during the whole germination process (the difference was not significant). With longer periods of imbibition the responses decreased. Concerning the imbibition periods shorter than 6 hours it may be observed that an irradiation of the seeds without any preceding imbibition (the seeds were irradiated before they were placed on the moist germination beds—air-dry seeds with about 5 per cent moisture content, cf. also table 7) could increase the following germination in darkness from 10 (dark control) to 70 per cent. Thus, even the unimbibed Scots pine seeds were sensitive to this irradiation with white light (see further below in section 2). The ex-

periment described above was carried out with seeds of provenance No. 3. A corresponding experiment, in which seeds of provenance No. 2 were used showed consistent results. In this connection it may be observed that in a corresponding experiment with a red irradiation (figure 23, provenance No. 3) the optimal period of imbibition was 12 hours. However, the differences in these three experiments between the germination responses obtained with irradiations after 3, 6 or 12 hours of imbibition were small and unsignificant, so an imbibition period of 6 hours was chosen as a convenient standard period for the imbibition in following experiments with limited irradiations on imbibed seeds (see further in Chapter IV).

2. Effect of Irradiations on Unimbibed Seeds

a. Effect on seeds of different provenances. Simultaneously with the experiments with seeds of different provenances (seeds from year 1958, provenance Nos. 18—36, cf. table 1) also the effects of irradiations on unimbibed seeds of the same provenances were investigated. These effects were studied in simultaneous laboratory and field experiments. The seeds used in both these types of experiments were irradiated at the same time (8×100 seeds of each provenance, 4×100 for the laboratory and 4×100 for the field experiment) with white light from a bank with fluorescent tubes. All individual samples were randomized under the bank and irradiated with 2000 lux for 24 hours. Directly after the end of this irradiation the experiments were started. In the laboratory experiment the germination responses of seeds under continuous irradiation and of seeds in darkness with and without the above described preirradiation were compared. In the field experiment the emergence responses were studied in seeds with and without this preirradiation. In the following figures (figures 11, 13) only the results from the experiments on seeds of provenances A (10348)—K (10350) (provenance Nos. 18—28, see table 1) have been given, but in the relevant tables (tables 7, 8, 9) results from all the investigated provenances have been included.

The results from the laboratory experiments are given in figure 11, in which the same results as in figure 8 (dark and empty columns) are reproduced again to permit an easier comparison.

These results showed that in all cases the germination percentages in darkness of the preirradiated seeds were at least three to four times greater than those for the unirradiated ones (see also table 7). All the samples irrespective of provenance reacted to the preirradiation. No evident trend could be traced in the responses, *i.e.*, there was no apparent difference in sensitivity to the irradiation in relation to the origin of the seeds. A com-

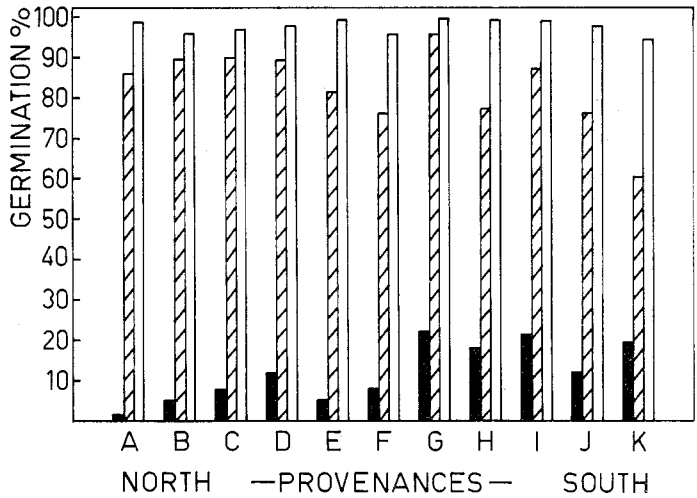

Figure 11. Germination responses of Scots pine seeds of different provenances under continuous irradiation (empty columns), in darkness (filled columns) and in darkness after an irradiation (white fluorescent tubes TL/33, 2000 lux, 24 hours) of the unimbibed seeds (lined columns). The same provenances as in figure 8. Cf. further table 7.

Table 7. Effect of irradiation on unimbibed Scots pine seeds of different provenances in relation to the water content of the seeds at the irradiation. Irradiation as in figure 11.

Provenance[1]	Per cent water of dry weight	Relative germination in darkness[2]	
		Irradiated seeds	Unirradiated seeds
A (10348)[3]	3.3	87	1
c (10317)	3.6	66	3
G (10321)[3]	3.7	95	22
H (10328)[3]	3.7	78	18
e (10366)	3.8	63	6
F (10327)[3]	4.6	80	8
C (10318)[3]	4.8	94	8
I (10304)[3]	5.0	89	22
E (10338)[3]	5.2	82	5
cc (10364)	5.2	71	20
J (10362)[3]	5.3	79	12
aa (10345)	5.3	80	27
d (10367)	5.5	48	4
B (10347)[3]	6.2	94	5
ee (10315)	6.2	85	5
D (10319)[3]	7.0	91	12
K (10350)[3]	7.0	64	21

[1] Cf. table 1.
[2] Per cent of the germination in continuous irradiation.
[3] The same as in figures 8 and 11.

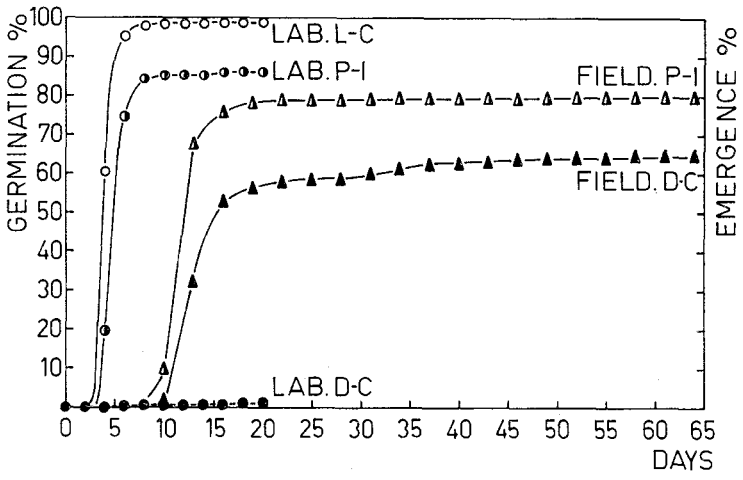

Figure 12. Effect of irradiations on the time course of germination and emergence in comparative laboratory and field experiments with Scots pine seeds. Germination under continuous irradiation (LAB. L-C), in darkness (LAB. D-C) and in darkness after an irradiation (white fluorescent tubes TL/33, 2000 lux, 24 hours) of the unimbibed seeds (LAB. P-I). Emergence of seeds irradiated as above in unimbibed condition (FIELD. P-I) and of unirradiated seeds (FIELD. D-C). Provenance No. 18 (A in figures 8 and 11).

parison between the effects of the preirradiation and continuous irradiation showed that in none of the cases could the preirradiation induce complete germination. The results from an attempt to correlate the responses to this preirradiation with the water content of the seeds (determined as per cent water of the dry weight in separate samples) are given in table 7. No connection between them could be found.

The corresponding field experiment was performed in a nursery. The experiment was arranged in four blocks with randomized distribution of the separate seed provenances in each block. From each provenance 2×100 seeds were sown in each block, 100 preirradiated and 100 control seeds. These samples were sown side by side. The seeds were sown directly from dark glass tubes into the seed furrows ($1 \times 1 \times 50$ cm) and immediately covered with sand. The sowing was performed on July 10, 1959 and the experiment was ended on September 15 of the same year. During the first 10 days the sowing area was covered with mats of reed. Cautious and repeated irrigation was performed during this period. After 10 days the first seedlings had emerged and the results were then registered every third day till the end of the experiment. During the following growing season the experiment was followed till June 10 for registration of delayed germination.

The purpose of this experiment was to investigate whether any effect of

Table 8. Effect of irradiation on unimbibed Scots pine seeds of different provenances. Rate of emergence in a field experiment. For further details, see figure 12.

Provenance[1]	Required number of days for an emergence up to 50 per cent in		Difference
	Irradiated seeds	Unirradiated seeds	
A (10348)[2]	12	17	5
B (10347)[2]	12	14	2
C (10318)[2]	12	13	1
D (10319)[2]	12	13	1
E (10338)[2]	11	13	2
F (10327)[2]	11	12	1
G (10321)[2]	9	11	2
H (10328)[2]	11	12	1
I (10304)[2]	11	11	0
J (10362)[2]	11	12	1
K (10350)[2]	11	11	0
c (10317)	13	58	45
d (10367)	14	46	32
e (10366)	15	> 65	> 50
aa (10345)	11	14	3
cc (10364)	12	15	3
dd (10303)	11	12	1
ee (10315)	12	14	2
ff (10314)	14	22	8
Mean value	12	20 (minimum value)	8 (minimum value)

[1] Cf. table 1.
[2] From the same experiment as in figure 13.

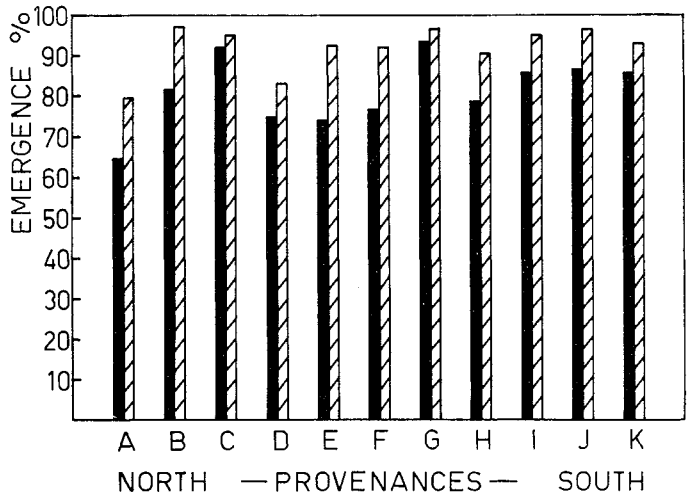

Figure 13. Emergence responses in field experiment with Scots pine seeds of different provenances after irradiation of the unimbibed seeds (lined columns). Control seeds unirradiated (filled columns). The same irradiation and provenances as in figure 11.

the preirradiation could be found under field conditions or whether certain uncontrolled factors in a field experiment (irradiation at the sowing, penetration of light through the cover, action of light-substituting factors) would decrease or eliminate the manifestation of this.

A comparison between the time course of the germination (in the laboratory) and of the emergence (in the field) is given in figure 12 for seeds of one of the investigated provenances (provenance No. 18, A in figures 8, 11, 13). These results showed that both the germination and the emergence of the preirradiated seeds (P-I) were greater than that of the unirradiated seeds (D-C). However, the greater emergence percentage of the unirradiated seeds resulted in a smaller difference between the treatments in the field than in the corresponding laboratory experiment. The reason for this increased emergence cannot be explained, depending on the nature of this special experiment (action of one or more of the above-mentioned uncontrolled factors). Beside the difference between the final emergence percentages in the pre- and unirradiated seeds the results in figure 12 also showed that the emergence was more rapid in the preirradiated seeds. An estimation of the required time for an emergence to 50 per cent in the preirradiated seeds gave 12 days and 17 days for the unirradiated ones (interpolation from the germination curve). Corresponding estimations from the results with all the investigated provenances are given in table 8. These showed that with two exceptions (provenances I and K) all the preirradiated seeds emerged to 50 per cent in a shorter time than the unirradiated ones. The preirradiation caused a stimulation of the germination rate. However, in most of the cases the stimulations were small. Exceptions were the seed provenances c, d, e, and f (provenance Nos. 29, 30, 31, 36, respectively, cf. table 1), which came from localities at higher altitudes (cf. above p. 27).

Beside the emergence rate also the final emergence percentages varied between the pre- and unirradiated seeds. This is shown in figure 13, for the experiments with the provenances A—K (provenance Nos. 18—28 in table 1). In all cases the mean values for the preirradiated seeds were greater than for the control seeds. A statistical test of the significance of the difference irrespective of provenance showed that the effect of the preirradiation was significant ($0.001 > P$). However, the differences between the emergence percentages of preirradiated and control seeds were much less than the corresponding ones in the laboratory experiment (cf. figure 11).

The mean differences between the emergence percentages of pre- and unirradiated seeds are shown in table 9, in which the results from all the investigated provenances are included. These results showed greater differences for seed provenances from localities at higher altitudes (cf. above p. 27).

Table 9. Effect of irradiation on unimbibed Scots pine seeds of different provenances. Effects during subsequent years in a field experiment. Cf. figure 13.

Provenance[1]	Difference in emergence percentage between irradiated and unirradiated seeds during the first year[2]	Difference in emergence percentage between the first and the second year in[3]	
		Irradiated seeds	Unirradiated seeds
A (10348)[4]	15	0	3
B (10347)[4]	16	0	5
C (10318)[4]	3	0	3
D (10319)[4]	8	0	4
E (10338)[4]	18	0	6
F (10327)[4]	16	2	5
G (10321)[4]	3	0	2
H (10328)[4]	12	0	6
I (10304)[4]	10	1	3
J (10362)[4]	10	0	2
K (10350)[4]	8	1	4
c (10317)	27	2	16
d (10367)	20	4	10
e (10366)	26	4	30
aa (10345)	19	1	7
cc (10364)	19	1	6
dd (10303)	18	0	2
ee (10315)	16	0	11
ff (10314)	19	0	10
Mean value	15	1	7

[1] Cf. table 1.
[2] Final observation 15/9-59.
[3] Final observation 10/6-60.
[4] The same provenances as in figures 11 and 13, tables 7 and 8.

Thus these seeds exhibited greater effects both concerning the emergence rate and the final emergence percentage.

In the same table (table 9) also results are given from registrations of the delayed emergence in the following year. No delayed emergence could be found in preirradiated seeds of 11 of the 19 investigated provenances and in the remaining 8 this did not exceed 5 per cent. Contrary to this there was delayed emergence in all the unirradiated seed provenances. The observed differences between the emergence percentages of pre- and unirradiated seeds during the first year were partly eliminated through the delayed emergence in the following year. A probable explanation of this seems to be that the seeds which were ungerminated at the end of the first year depending on shortage of light or light-substituting factor, were naturally stratified during the colder part of the year and in this way lost their light dependence (cf. the results in figure 9).

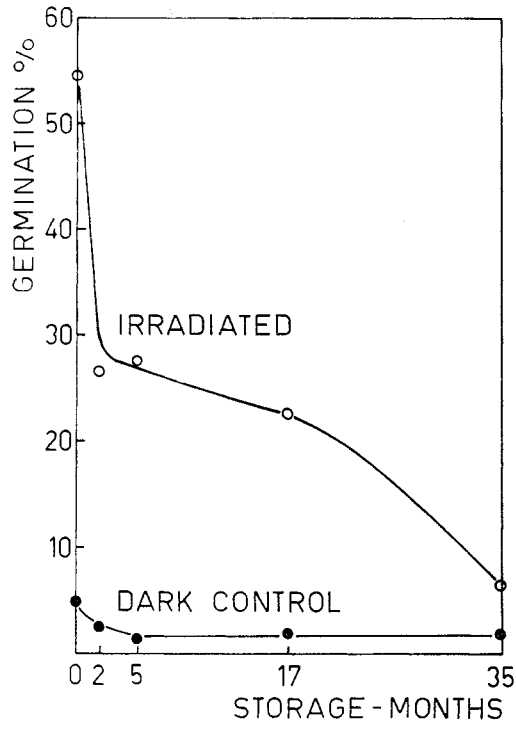

Figure 14. Effect of storage on the light-induced germination of Scots pine seeds. Irradiation of the unimbibed seeds (white fluorescent tubes TL/33, 4000 lux, 24 hours), germination in darkness. The same dark control as in figure 9. Provenance No. 2.

b. Permanence of the effect. At the start of the previously described storage experiment (see p. 27) 20×100 seeds (provenance No. 2, cf. table 1) were irradiated in unimbibed condition (5.7 per cent moisture content of the dry weight) with 4000 lux for 24 hours from white fluorescent tubes and immediately afterwards transferred to storage (in darkness, + 3—4° C). Repeated germination tests in darkness were performed with these seeds at the same time as with the control material during a period of 35 months. The results are given in figure 14.

These show that the preirradiation effect, which was found in the test performed immediately after the irradiation, decreased with longer period of storage. However, the preirradiated seeds had a greater germinative capacity in darkness than the control seeds even after a storage for 17 months. The difference was significant ($0.001 > P$). Eighteen months later, however, the difference had decreased further and was no longer significant ($0.2 > P > 0.05$). These results showed that the preirradiation of the unimbibed seeds induced an effect which could be manifested as a stimulated germination in darkness for a long time after the irradiation.

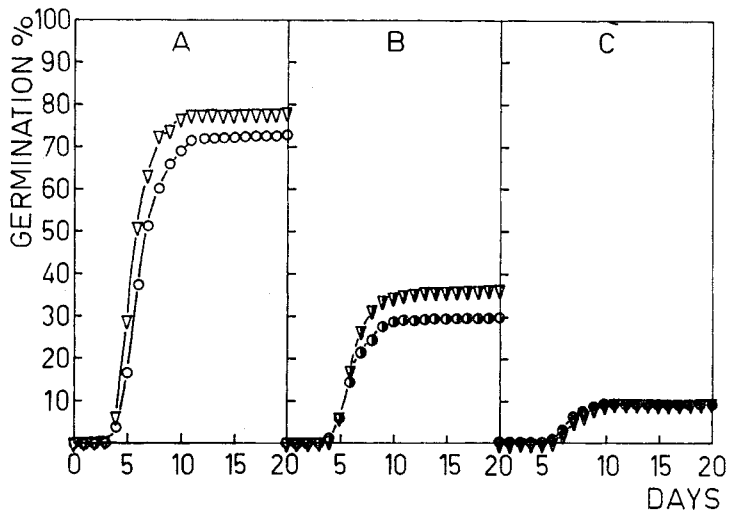

Figure 15. Effect of an X-ray irradiation on the germination course of Scots pine seeds. Circles: X-ray irradiated seeds (10 kV, 20 mAs, f = 25 cm). Inverted triangles: control seeds. A: germination under continuous irradiation (white fluorescent tubes TL/33, 2800 lux, unfilled symbols) of seeds irradiated in unimbibed condition as in B. B: germination in darkness after an irradiation of the unimbibed seeds (the same light-source, 3600 lux, 6 min., half-filled symbols). C: dark control (filled symbols). Provenance No. 1.

3. Effect of Embryo Development on the Irradiation Responses

The irradiation effects on the germination in seeds of different provenances have been studied on seed materials which have shown high germinative capacities in continuous white light (> 90 per cent), *i.e.*, indicating a good morphological ripeness (development of the embryo in relation to the embryo cavity, cf. p. 24). However, in order to get more detailed information concerning eventual relations between the light responses and the morphological ripeness of the seeds experiments were carried out on a seed material with lower germinative capacity (provenance No. 1). For this purpose an X-ray technique (see p. 25) was used.

The germination tests were performed with seeds which were preirradiated in unimbibed condition for 6 minutes with 3600 lux from white fluorescent tubes. Half of these seeds were germinated in continuous white light (experiment A in figure 15) and the rest in darkness (experiment B). Unirradiated seeds were also germinated in darkness (experiment C). In each of these experiments 16 × 50 seeds were used. The seeds were X-ray photographed after the above-mentioned preirradiation but before the start of the germination tests. The total germinative capacities of these seeds were compared with seeds which were not X-ray photographed but irradiated and

germinated under the same circumstances (3×100 seeds in each experiment). The results are given in figure 15, which shows that the X-ray photographed seeds (circles) had slightly lower germinative capacities in experiments A and B than the control seeds (inverted triangles) but these differences were not significant. The X-ray dose did not influence to any greater extent the germination responses. Thus these results permitted a further analysis of the germination responses concerning the relations between the light factor and the degree of morphological ripeness in the individual seeds.

In the X-ray photographing the seeds were placed in seed frames (cf. MÜLLER-OLSEN and SIMAK 1954) and afterwards transferred to the germination beds still preserving the mutual positions of the seeds. In this way the germination responses could be correlated with the results from the analysis of the X-ray films. In this the seeds were referred to different embryo-classes, which were defined in the following way:

Embryo-class 0: No endosperm and no embryo present (empty seed).
I: Endosperm but no embryo present.
II: Endosperm and embryo (1—several) present. The embryo less than half the length of the embryo-cavity.
III: Endosperm and embryo present. The embryo as long as or longer than half but shorter than the whole length of the embryo-cavity.
IV: Endosperm and embryo present. The embryo of the same size as the whole embryo-cavity.

Table 10. Embryo-class distribution of the X-ray photographed seed material of Scots pine and the corresponding total germinative capacities under different light conditions. Provenance No. 1.

Pretreatment[1]	Germination in	Total number of investigated seeds in each embryo-class	Total number of germinated seeds after	
			10 days	20 days
3600 lux for 6 min.	Continuous white light (2800 lux)	II 149	21	28
		III 223	169	178
		IV 395	361	375
	Darkness	II 134	17	20
		III 225	66	66
		IV 399	148	154
—	Darkness	II 122	3	3
		III 213	23	25
		IV 429	49	50

[1] Irradiation of the unimbibed seeds (white fluorescent tubes TL/33).

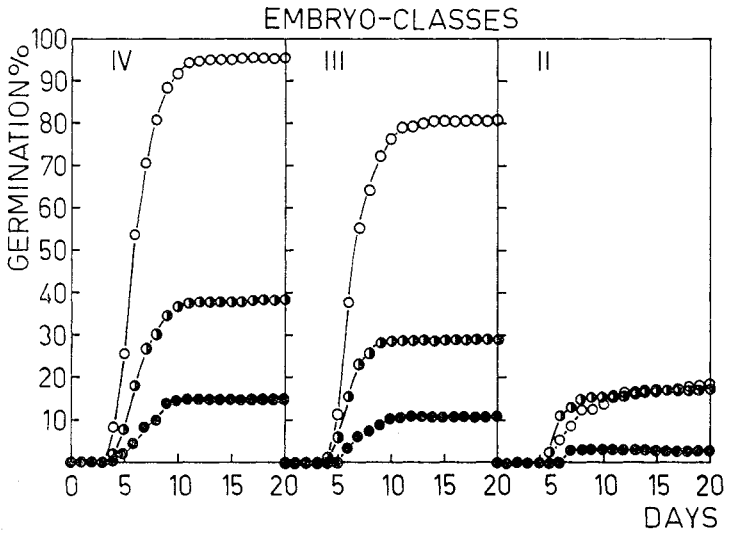

Figure 16. Connection between embryo development and irradiation effects on the germination course of Scots pine seeds. For definitions of embryo-classes, see the text. Irradiations and symbols as in figure 15. Provenance No. 1.

In some cases seeds germinated which from the X-ray photographs had been referred to embryo-class I, *i.e.*, as seeds without embryo. These incorrect classifications depended on the position of the embryos in close contact with the wall of the embryo-cavity. Such germinated seeds were excluded from the analysis.

A comparison of the distribution of embryo-classes in the seeds which were used in this experiment is given in table 10, comprising also the total number of respective embryo-classed seeds which germinated under the different light conditions. The time course of the germination in corresponding seeds is shown in figure 16.

The results, which are presented in figure 15, show that a maximal response was obtained with continuous irradiation (experiment A) and that the preirradiation increased the following germination in darkness (experiment B) in comparison with that of the dark control (experiment C). Both a comparison between experiments A and B and between B and C gave significant differences ($0.001 > P$). Corresponding comparisons of seeds belonging to embryo-classes IV and III in figure 16 gave also significant differences ($0.001 > P$) and the germination curves showed the same general trends as for the total seed populations. Concerning seeds of embryo-class II the same effects were obtained with continuous irradiation as with a preirradiation. A comparison between the germinative capacities of seeds

in the different embryo-classes with continuous irradiation also showed an evident decrease with decreased development of the embryos (IV: 94.9; III: 79.8; II: 18.8 per cent; cf. also corresponding values in MÜLLER-OLSEN and SIMAK l.c., table 3).

The foregoing results showed that light influenced the germination irrespective of the morphological ripeness of the seeds. Thus the presence of a greater or lesser discrepancy between the germinative capacities in light and darkness does not seem to be correlated with the special form of ripeness studied here, *i.e.*, the developmental degree of the embryos in the seeds. This statement is also indirectly confirmed by the results in figure 8 and partly also by the results in figure 7.

That a high percentage of completely developed seeds (embryo-class IV) in a seed sample is a requisite for a high germinative capacity in combination with irradiation in viable Scots pine was shown above. It seems reasonable to conclude that the same is valid for the investigated seed provenances in figures 7 and 8, which all had great germinative capacities with continuous irradiation. However, specially the provenances in the last-mentioned figure showed small germination percentages in darkness. From this it follows that also seeds with completely developed embryo in these cases could not germinate without irradiation with white light, *i.e.*, the same behaviour that was found from the investigation with the X-ray analysis. These results together seem to confirm the conclusion that the light dependence for the germination of Scots pine seeds is not correlated with the morphological ripeness of the seeds.

4. Effect of Seed Coat on the Irradiation Responses

Another attempt to correlate the light dependence of the germination with a morphological structure in the seeds was made in experiments in which the seed coats were partially or completely removed.

A first experiment was done with four different treatments in this respect. The used seeds had been previously dewinged as cautiously as possible (seed by seed with a preparatory needle). The control seeds were left intact (treatment A). Other seeds were intensively rubbed in a satchel for 5 minutes. This treatment completely removed the dark superficial layer of the coats (treatment B). In a third type of the experiment a piece of the seed coat was removed from the central part of the edge of the seed (punctured seeds, average size of the removed part was 5 per cent of the total seed surface, treatment C). From other seeds the coats were completely removed (decoated seeds, treatment D). After these treatments the seeds were germinated

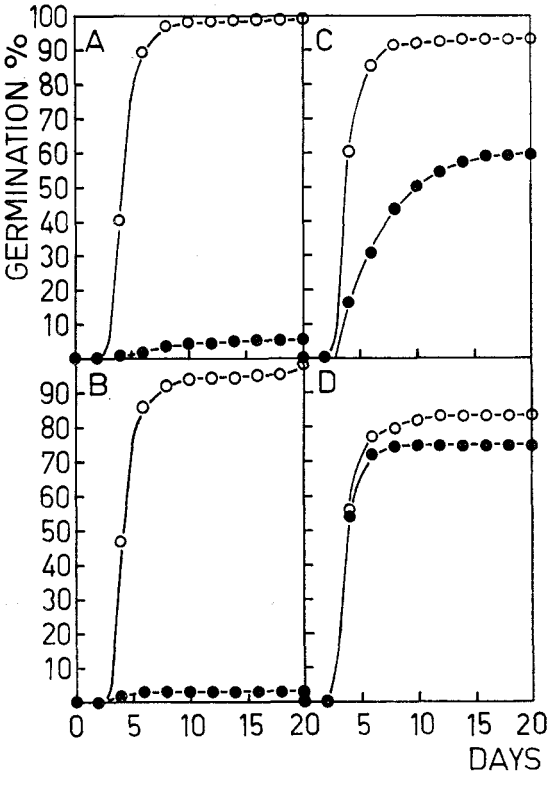

Figure 17. Connection between the seed coat and germination course of Scots pine seeds under continuous irradiation (empty circles) and in darkness (filled circles). A: intact seeds. B: rubbed seeds. C: punctured seeds. D: decoated seeds. Irradiation as in figure 7. Provenance No. 2.

under continuous irradiation and in darkness (in treatments A, B and C with 4×100 seeds, in treatment D with 4×50 seeds in light and darkness, respectively). The results are given in figure 17 A—D.

The results showed that the intensive rubbing did not influence the germinative capacities either in light or in darkness. Contrary to this the punctured seeds showed a stimulated germination in darkness. A slight decrease of the germination in light was found after this treatment. This was more pronounced in the decoated seeds, in which, however, no significant difference could be found between the germination in light and darkness. These results showed that a puncturing of the seed coats could increase the germination in darkness and that a complete removal of the seed coats nearly completely abolished the light requirement. See also in other experiments the influence of the seed coat on the red—far-red controlled germination (figure 31), on the occurrence of embryo-mitosis (figure 41) and on the respiration (figures 43, 44, 46).

In further experiments only puncturings were done on seeds which had been under germinating conditions for different long periods in darkness. Re-

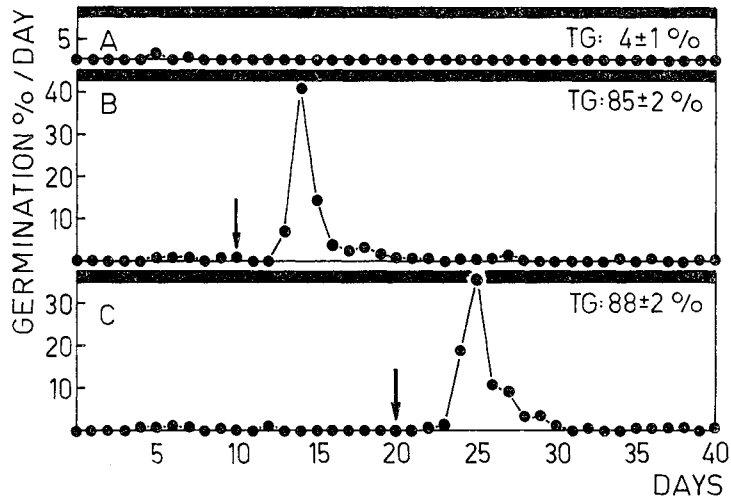

Figure 18. Effect of puncturing of the seed coat on the germination rate in darkness of Scots pine seeds. A: no puncturing. B: puncturing after 10 days. C: puncturing after 20 days. TG: total germination percentage after 40 days. Provenance No. 2.

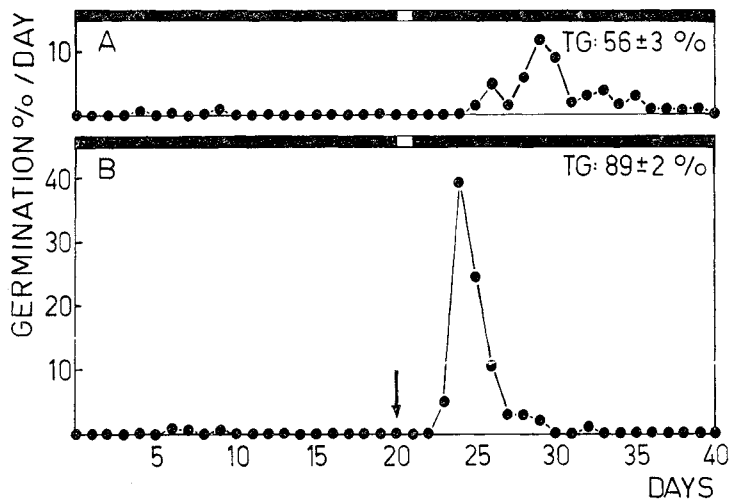

Figure 19. Effect of puncturing of the seed coat and irradiation (white fluorescent tubes TL/33, 4000 lux, 24 hours) on the germination rate of Scots pine seeds. Treatments performed after 20 days. A: irradiation. B: irradiation and puncturing. TG: total germination percentage after 40 days. Provenance No. 2.

sults from such experiments are given in figure 18. The puncturings were done after 10 and 20 days (4 × 50 seeds per experiment). In both cases a stimulated germination was obtained, the magnitude of which was not influenced by the length of the preceding dark period. In a corresponding experiment, the results of which are given in figure 19, a puncturing of the

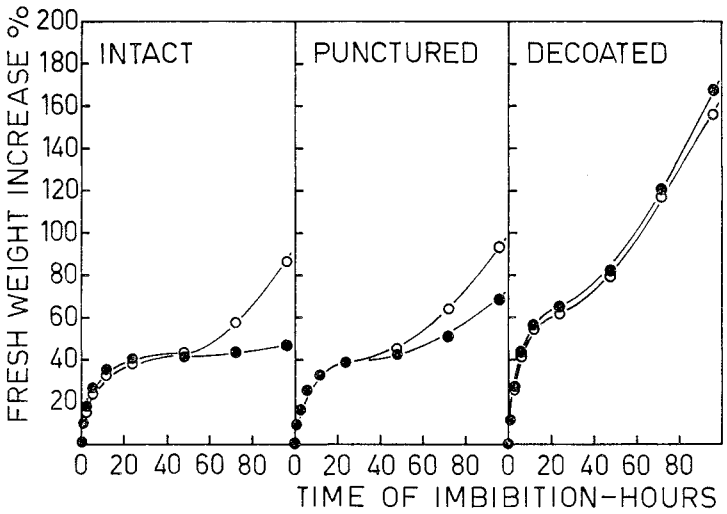

Figure 20. Influence of the seed coat on the imbibition course of Scots pine seeds under continuous irradiation (empty circles) and in darkness (filled circles). Irradiation as in figure 7. Provenance No. 3.

seeds after 20 days was combined with an irradiation with white light for 24 hours. The combination of irradiation and puncturing resulted in a greater stimulation of the germination than only an irradiation but compared with only puncturing after the same time (in figure 18) the same response was received. Even after a prolonged stay of the seeds under germinating conditions in darkness a disturbance of the seed coat could induce germination without irradiation (the intact control seeds in the above experiment were irradiated with safelight during the same time as the one employed in the puncturing experiments) and this to a greater extent than only an irradiation with white light on the intact seeds.

These results raised the question of whether the presence of an intact seed coat in Scots pine seeds constituted an obstacle for either the imbibition process or the exchange of gases and whether such an eventual obstacle could be eliminated by the irradiation of the intact seed coat. These possibilities were tested in following experiments.

Under the same conditions as in the germination tests with continuous irradiation and in darkness the time course of the imbibition was followed. Punctured and decoated seeds (treated in the same way as described above) together with intact seeds were weighed and placed on the germination beds in light and darkness. After different periods the seeds were reweighed after cautious wiping between filter papers and then replaced for continued imbibition. All weighings were performed with the seeds in closed aluminium containers, 2×50 seeds in each experiment. The results are given in figure 20.

The results with intact seeds showed that the imbibition was the same in light as in darkness. Thus the inhibited germination in darkness could not be referred to an inhibited imbibition under such circumstances. A comparison with the results on punctured seeds (with partially eliminated light-control, cf. figure 17 C) further confirmed that the intact and unirradiated seed coats did not constitute a limiting factor for an optimal imbibition. In the decoated seeds the imbibition process was more rapid and greater, but this does not seem to disapprove the above statement that the intact seed coats of Scots pine should restrict the germination in darkness by constituting an obstacle for the imbibition.

An attempt was also made to find out whether the intact and unirradiated seed coats constituted any obstacle for the permeation of oxygen required for a complete germination. For this purpose germination experiments were done in different oxygen concentrations. In each one of flat, rectangular, glass bottles ($25 \times 12 \times 6$ cm) three germination beds were placed, each with 100 intact seeds, together with 25 ml distilled water. The bottles were flushed with different gas mixtures of oxygen and nitrogen. These were made up with gas from steel containers with compressed gas (AGA, Lidingö, Sweden) according to the displacement method (UMBREIT *et al.* 1959). Each bottle was flushed with about 5 l of the appropriate gas mixture. Bottles with the same gas mixtures were made up in duplicate, one of them was afterwards placed under continuous irradiation (1700 lux), the other one in darkness. Closed bottles with ordinary air (21 vol. per cent oxygen) were also used together with the flushed and closed bottles. The experiment was ended after 10 days.

The results, which are given in figure 21, showed that an increased oxygen concentration could not substitute the stimulating action of the irradiation. Contrary to this, greater oxygen concentrations than the one in ordinary air caused for some unknown reason a decrease of the germination in darkness, an effect which did not appear in light. It may also be observed that in darkness the germination percentage in 10 vol. per cent oxygen was not significantly different from that in ordinary air but significantly different ($0.001 > P$) from the germination in pure nitrogen. These results do not seem to be consistent with the idea that the unirradiated seed coats should not permit a sufficient permeation of oxygen, *i.e.*, that the stimulated germination in darkness after puncturing or decoating should depend on an elimination of a diffusion barrier for oxygen.

The same consideration for carbon dioxide was also tested in an experiment, in which the seeds were germinated in darkness in the presence of phosphate buffers ($pH > 7$). No effects were obtained. See further experiments in Chapter VI concerning the interactions of irradiation, seed coat

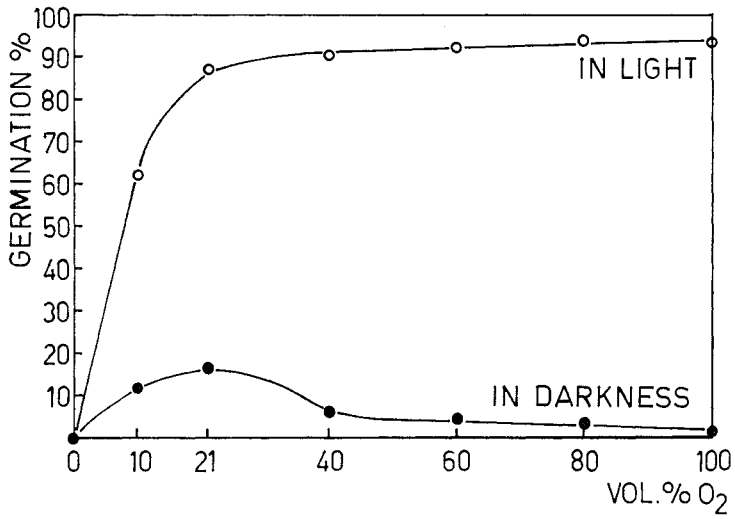

Figure 21. Effect of different oxygen concentrations on the germination responses of Scots pine seeds under continuous irradiation and in darkness (after 10 days). Irradiation as in figure 7. Provenance No. 4.

and respiration, which could not be evaluated from the point of view that the intact seed coat should be a diffusion barrier for the exchange of oxygen and carbon dioxide between the respiring seed and the surroundings.

Finally it may be mentioned that some experiments were done concerning the presence of water-soluble germination inhibitors in the Scots pine seeds. These were germinated in light and darkness, with and without a preceding irradiation of the unimbibed seeds. Unirradiated seeds were germinated in the presence of a leachate (400 seeds imbibed in 100 ml distilled water for 24 hours at + 25° C) prepared under continuous irradiation. A corresponding leachate prepared in darkness was used at the germination of the preirradiated seeds. The germination tests were performed in Petri dishes with 15 ml leachate per dish. No evidence was obtained indicating the occurrence of any water-soluble germination inhibitor connected with the light dependence of the germination.

D. Discussion

As evident from the results presented in the foregoing most of the experiments have been devoted to studies on the effects of special factors on the light-darkness responses of the Scots pine seeds and not to studies on the effects of the irradiations *per se*, which is the case in experiments with

red and far-red irradiations in the next chapter. This has been done in order to get a more general idea of the importance of the light factor for the germination and some introductory clues to a following study of the mechanism of the light action.

The establishment of light sensitivity in unimbibed Scots pine seeds by NORDSTRÖM (1953 a and b) under irradiations with direct sunlight or light from an UV-lamp (cf. also BERGMAN 1957, HUSS 1961) was confirmed here both in laboratory and in field experiments with the use of a moderate irradiance from white fluorescent tubes (figures 11, 12, 13, see also NYMAN 1957). The responses of the irradiation on the unimbibed seeds of different provenances did not show any connection with the origin of the seeds. This is not in accordance with the statement by NORDSTRÖM (l.c.) that Swedish Scots pine seeds from more northern localities should react to a greater extent on such an irradiation. Neither could the supposition of the same author (l.c.) that the effect of such an irradiation should give greater responses in field than in laboratory tests be confirmed. Contrary to this the responses were greater in the laboratory tests, which also has been found by HUSS (l.c.). However, contrary to the results by HUSS, all the 19 investigated seed provenances studied here showed positive differences between the emergence percentages of preirradiated and unirradiated seeds, a case found only in older seeds by HUSS (l.c., table 13) in a corresponding nursery experiment. Beside a prevailing greater emergence percentage here established after the preirradiation also a greater rate of emergence could be found in most of the provenances (table 8).

The sensitivity to irradiation in the unimbibed Scots pine seeds—also found by FURUKAWA (1956) in seeds of *Pinus thunbergii* Parl. and *P. densiflora* Sieb. & Zucc. from irradiations with sunlight—is not consistent with the earlier general conception that seeds with light-dependent germination react to irradiations only in imbibed condition. See also the introduction to this chapter (p. 22). EVENARI and NEUMANN (1953 b) have shown that lettuce seeds stored in an atmosphere with high relative humidity were influenced by irradiation. In connection with these last-mentioned findings it may be observed that the responses of air-dry Scots pine seeds of different provenances did not show any relation to the actual moisture content of the seeds at the irradiation (table 7).

Concerning the permanence of the preirradiation effect this was significant even after a storage for 17 months (figure 14) in spite of a decreasing trend with increasing period of storage, thus indicating a relative slow deterioration of the effect. This was also suggested by the results of NORDSTRÖM (l.c.) in seeds stored for 5 months. Compare the findings of FURUKAWA (l.c.) who could observe an effect in seeds stored for 2 to 3 months.

However, in spite of the occurrence of sensitivity to irradiations in the unimbibed Scots pine seeds there was an evident increase of the sensitivity after the start of the imbibition. After an imbibition for 6 hours the maximal sensitivity was attained (figure 10). Irrespective of the sensitivity in unimbibed seeds and the time required for reaching a maximal sensitivity, which can be different in different species, these results were in conformity with the general trend for other light-sensitive seeds in this respect (cf. reviews by EVENARI 1956 and in press). These results were the basis for the choice of an appropriate period of imbibition in the irradiation experiments on imbibed seeds in following chapters. See also the discussion about red—far-red sensitivity and imbibition in the next chapter (p. 83). This possibility of inducing a complete germination with a single exposure after an appropriate period of imbibition also may be compared with the stimulating effects of longer irradiations for only restricted periods on Scots pine seeds (HAACK 1906, 1912, ELIASON and HEIT 1940, SARVAS 1950, HUSS 1961) and on seeds of *Pinus thunbergii* Parl. (ISIKAWA and SHIMOGAWARA 1954, HASEGAWA and FURUKAWA 1953). For other pine species, see the next chapter.

In this connection it may be observed that even irradiations for longer periods on unimbibed seeds (figure 11, see also figure 22, no imbibition, and corresponding results with irradiations in sunlight for 3 days in NORDSTRÖM l.c. and HUSS l.c.) could not induce a complete germination.

As pointed out in the introduction to this chapter (p. 23) the light dependence expressed as the difference between the germinative capacities under continuous irradiation and in darkness for Scots pine seeds has been shown to be different depending on the origin of the seeds. For Swedish Scots pine NORDSTRÖM (l.c.) has pointed out that this difference should be specially pronounced in seeds of northern provenances, a finding which could not be confirmed in the seed provenances investigated here (figures 7, 8). The same author has suggested that the light dependence should be connected with the often poorly developed embryos in such seeds. SIMAK and GUSTAFSSON (1954) have also deduced from sowing experiments with embryo-classed seeds that only seeds with uncompletely developed embryos (embryo-classes II and III) reacted to irradiations. This could not be confirmed here (figure 16, table 10). It was found that the seeds were dependent on irradiation for the germination regardless of their degrees of morphological ripeness. Indirectly this could be confirmed from the large discrepancies between the germination responses under continuous irradiation and in darkness in seeds of different provenances (figure 8) under simultaneous consideration of a good morphological development of the embryos as a requisite for such great germinative capacities in light (table 10, embryo-class IV, see also MÜLLER-OLSEN and SIMAK 1954).

Stratification as a method for elimination of both morphological and physiological kinds of unripeness in seeds (cf. reviews by CROCKER 1948 and BARTON 1961) also has been shown to eliminate the light dependence of germination. For Scots pine seeds this has been shown by ELIASON and HEIT (1940) and HEIT and ELIASON (1940) and has also been confirmed in this investigation (figure 9). The same phenomenon has been described for seeds of other pine species (HASEGAWA and FURUKAWA 1953, 1955, ASAKAWA 1956, 1959, see also in the following chapters). For further discussion of the stratification, see Chapter VI.

This light-substituting effect of a stratification seems to be the probable cause of the greater delayed emergence of the control seeds than that of the preirradiated seeds in the field experiment (table 9), a fact also partly observed by HUSS (l.c.) in corresponding experiments.

Like several other species in which there is a light-influenced germination (EVENARI 1956 and in press) also the seeds of Scots pine showed a pronounced dependence on the presence of intact seed coats as a requisite for light to be a controlling factor in the germination (figures 17—19). Attempts to determine the physiological basis for this were made here along two different lines: the seed coat as a hindrance for a sufficient imbibition or as a hindrance for gas exchange of the seeds.

Concerning the first case it was found that the uptake of water was the same under continuous irradiation as in darkness in intact seeds until the time when the directly visible germination started ($>$ 48 hours, figure 20). Also the punctured seeds showed corresponding results, but in the decoated seeds the uptake of water was more rapid and somewhat greater. These results seem to show that the inhibited germination of intact seeds in darkness did not depend on an insufficient imbibition, because the uptake of water was the same in seeds irradiated or punctured, *i.e.*, under conditions not restricting the germination.

Concerning the second case with intact and unirradiated seed coats as a possible hindrance for exchange of oxygen required for the germination the experiments with increased oxygen concentrations (figure 21) did not confirm this supposition (cf. in Chapter VI results also supporting this idea). Contrary to this there was an inhibiting interaction between higher oxygen concentrations and darkness. The reason for these results is not obvious.

In other species with light-dependent germination, where the germination in darkness could be increased after a decoating, it has been found that the germination of the intact seeds in higher oxygen concentrations was increased, *e.g.*, for *Rumex crispus* L. by GARDNER (1921) and AXENTIEFF (1929). In this species also KOLK (1961) has found the same effect after decoating but only a smaller effect of the oxygen factor. In seeds of *Betula verrucosa*

Ehrh. BLACK and WAREING (1959) could not induce any germination in darkness of intact seeds in higher oxygen concentrations, in spite of the fact that opening of the seed coverings increased the germination in darkness in air and that the isolated embryos germinated independently of the light factor. These authors could correlate their results with the occurrence of a water-soluble inhibitor in the seed coverings, the inhibiting action of which was dependent on the light and oxygen factors. This inhibitor could not be destroyed by irradiations *in vitro*, a case which has been shown by REDMOND and ROBINSON (1954) for a water-soluble inhibitor from seeds of *Betula lutea* Michx. These latter seeds did not germinate either in light or in darkness with intact or opened seed coats. However, after a complete removal of the coats the germination was great in distilled water but completely inhibited in the presence of a water extract from seed coats, an inhibition partially eliminated if the extract had been irradiated. Also several other results indicate a correlation between a light-influenced germination and the presence of germination inhibitors (MAGNUS 1920, PETERS 1924, AXENTIEFF 1929, TILLY 1935, COX *et al.* 1945). GASSNER (1915) has also stated that the stimulating action of irradiation on the germination of *Chloris ciliata* Sw. was caused by light acting as an inhibitor of an inhibitor. EVENARI (1949) has supposed a correlation between light action on the germination and germination inhibitors. In this connection it may be observed that NUTILE (1944, 1945) succeeded in making a nonlight-dependent strain of lettuce seeds light-dependent in the presence of a coumarin solution.

For the Scots pine seeds no corresponding results could be found in experiments where seeds were germinated in leachates from dark control seeds and from irradiated seeds (p. 45). These results do not disprove the eventual importance of germination inhibitors affecting the light dependence of the germination in this species but can be observed and compared with the results in puncturing experiments (figures 17 C, 18, 19). In these about 5 per cent of the total seed coat were removed from the central part of the edge of the flat seeds, *i.e.*, a part of the germinating seed which was not in contact with the germination bed, a condition which does not seem to facilitate a leaching to the germination beds of eventual water-soluble inhibitors through the puncturings. These circumstances may possibly suggest an interaction between the occurrence of a gaseous inhibitor and the light dependence of the germination in the intact seeds. However, in this connection it may also be pointed out that the stimulating effects of puncturing or decoating may depend on a wounding phenomenon, which has been found by BELDEROCK (1961) to cause a part of the stimulating action on the germination of dormant wheat grains on piercing the covering layers over the embryos. TOOLE *et al.* (1956 a) have pointed out that the decreased or elim-

inated light control for the germination in seeds connected with puncturings or decoatings need not depend on the treatments *per se* but can be influenced by the way in which the treatments have been performed, *i.e.*, can be dependent on associated internal physical changes in the seeds. This can be compared with the stimulating effects on the germination in darkness after mechanical shocks (NORDSTRÖM 1953 a and b) and also be connected with the observed decreased germinative capacity in light in Scots pine seeds after mechanical treatments (HUSS 1950, NILSSON 1963), a circumstance suggesting the existence of a sensible structural system in these seeds, which easily can be destroyed by mechanical treatments and indirectly influences the mechanism of the light action. Compare, however, the results in figure 17 B, which are not consistent with this idea.

Chapter IV. Studies on the Germination — Effect of Irradiations with Coloured Light

A. Introduction

The extensive literature on the effect of light on seed germination (see reviews quoted in the introduction to Chapter I) shows that most of the older investigations in this field have been devoted to studies on the effects of white light. The investigations which include studies on the effect of coloured light (cf. EVENARI 1956) indicate that it is the red part of the visible spectrum, which is the most effective one for promotion of the germination. For the seeds of *Pinus silvestris* L. this has been shown by HAACK (1906, 1912), PITTAUER (1912) and ELIASON and HEIT (1940). Corresponding results were obtained in *P. nigra* Arnold and *P. strobus* L. by PITTAUER (l.c.) and in *P. densiflora* Sieb. & Zucc. and *P. thunbergii* Parl. by HASHIMOTO *et al.* (1954) and IWAKAWA and KOTANI (1954). Inhibition by irradiations with blue light, *i.e.*, less germination than in the dark control seeds, also was observed by ELIASON and HEIT (l.c.) and PITTAUER (l.c.). It is also interesting to notice that GERLAI (1937) found an inhibiting action by irradiations of high irradiance (from incandescent lamp) on the germination in *P. silvestris* L., but this was not obtained with the use of a filter absorbing wavelengths longer than those in the red part of the spectrum. At wavelengths longer than red (far-red, 7000—8000 Å, for a discussion of the nomenclature cf. WASSINK and STOLWIJK 1956) FLINT and MCALISTER (1935), FLINT (1936) and FLINT and MCALISTER (1937) found an inhibition of the germination in seeds of *Lactuca sativa* L. These last-mentioned findings were the starting point for the classical investigations by

BORTHWICK *et al.* (1952 a), TOOLE *et al.* (1953) and BORTHWICK *et al.* (1954), which showed that the germination of lettuce seeds (var. Grand Rapids) was regulated by light through the mediation of a repeatedly reversible red—far-red sensitive mechanism (cf. also EVENARI and NEUMANN 1953 a and EVENARI *et al.* 1953). The action spectrum for this process has been found to be equivalent to the corresponding ones for a series of other photomorphogenic processes (cf. reviews by VIRGIN 1958, LIVERMAN 1960, MOHR 1960 a, 1961, 1962, see also WITHROW 1959 and p. 142 below). This has also been confirmed for the germination of other light-sensitive seeds: in *Arctium lappa* L. (NAKAMURA 1954, q.f. TOOLE *et al.* 1956 a), in different vegetable seeds (NAKAMURA *et al.* 1955, q.f. TOOLE *et al.* l.c.), in *Lepidium virginicum* L. (TOOLE *et al.* 1955) and in *Arabidopsis thaliana* (L.) Eastland (SHROPSHIRE *et al.* 1961). Also the germination of fern spores regulated by light follows the corresponding action spectrum as shown by BÜNNING and MOHR (1955) and MOHR (1956).

Beside the establishment of equivalent action spectra for these species a reversible red—far-red controlled germination has been shown in several other species, thus suggesting the occurrence of the same action spectrum. See table 11.

Also for the following species TOOLE (1959, 1961) has mentioned that their germination is controlled by a reversible red—far-red mechanism: *Brassica nigra* (L.) Koch., *B. juncea* (L.) Coss., *Fragaria virginiana* Duchesne, *Lepidium campestre* (L.) R. Br., *Lycopersicum esculentum* Mill., *Lythrum salicaria* L., *Oenothera biennis* L., *Puya berteroniana* Mez., *Thlaspi arvense* L., *Ulmus americana* L. and *Verbascum thapsus* L. In moss spores (*Funaria hygrometrica* Hedw.) the same mechanism has been shown by BAUER and MOHR (1959).

Thus, concerning the red—far-red part of the spectrum there is a general agreement between the results from studies on divergent types of seeds. It is also interesting to notice that the germination of seeds which is inhibited by white light also is regulated by the participation of the same red—far-red reversible system. The opposite effects of white light on the germination of light-stimulated and light-inhibited seeds can be referred to a different relative sensitivity to the red and far-red part of the white light (see a review by EVENARI in press).

However, the agreement between the results in different species concerning the red—far-red part of the spectrum is not valid for the blue part (4000—5000 Å). Both inhibition and stimulation as well as the absence of effects after irradiations with blue light have been described (cf. EVENARI l.c., see also MOHR 1961 b). At the same time the effect of the blue light is confused by the participation of a "high-energy reaction" (MOHR 1960 a,

Table 11. A survey of species with reversible, red—far-red controlled germination.

Species	References
Amaranthus blitoides S. Wats.	Kadman-Zahavi 1955
A. retroflexus L.	Kadman-Zahavi 1957, 1960
Barbarea vulgaris R. Br.	Toole *et al.* 1957
Berteroa incana (L.) DC.	Toole *et al.* 1957
Betula pubescens Ehrh.	Black & Wareing 1955
Chenopodium rubrum L.	Cumming 1959
Ch. salinum Standley	Cumming 1959
Ch. glaucum L.	Cumming 1959
Ch. album L.	Cumming 1959
Carnegia gigantea (Engelm.) Britt. & Rose	Alcorn & Kurtz 1959
Camelina microcarpa Andrz.	Toole *et al.* 1957
Capsella bursa-pastoris (L.) Medic.	Toole *et al.* 1957
Epilobium cephalostigma Hausk	Isikawa 1962
Eragrostis ferruginea Beauv.	Isikawa *et al.* 1961, Fujii 1962
Lamium amplexicaule L.	Jones & Bailey 1956
Lepidium densiflorum Schrad.	Toole *et al.* 1957
Lysimachia mauritiana Lam.	Isikawa 1962
Nicotiana tabacum L.	Toole *et al.* 1953
Paulownia tomentosa (Thunb.) Steud.	Toole *et al.* 1958
Picea glehnii Mast.	Asakawa & Inokuma 1961
Pinus silvestris L.	Nyman 1957, 1961
P. strobus L.	Toole *et al.* 1962
P. taeda L.	Lane 1957, Toole *et al.* 1958, 1962
P. thunbergii Parl.	Asakawa & Inokuma 1961
P. virginiana Mill.	Toole *et al.* 1956 b, 1961, Lane 1957
Rumex obtusifolius L. subsp. *agrestis* Danser	Isikawa & Fujii 1961
Salsola volkensii Schweinf. & Aschers.	Negbi 1959 (q.f. Evenari 1961)
Sisymbrium altissimum L.	Toole *et al.* 1957
S. officinale (L.) Scop.	Toole *et al.* 1957

1962) in addition to the "low-energy reaction" mediated by the red—far-red reversible system dealt with here.

From their studies on the action spectrum of lettuce seeds Borthwick *et al.* (1952, 1954) concluded that the red—far-red light, which controlled the germination of this species, was mediated by two pigments, mutually convertible through irradiations preferably in the red (6400—6700 Å) and in the far-red (7200—7500 Å) region. These results and the widespread occurrence of the same type of photomorphogenic processes (cf. reviews above) showed that this pigment system must be present in many different types of plants. The postulated properties of this pigment system afterwards have been verified. With a special spectrophotometer (Birth 1960) the pigment has been detected *in vivo* and partially purified by Butler *et al.* (1959). It has been called *phytochrome* by Borthwick and Hendricks (1960), Butler *et al.* (1960) and Hendricks (1960 a and b) and further studied by Butler (1961), Butler *et al.* (1961), Siegelman *et al.* (1961, 1962), Bonner (1960, 1961, 1962), Gordon (1961) and Lane *et al.* (1962). The pigment is of a protein nature with a blue-green colour and in experi-

ments both *in vivo* and partially *in vitro* it showed the properties postulated from the primary physiological studies.

These results and the hitherto presented preliminary reports on the red—far-red controlled germination in the seeds of *Pinus silvestris* L. (NYMAN 1957, 1961) make it probable that the primary absorption of light occurs in this red—far-red reversible pigment system (phytochrome). The light absorption (in red) then is followed by a series of unknown processes leading to the start of growth of the embryos with protruding rootlets as a consequence, *i.e.*, a visible germination. However, further studies on the effects of red—far-red irradiations and on the action spectrum of the directly visible germination will be given in this chapter contributing more details about the mechanism of the light action in this species. In the two following chapters an attempt will be made to describe other light-controlled phenomena (embryo-mitosis and respiration) correlated with the directly visible germination, which possibly can give some clues to the understanding of the processes intervening between the primary light absorption and the final, visible germination, *i.e.*, in what way this pigment system influences the germination of the Scots pine seeds.

B. Methods

The germination experiments described in this chapter were performed in the same way as described in Chapter II (p. 12). In all the experiments the temperature has been + 25° C if not otherwise stated.

The equipment for irradiations with red or far-red light used in the experiments on the red—far-red reversibility (section 2, see below) has been described earlier (p. 17). The irradiances were 350 $\mu W/cm^2$. An irradiation with red or far-red light carried out with this equipment for 30 minutes on the seeds, which, if not otherwise stated, had imbibed for 6 hours in darkness at + 25° C, has been called a standard red or far-red irradiation.

For the studies on the action spectra (section 3, see below) the irradiations were administered with another apparatus (with interference filters) also earlier described in detail (p. 18).

For further details about the irradiations, see corresponding experiments.

C. Results

1. Preliminary Investigations

The results in the previous chapter showed that the stimulating action of white light given continuously during all the germination process could be partially or completely substituted by a limited irradiation depending on the state of the imbibition at the irradiation. These results were further

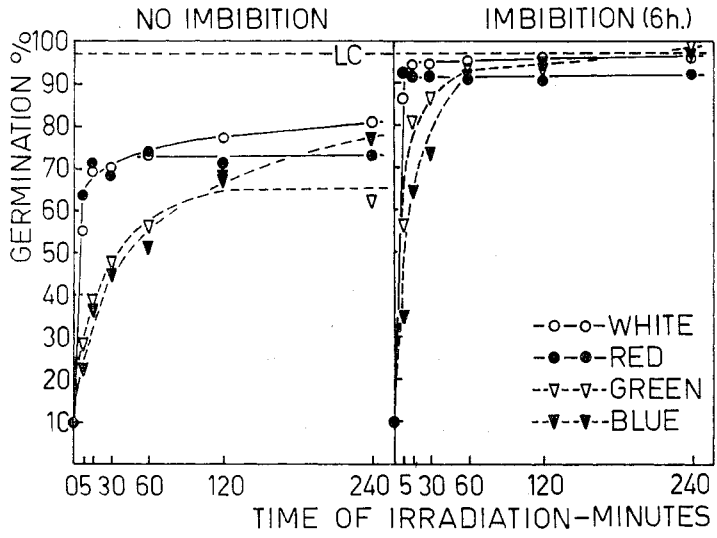

Figure 22. Effect of irradiation time, light quality and state of imbibition during the irradiations on the germination of Scots pine seeds. Irradiation with white (TL/33), red (TL/15), green (TL/17) and blue (TL/18) fluorescent tubes. Spectral composition, see table 5 and figure 4. Irradiance 350 $\mu W/cm^2$. LC: light control as in figure 10. Provenance No. 3.

investigated and combined with experiments in which the irradiations were done with light from red, green and blue fluorescent tubes.

The irradiation sources consisted of four different banks, each with eight fluorescent tubes (Philips 20 W: white TL/33, red TL/15, green TL/17 and blue TL/18). Spectral composition of the light from these tubes is given in table 5 and figure 4. The banks were placed above Jacobsen apparatuses. The distances between the individual banks and the seed beds were arranged in such a way that the irradiances (measured under the glass bell jars covering the seeds) were the same in all cases (350 $\mu W/cm^2$). In half of the experiments the irradiations were performed with the seeds placed on dry germination beds, in the other half the seeds had imbibed in darkness for 6 hours before the start of the irradiations. The results are given in figure 22.

The results showed that the irradiations caused a stimulated germination irrespective of the used light quality and the state of imbibition. However, even after an irradiation for 4 hours of the unimbibed seeds the stimulation was not complete. In the imbibed seeds an irradiation with white or red light for 15 minutes was sufficient for full stimulation. The light sensitivity of the seeds increased with the imbibition but was also present before the start of the imbibition. Compared with the white and red light, the green and blue light had a less stimulating effect after shorter periods of irradia-

tions. These results suggested that the part of the spectrum most effective for promotion of the germination is to be found in the red. The stimulating actions of light from both the green and the blue fluorescent tubes can, as a matter of fact, be explained merely by the content of red light in both types of irradiations (5 per cent and 0.5 per cent of the total emission from green TL/17 and blue TL/18 tubes, respectively, is to be found in the region 6000—7000 Å). With the used irradiance 350 $\mu W/cm^2$ this gave an irradiance in the region 6000—7000 Å sufficient for being the only light quality in these irradiations causing the stimulating germination (cf. figure 33). Below a study of the action spectrum (p. 74) performed with more monochromatic light (interference filters) is given indicating that under the conditions of irradiation used the blue part of the spectrum had no effect but the green caused a stimulation, which however was less than in the red part.

Studies on the Red—Far-Red Reversibility

a. Effect of imbibition. The results in figure 22 showed that an irradiation with red light (350 $\mu W/cm^2$) for 30 minutes was more than sufficient (saturation dose) for induction of full germination, when the seeds had imbibed in darkness for 6 hours. The use of the same irradiation but different periods of imbibition gave the results in figure 23 (R). Also in this case the red irradiation of the unimbibed seeds caused a germination corresponding to that in figure 22 (No imbibition, red, 30 min.). A light sensitivity was present before the start of the imbibition. However, during the course of the first 12 hours of imbibition the sensitivity increased, but after this period it decreased with increasing time of imbibition. These results were principally in accordance with the results from a corresponding experiment performed with white light (figure 10).

In another part of this experiment the same red irradiations were immediately followed by a far-red irradiation of the same irradiance. As a period of irradiation 30 minutes were chosen. The results in figure 23 (R+FR) showed that far-red light reversed the red induction. After 3—6 hours of imbibition a far-red irradiation could completely nullify this induction. Thus the responses to the far-red irradiation increased simultaneously as the responses to the red irradiation increased. However, the decreasing sensitivity to red light after more than 12 hours of imbibition could not be refound as a corresponding decrease of the far-red sensitivity after the same time. After more than 12 hours of imbibition the far-red irradiation inhibited the germination to a constant level, the same level as was shown by the unirradiated control seeds. Thus these experiments showed a cor-

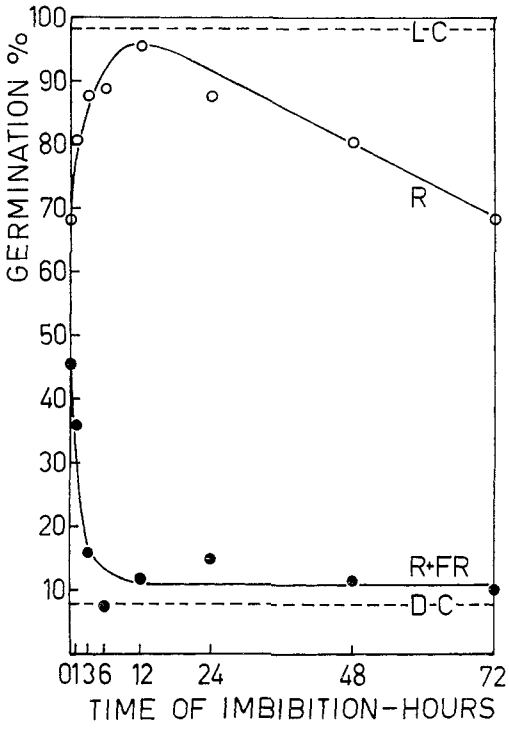

Figure 23. Effect of imbibition time on the red induction (R) and far-red inhibition (R + FR) of the germination in Scots pine seeds. Red and far-red irradiations with the standard equipments (350 μW/cm^2, 30 min. of each). LC: light control. DC: dark control. Provenance No. 3.

related change of the sensitivity (as it is expressed here as the germination response to a fixed irradiation) to red and far-red irradiations after periods of imbibition less than 12 hours.

As a consequence of these results a period of 6 hours of imbibition before the start of the irradiations has been chosen as a convenient standard period in the following experiments.

In the last experiment described above an irradiation of 30 minutes (in far-red) was randomly selected, which gave a full inhibition of the red induction. To investigate the importance of the period of irradiation with far-red for this inhibition, an experiment was done in which also the influence of the imbibition was studied. In one part of the experiment all irradiations were performed on unimbibed seeds immediately before the start of the imbibition. In the other part the irradiations were started after the standard period of imbibition (6 hours). Before the far-red irradiations all seeds were red irradiated for 30 minutes. The results from this experiment are given in figure 24.

The results with the imbibed seeds showed that the red induction, which gave the same germination percentage as at germination in continuous white

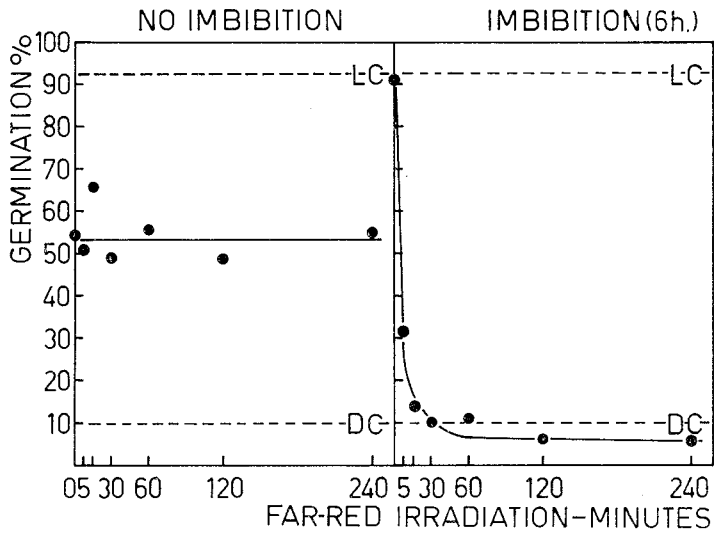

Figure 24. Effect of irradiation time and imbibition on the far-red inhibition of the red-induced germination in Scots pine seeds. Red and far-red standard equipment. Induction with red irradiation (30 min.). No imbibition: irradiations performed immediately before the start of the imbibition. Imbibition (6 h.): irradiations performed after 6 hours of imbibition. LC: light control. DC: dark control. Provenance No. 3.

light (LC), could be completely nullified with a far-red irradiation for 30 minutes, *i.e.*, with the same period of irradiation as previously used. As a consequence of this 30 minutes of irradiation with far-red light has been chosen as a standard period for irradiations with this light quality.

In contradiction to the results on imbibed seeds the unimbibed ones could not be inhibited with the far-red irradiations. Even as long an irradiation as 4 hours had no effect. These results demonstrated a pronounced effect of the imbibition on the far-red sensitivity of the seeds.

The results from figures 22—24 may be summed up in the following way. Red light induced the germination of the Scots pine seeds. This induction appeared also after irradiations of the unimbibed seeds. The sentitivity to red light increased with increasing period of imbibition up to 12 hours, afterwards decreasing. Far-red light nullified the inductive action of the red light, but this effect was not observed after irradiations on the unimbibed seeds. The sensitivity to far-red light increased with increasing period of imbibition up to 6 hours, after that time no changes could be observed with the used method.

From these results a standard scheme for inductive red irradiations and inhibitive far-red irradiations was adopted: with the standard equipment for red irradiation (red fluorescent tubes TL/15, irradiance 350 $\mu W/cm^2$) inductive irradiations were given for 30 minutes after the seeds had imbibed

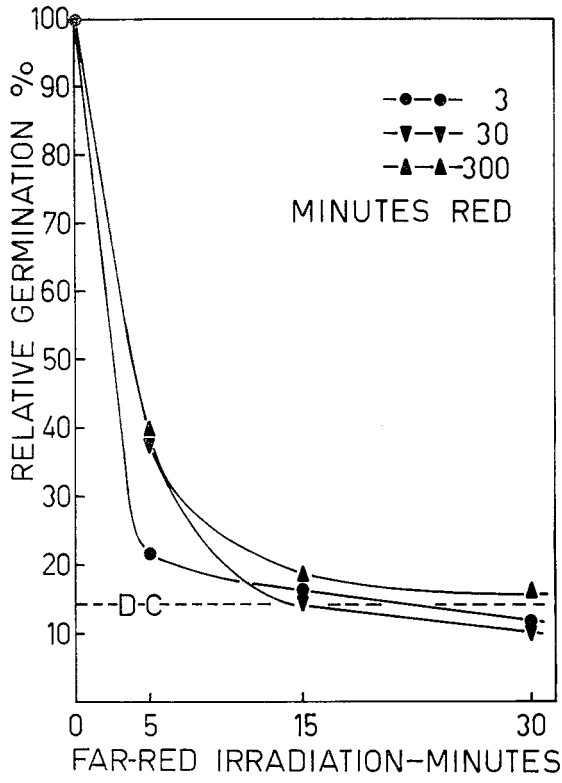

Figure 25. Effect of the red induction on the far-red inhibition of the germination in Scots pine seeds. Red and far-red standard equipment. Irradiations performed after 6 hours of imbibition. DC: dark control. Provenance No. 3.

in darkness for 6 hours; with the standard equipment for far-red irradiation (incandescent lamp, blue-red and water filters, irradiance 350 $\mu W/cm^2$) inhibitive irradiations were given for 30 minutes after standard induction with red light.

b. Effect of red induction on the far-red inhibition. As shown in the preceding part the inhibiting action of a far-red irradiation was dependent on the imbibition and in the imbibed seeds on the period of irradiation with the far-red light. These experiments were carried out with seeds which had been induced to full germination with an irradiation in red light of a fixed energy. To investigate whether the far-red inhibition was dependent on the energy used for the red induction of the germination the following experiments were done.

After 6 hours of imbibition the seeds were irradiated for 3, 30 and 300 minutes, respectively, in red light from the same source as above. Without following far-red irradiation the corresponding germination percentages were 89, 95 and 95, respectively. With far-red irradiations for different periods inhibitions appeared. The results are presented in figure 25.

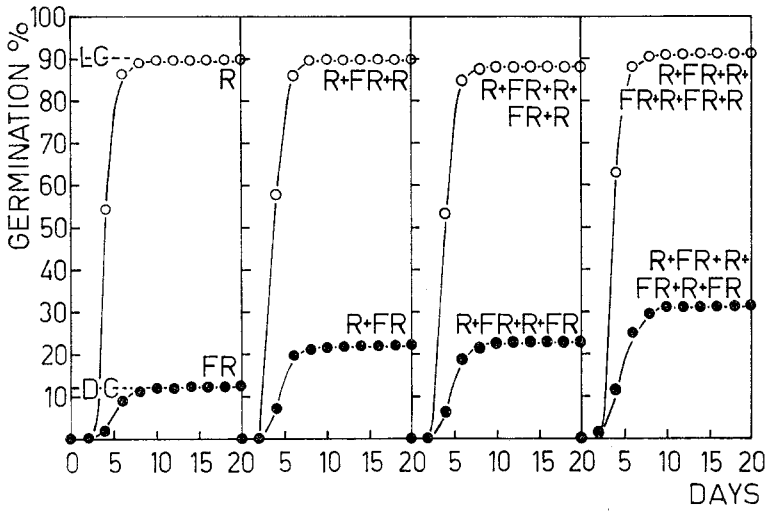

Figure 26. Reversible effect of repeated red—far-red irradiations on the germination course of Scots pine seeds. Standard irradiations (irradiations with red and far-red standard equipment, each for 30 min. after 6 hours of imbibition). LC: light control. DC: dark control. Provenance No. 3.

The results showed that with far-red irradiations for 15 minutes or longer the produced inhibitions were independent of the magnitude of the red energy used for the induction. This was in conformity with the idea developed by BORTHWICK *et al.* (1952 a), that the red light caused a shift of an equilibrium between a red and a far-red absorbing form of the pigment system, which controlled the germination.

c. Repeated red—far-red reversibility. In the experiments described above the inhibiting action of a far-red irradiation on the red induction was demonstrated by a single far-red irradiation of the seeds. It was investigated whether this red—far-red reversibility could be repeated in an experiment with a longer series of alternating red—far-red irradiations. This was done by means of standard irradiations (see p. 57). All irradiations followed immediately after each other. The results are given in figure 26.

As above a single red irradiation induced the same germination percentage as a continuous irradiation with white light (cf. figures 22—24). A far-red irradiation alone had no effect, but given after a red one it nullified the effect of the latter. However, this inhibition could be eliminated by a following red irradiation. In all experiments in which the irradiation sequences ended with a red one the germination was complete. On the other hand, if the sequences ended with a far-red irradiation the germination was inhibited. A slight decrease of the inhibition was found with an increased sequence of

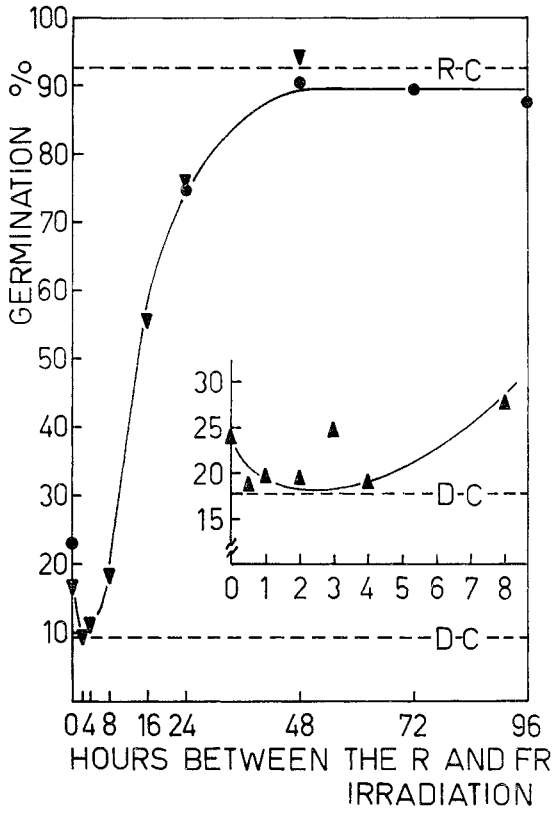

Figure 27. Reduction of the far-red inhibition of the germination in Scots pine seeds with an increasing dark period between a red and a far-red irradiation. Standard irradiations (cf. figure 26). RC: only red irradiated seeds. DC: dark control. Provenance No. 3.

irradiations. However, this decrease was only slightly significant ($0.05 > P > 0.10$) for the used number of repeated irradiations.

These results showed that the germination of Scots pine seeds was regulated by red—far-red irradiations through the mediation of a repeatedly reversible mechanism.

d. Loss of far-red inhibition with a dark period between red and far-red irradiations. In the experiments described above the far-red irradiations were given immediately after the end of the inductive red irradiations. In order to study how long after a red irradiation the inhibition of the far-red light could be found experiments were performed in which a dark period of different length was introduced between a standard red and a far-red irradiation. The results from these experiments (three repeated ones: dots, inverted triangles, triangles) are given in figure 27.

The results showed that with increasing time of the dark phase between an inductive red irradiation and a subsequent far-red irradiation the effect

of the last-mentioned decreased. With a dark period of 48 hours the inhibiting action of the far-red irradiation completely disappeared (concerning the effect of the temperature during this dark period, cf. below p. 64).

However, the gradually decreasing far-red inhibition with longer intervening dark periods was substituted by an opposite, increased far-red inhibition with shorter dark periods of 2 and 4 hours (figure 27, inverted triangles). A statistical comparison between, on the one hand, the results from the experiment with no intervening dark period between red and far-red irradiations and, on the other hand, with 2 and 4 hours revealed a slightly significant difference ($0.05 > P > 0.01$). In a repeated experiment for a more detailed study of this period (figure 27, triangles) a comparison of the results with no intervening dark phase and 0.5, 1 and 2 hours did not give any significance ($0.2 > P > 0.05$) for such a trend. See further on in the discussion of this chapter (p. 81).

e. Effect of temperature at red—far-red irradiations. In the earlier investigations both the irradiations and the following germination experiments were carried out at the same temperature (+ 25° C). In this experiment the temperature at the irradiations was varied but the conditions for the germination tests were unchanged.

The seeds were given red or red + far-red irradiations with the same standard methods as above. The irradiation equipment was placed in a room, where the temperature could be varied (+ 5— + 25° C). Before the seeds were irradiated, they had imbibed in darkness for 6 hours at + 25° C. The temperature of the seed beds was controlled and the irradiations were started after the seeds had been at the indicated temperatures for 30 minutes for adaptation. Dark control seeds were kept under the corresponding conditions for one hour but were left unirradiated. After the end of the irradiations the seeds were transferred to + 25° C for germination.

The results showed (figure 28, empty columns), that irrespective of the temperature employed the red irradiations induced full germination. The same independence of the temperature was valid for the far-red inhibition (figure 28, lined columns). The red + far-red irradiations caused the same general level of the germination percentages as only darkness. The results in darkness did not show any significant trend indicating an effect of the temperature *per se* (figure 28, filled columns). These results suggested that the effects of the red—far-red irradiations were independent of the temperature at the irradiations.

A corresponding experiment with white light, in which the irradiations were administered to unimbibed seeds, was also performed. Owing to the arrangement with the irradiation equipment in the room, the temperature of which was varied and the temperature dependence for the emitted radiant

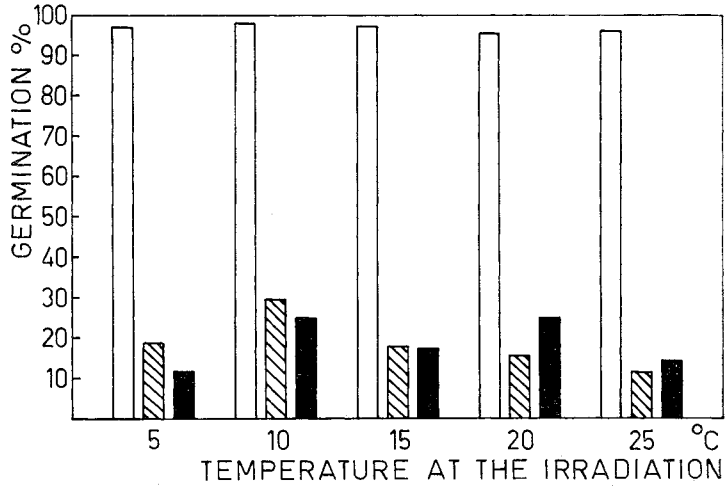

Figure 28. Effect of the temperature at red (unfilled columns) and red + far-red (lined columns) irradiations on the subsequent germination at + 25° C of Scots pine seeds. Standard irradiations (cf. figure 26). Dark control (filled columns). Provenance No. 3.

energy from fluorescent tubes (WITHROW and WITHROW 1956), a case which was neglected in the previous experiment, the radiation source (five white fluorescent tubes TL 40 W/33) was placed above a refrigatory box, the temperature of which was varied and in which the seeds were placed during the irradiations. The unimbibed seeds were irradiated with 1100 lux for 2 hours. For the rest of the experiment the conditions were unchanged. The results are presented in table 12.

A comparison of the germination in the irradiated and the control seeds regardless of the temperature showed a statistically significant stimulation ($0.001 > P$). The dark control seeds, which were kept under corresponding conditions but not irradiated, were not affected by the used temperatures. The irradiated seeds showed a proportionally increased germination with increased temperature at the irradiations. The regression coefficient (BON-

Table 12. Effect of the temperature at the irradiation of unimbibed Scots pine seeds (white fluorescent tubes TL/33, 1100 lux, 2 hours). Provenance No. 2.

Temperature at the irradiation °C	Germination percentage	
	Irradiated seeds	Dark control seeds
— 14	12 ± 2	5 ± 1
— 5	15 ± 2	3 ± 1
+ 6	15 ± 2	5 ± 1
+ 18	22 ± 2	4 ± 1
+ 33	22 ± 2	3 ± 1

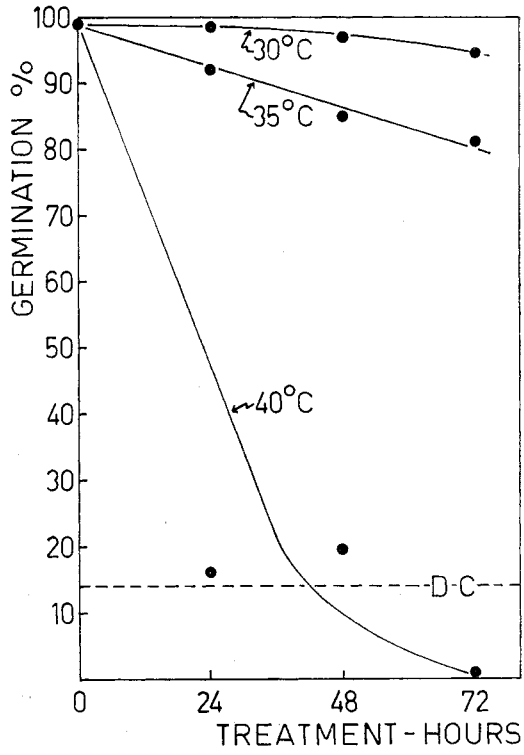

Figure 29. Effect of an increased temperature after a red induction of the germination in Scots pine seeds. Standard red irradiation (cf. figure 26) at + 25° C and completed germination at the same temperature after the end of the treatments. DC: dark control at + 25° C. Provenance No. 3.

NIER and TEDIN 1957) for this dependence (b = 0.22 ± 0.04) was significantly different from zero (0.001 > P), thus indicating a temperature dependence, the magnitude of which, however, was slight (an increased germination response of 2 per cent per increase of 10° C).

f. Effect of temperature after a red irradiation. In order to study whether an increased temperature reversed the induction after a red irradiation (performed at + 25° C) in the same way as a far-red irradiation the following experiment was done.

A complete induction of the germination was produced by a standard red irradiation after the seeds had imbibed in darkness for 6 hours at + 25° C. Immediately after this irradiation, the seeds—placed on the moist germination beds—were transferred to dark Petri dishes. These were kept during the indicated periods at different constant temperatures in thermostats. After the end of the treatments the seeds were returned to the Jacobsen apparatus (in darkness at + 25° C). Dark control seeds, which were left unirradiated, were kept all the time at + 25° C in the germinator. The results are presented in figure 29.

The results showed that the treatments with + 30 and + 35° C even

for as long a period as 72 hours had only a minor depressing effect on the germination of the red-induced seeds. On the contrary, the treatments with + 40° C had a pronounced depressing effect.

In order to test whether these decreases of the germination were caused by a non-photochemical conversion of the red—far-red reversible pigment system the remaining ungerminated seeds were reirradiated with red light. This irradiation was identical with the first one used and given after the seeds had been under germinating conditions for 20 days. Also the dark control seeds were included in the irradiation. After 10 more days the germination responses were determined. The results showed that—except for the original dark control seeds—in no case was an increased germination percentage exceeding 2 per cent obtained. However, the germination of the original dark control seeds was increased in additional 20 per cent. Concerning the last case it may be observed that the same red irradiation of the seeds after 6 hours of imbibition gave a complete induction of the germination at + 25° C. That this irradiation of the seeds, after they had been for 20 days under germinating conditions, did not induce full germination was in conformity with the results in figure 23, which showed that the germination response after such an irradiation decreased with an increasing time of imbibition (longer than 12 hours).

These results indicated that a period of increased temperature (+ 30—+ 35° C) only decreased the red induction of the germination to a slight extent. That this decrease was not caused by a thermal reversal of the red—far-red reversible pigment system was suggested by the slight stimulating effect of a repeated red irradiation. This was also incapable of reversing the more pronounced inhibiting effect of a still higher temperature (+ 40° C). In experiments with different germination temperatures (figure 2) it was shown that temperatures above + 25° C were supraoptimal for the germination of Scots pine seeds. This combined with the different magnitude of the response of the original dark control seeds, which had not been exposed to temperatures above + 25° C, and of the high-temperature treated seeds, instead suggested that the responses obtained with the higher temperatures were caused by a harmful effect on the viability of the seeds.

g. Effect of temperature during a dark period between red and far-red irradiations. In an earlier experiment (figure 27) it was shown that the inhibiting action of far-red light on the inductive effect of a red irradiation decreased with increasing time between the red and the far-red irradiation. After an intervening dark period of 48 hours the far-red irradiation was ineffective for inhibition of the germination. That experiment was performed at + 25° C. With these results as a starting point an experiment was undertaken for studying the importance of the temperature during this dark period.

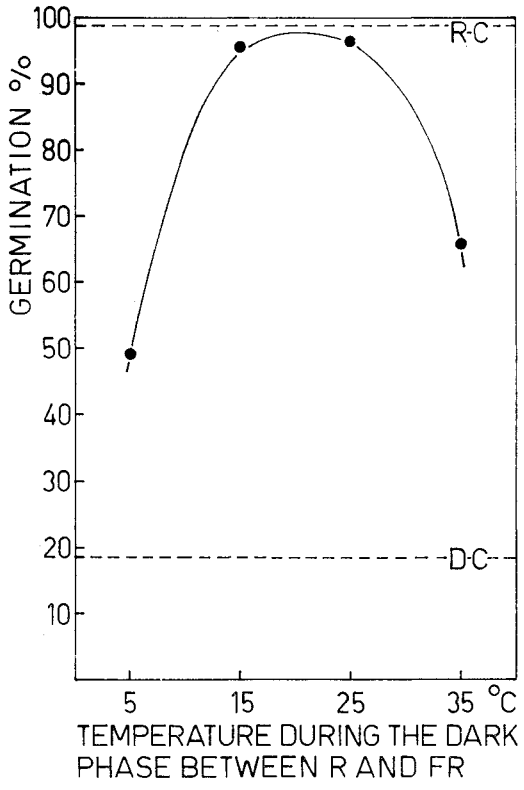

Figure 30. Effect of the temperature during a dark phase of 48 hours between a red and a subsequent far-red irradiation on the germination of Scots pine seeds. Standard red and far-red irradiations (cf. figure 26). Temperature before and after the dark phase + 25° C. RC: only red irradiated seeds at + 25° C. DC: dark control at the same temperature. Provenance No. 3.

After 6 hours of imbibition at + 25° C in darkness the seeds were given a standard red irradiation. Immediately after the end of this the seeds were exposed to different temperatures for 48 hours in darkness (for details about this, see the previous experiment). After the end of these treatments the seeds were transferred back to + 25° C, irradiated with a standard far-red irradiation and returned to the germinator (+ 25° C, darkness). The results from this experiment are given in figure 30.

In the range of temperature above + 25° C there was an inhibition of the germination. Compared with the results in the previous experiment (figure 29, + 35° C, 48 hours) the same treatment here combined with a subsequent far-red irradiation gave a somewhat greater inhibition, suggesting a further inhibition caused by the far-red irradiation. Owing to the scanty experimental material on this point the only possible conclusion is that the results are consistent with the results in the previous experiment, suggesting that treatments with temperatures above + 25° C do not seem to accentuate the dark reversal of the red—far-red pigment system.

Contrary to this the experiments within the range of temperatures below + 25° C indicated a positive dependence on the temperature. Compared with the seeds kept all the time at + 25° C the seeds subjected to + 5° C for 48 hours still could be inhibited with the subsequent far-red irradiation. A comparison of the results with + 15 and + 25° C, however, did not show any difference. This suggested that with the period of darkness employed (48 hours) even at + 15° C the reactions following the red irradiation had advanced to a point, where the regulating capacity of the far-red light had disappeared. In spite of this, the general trend of the results indicated that the dark reactions following a red irradiation were positively dependent on the temperature. This was contrary to the temperature independence of the irradiations earlier described (cf. figure 28, table 12). The temperature-independent photoreaction was followed by temperature-dependent, dark reactions. If these were permitted to proceed for 48 hours at + 25° C, they resulted in the complete loss of controlling power of the light mechanism.

h. Effect of oxygen on the red induction. In order to establish whether the induction of the germination which in the previous experiments was produced by irradiations with red light in air was dependent on the presence of oxygen, the following experiments with red irradiations of the seeds in different oxygen concentrations were carried out.

In the performance of the experiments the same glass containers were used as in an earlier experiment with germination in different oxygen concentrations (p. 44). In each container 3×100 seeds were placed, 100 seeds per germination bed (the germination beds were the same as used in the germinator). In one part of the experiment the containers were flushed with pure oxygen, in the other with pure nitrogen, which had been conducted through an alkaline solution of pyrogallol (RUGE 1951) and over redhot sheet-copper for elimination of traces of oxygen and after cooling also through concentrated sulphuric acid for drying. The gases were taken from commercial steel containers (AGA). The flushings were started one hour before the start of the irradiations. When the flushings were ended, the containers were closed and placed below the same bank with red fluorescent tubes as used in the standard red irradiations above. The period of irradiation was 30 minutes. Until the start of the irradiations the containers were wrapped in dark plastic foil. Afterwards the containers were placed in darkness at + 25° C. All the containers were flushed with ordinary air in darkness 24 hours after the end of the irradiations. This was also done with the controls containing ordinary air. After these last flushings were completed the containers were closed again and the germination percentages were determined after the seeds had germinated for 10 days in darkness.

In addition to the influence of different gases the effect of the variation

Table 13. Effect of different oxygen concentrations on the red induction of the germination in Scots pine seeds. For further details, see the text. Provenance No. 4.

Per cent oxygen volume at the irradiation	Germination percentage			
	Unimbibed seeds in the different oxygen concentrations		Imbibed seeds in the different oxygen concentrations	
	Irradiated	Dark control	Irradiated	Dark control
0	37 ± 2	16 ± 2	74 ± 2	33 ± 2
21	51 ± 2	19 ± 2	69 ± 2	20 ± 2
100	33 ± 2	3 ± 1	75 ± 2	20 ± 2

of the imbibition state of the seeds prior to the irradiations was also studied. In half of the experiments the unimbibed seeds were irradiated. In this case the imbibition was started after the end of the last flushing. In the other part the irradiations were done after the seeds had imbibed for 6 hours in darkness (in the containers). The corresponding dark control seeds were treated in the same way but were left unirradiated. The results from these experiments are given in table 13.

The results showed that both the irradiations of the unimbibed seeds and the imbibed seeds caused a stimulated germination. This was significantly higher ($0.001 > P$) than in the corresponding dark control seeds. Concerning the importance of the oxygen for the light effect the results showed no significant effects. In spite of certain variations the red induction was of the same magnitude regardless of whether the irradiations were given in nitrogen, air or pure oxygen.

With the experimental method employed it was not possible to show that the red induction was dependent on the presence of oxygen. This made it probable that the photoreaction, at least the red-sensitive one is not a photo-oxidation in the meaning that oxygen directly participates in the photoprocess.

i. Seed coat and red—far-red reversibility. The importance of an intact seed coat for the light dependence of the germination was earlier demonstrated in experiments with continuous white light and darkness (figure 17). These results showed that the light dependence diminished after a puncturing and completely disappeared after removal of the seed coats. In the following experiments this effect of the seed coat was investigated in combination with limited irradiations with red and far-red light.

For every type of treatment 4×50 seeds were used. Puncturing and decoating were done in the same way as previously described (p. 40). The control consisted of intact seeds. After 6 hours of imbibition in darkness one part of the seeds was given a standard red irradiation and the other part the same red irradiation and an immediately following standard far-

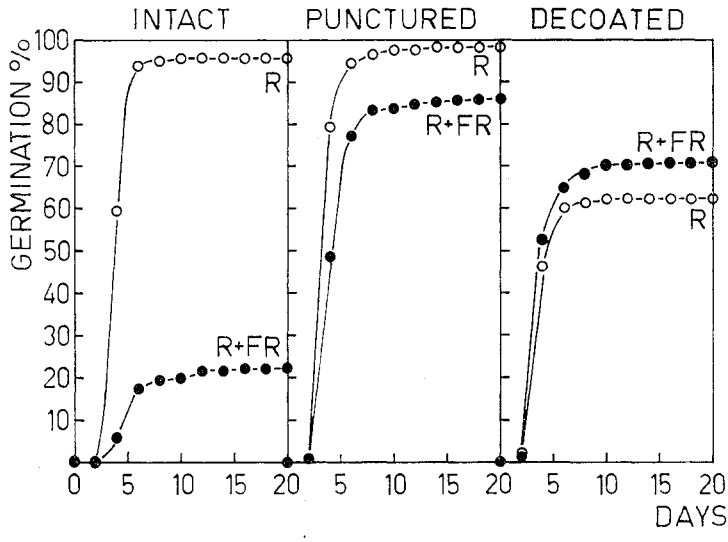

Figure 31. Influence of the seed coat on the germination course of Scots pine seeds in combination with red (R) and red + far-red (R + FR) irradiations. Standard irradiations (cf. figure 26). Provenance No. 2.

red irradiation. The germination course was followed and the results are presented in figure 31.

As in previous experiments the standard red irradiation induced complete germination. This red induction could be inhibited by a subsequent far-red irradiation of the intact seeds. The seeds with punctured coats also showed complete germination after a red irradiation, but in this case the far-red irradiation did not eliminate the red induction to the same extent. The difference between the final germination percentages of the red and the red + far-red irradiated, punctured seeds was only slightly significant ($0.05 > P > 0.01$). The effect was more pronounced in the decoated seeds, where also the final germination of the red + far-red irradiated seeds was somewhat higher than that of the red irradiated ones. However, the difference was not significant ($0.2 > P > 0.05$). These results also showed that a decoating caused a certain decrease of the viability in the seeds. The same observation was made in the corresponding experiment in figure 17.

These results showed that the light dependence of the germination in Scots pine seeds was correlated with the existence of an intact seed coat. The elimination of the seed coat not only abolished the stimulating action of the light but also made a normally inhibiting action of a far-red irradiation ineffective. For further experiments and discussion on this point, see Chapter VI.

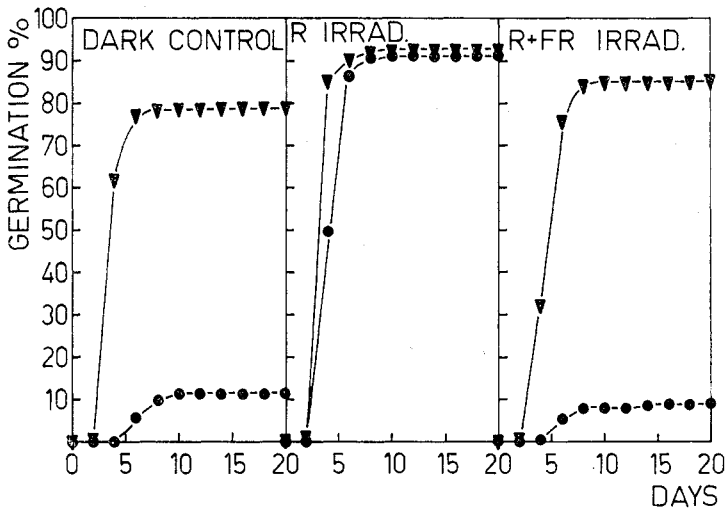

Figure 32. Effect of stratification on the germination course of Scots pine seeds in combination with red (R) and red + far-red (R + FR) irradiations. Standard irradiations (cf. figure 26). Inverted triangles: seeds stored on moist germination beds in dark Petri dishes for 2 weeks at + 3—4° C. Circles: seeds stored dry under the same conditions. Irradiations started after 6 hours of imbibition at + 25° C. Dark control unirradiated. Provenance No. 3.

j. Stratification and red—far-red reversibility. Besides the elimination of the seed coat also a treatment of the seeds in imbibed condition with a low temperature (stratification) was earlier shown to increase the ability of the seeds to germinate in darkness (cf. figure 9). These results were here combined in an experiment with red and red + far-red irradiations.

The seeds were placed on moist germination beds in dark Petri dishes and subjected to + 2—4° C for 2 weeks. The control seeds were treated in the same way but in unimbibed condition. After the end of this treatment the seeds were placed into the germinator for 6 hours at + 25° C and then divided into two groups, one of which was given a standard red irradiation, and the other a standard red and an immediately following standard far-red irradiation. Dark control seeds were left unirradiated. After the end of these irradiations the germination was continued in darkness at + 25° C. The time course of the germination was followed and the results are given in figure 32.

These results showed that the responses of the control seeds (unimbibed at the temperature treatment) to the red and red + far-red irradiations were the same as in corresponding previous experiments. As also shown earlier (figure 9) the germination in darkness increased after a stratification. After a combination of stratification and red irradiation the same germination appeared as after only a red irradiation of the unstratified seeds. However,

this red stimulation could be nullified by the far-red light only to the same level as the germination of the stratified, dark control seeds. This inhibition was significant ($0.01 > P > 0.001$), but a comparison between the germination of the stratified dark control seeds and the same red + far-red irradiated seeds showed no significance ($P > 0.2$). The stratification eliminated the dependence of stimulating irradiation (red light) and at the same time the dependence of inhibiting irradiation (far-red light). A corresponding fraction of the seeds, which germinated without light, was also independent of the far-red irradiation. The stratification evidently caused changes in the seeds which resulted in the red—far-red reversible pigment system losing its property as a controlling system for the germination.

3. Studies on the Action Spectrum

For further studies on the irradiance dependence in the red and far-red part of the spectrum and for studies on the action spectrum an irradiation apparatus adopted from MOHR and SCHOSER (1959) with interference filters was used. For details about this, see page 18 and the separate experiments. All irradiations were given after the seeds had imbibed in darkness for 6 hours at + 25° C. The germination tests were completed under the same conditions (in the germinators).

a. Effect of irradiance and period of irradiation in red light. In these experiments an interference filter which had a transmission maximum at 6600 Å was used (cf. also figure 6 for the complete transmission curve of this filter). The different irradiances were obtained by changing the power input of the lamp and by combinations with neutral filters.

In a first experiment the effects of irradiances from 25—400 $\mu W/cm^2$ and irradiation periods from 10—10000 seconds were studied. In a complementary experiment the irradiances and periods were 1—100 $\mu W/cm^2$, 100 and 1000 seconds, respectively.

The results, which are given in figure 33, showed that an irradiation with 100 $\mu W/cm^2$ for 10 seconds (1 mj/cm^2) was sufficient for the induction of the germination in about half of the light-requiring seeds. A more narrow estimation of the energy required for the promotion of the germination to 50 per cent was obtained with the following method adopted from BORTHWICK *et al.* (1954). The calculated per cents of seeds, the germination of which was induced by the red irradiations, were transformed to the corresponding probit values (BONNIER and TEDIN 1957). These were plotted against the logarithms of the corresponding red energies. From the linear part of this relationship the energy required at 6600 Å for a 50 per cent stimulation was estimated at 2 mj/cm^2.

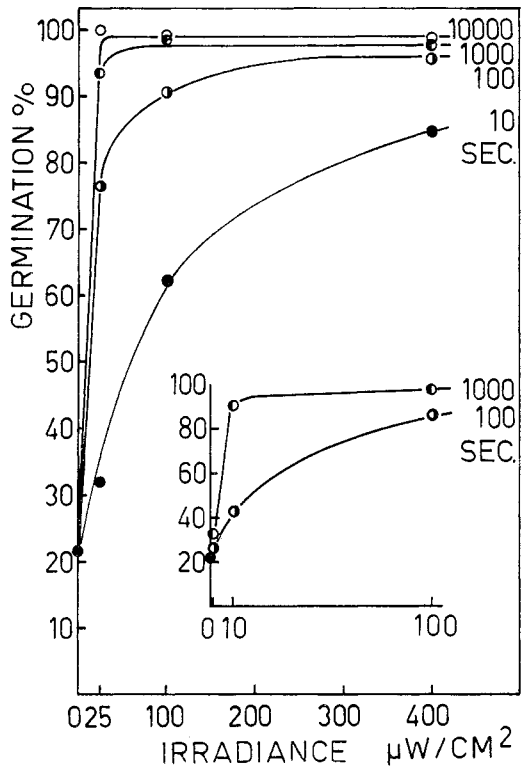

Figure 33. Effect of irradiance and irradiation time in red light (interference filter 6600 Å) on the germination of Scots pine seeds. Irradiations started after 6 hours of imbibition. Provenance No. 3.

b. Effect of irradiance and period of irradiation in far-red light. As in earlier experiments, where the reversible effect of a far-red irradiation was demonstrated, the seeds at first were given an induction irradiation. This was performed with red light with the use of the same interference filter as in the previous experiment (6600 Å). The irradiance was 100 μW/cm² and period of irradiation 1000 seconds. This energy (100 mj/cm²) was more than sufficient for full induction of the germination (saturation dose, cf. figure 33). Immediately after such an irradiation the seeds were given far-red irradiation, which was produced with an interference filter, which had its transmission maximum at 7300 Å (see figure 6). The irradiances were adjusted in the same way as above (25—400 μW/cm²). Irradiation periods were from 10—10000 seconds. The results are presented in figure 34.

The results showed that the energy 1 mj/cm² (100 μW/cm² for 10 sec.), which in red (6600 Å, cf. the previous experiment) induced germination in about half the light-requiring seeds, in far-red (7300 Å) was completely ineffective in causing any inhibition. Evidently the sensitivity to far-red light was lower than to red light. With the same method as in the previous

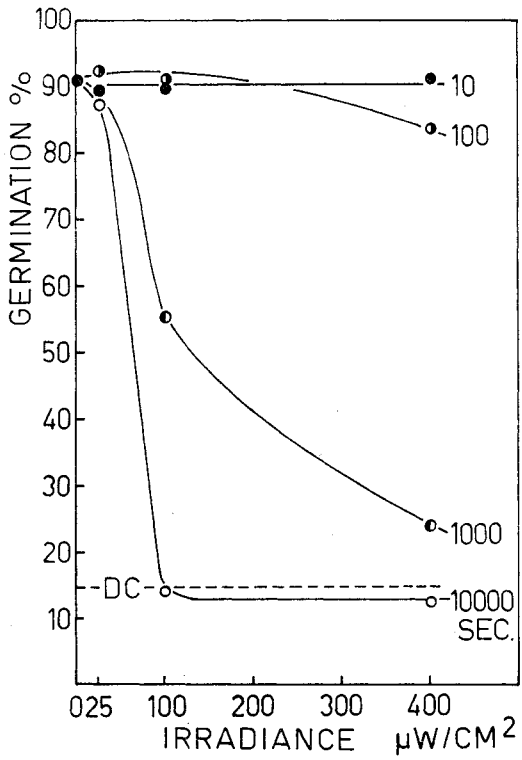

Figure 34. Effect of irradiance and irradiation time in far-red light (interference filter 7300 Å) on the germination of Scots pine seeds. Far-red irradiations immediately after red induction with 6600 Å (100 μW/cm², 1000 sec.). Imbibition as in figure 33. DC: dark control. Provenance No. 3.

experiment the energy required for the inhibition of the germination to 50 per cent was estimated at 100 mj/cm².

A comparison of the results in these last two experiments showed that the sensitivity of the Scots pine seeds was greater in red (6600 Å) than in far-red (7300 Å) light: 2 mj/cm² in red and 100 mj/cm² in far-red were required for the corresponding stimulation and inhibition of the germination to 50 per cent in the portion of the seeds that were light-requiring.

c. Effect of the irradiance at constant energy in red light. A further attempt to describe the quantitative relations between the energy in red light (6600 Å) and the germination response was made in an experiment for studying the validity of the product law for the induction of the germination.

The irradiations were performed with the same red interference filter (6600 Å) as used above. Adjustments of the irradiances were also made as above. The experiments were arranged in such a way that at the changes of the irradiances the periods of irradiations were changed in the opposite direction, *i.e.*, the total energy given to the seeds was kept constant. The experiments were carried out at two different energies with the following

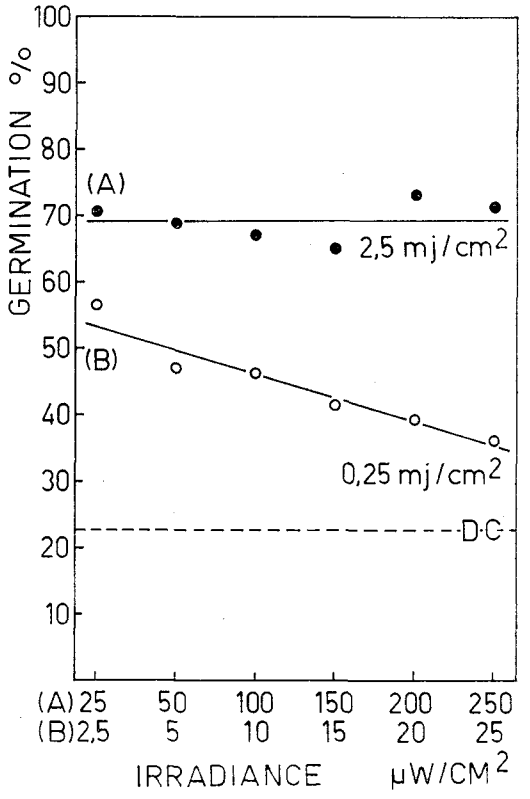

Figure 35. Effect of irradiance at constant energy in red light (6600 Å) on the germination of Scots pine seeds. Irradiation time 100—10 sec. Imbibition as in figure 33. Provenance No. 3.

variations of irradiances and irradiation periods: 2.5—25 μW/cm² for 100—10 seconds (0.25 mj/cm²) and 25—250 μW/cm² for 100—10 seconds (2.5 mj/cm²). The results from these two series are given in figure 35.

The results with 0.25 mj/cm² showed that the germination responses decreased with increasing irradiance and decreasing time of irradiation. In spite of constant energy the germination responses were evidently affected by a limiting time of irradiation. In this range the product law was not valid.

However, with 2.5 mj/cm² the results showed that the germination responses were independent of the combinations of irradiances and irradiation times employed and thus the results were in coordination with the requirements for the validity of the product law.

With a total energy nearly equivalent to the energy required for the induction of 50 per cent germination (cf. above p. 70) and with relative short periods of irradiation (10—100 sec.) it was possible to demonstrate that the product law was valid for the induction of the germination with red light, *i.e.*, for a certain germination response the required energy could

be given either through a smaller irradiance and a longer period of irradiation or the reverse.

d. Action spectrum. An extension of the above experiments on the dose-response relations and the validity of the product law as the basis for the establishment of a true action spectrum (the energy requirements at different wavelengths for a defined response, BLUM 1950, WITHROW *et al.* 1957, cf. also BORTHWICK *et al.* 1954, TOOLE *et al.* 1955, HAUPT 1959, SHROPSHIRE *et al.* 1961, VIRGIN 1962 and others) was substituted by a more direct method, in which the responses after irradiations with a defined energy at different wavelengths were used. According to MOHR (1956) this direct method gives as useful action spectra as with the first-mentioned method. The positions of the peaks in the action spectra are independent of the method employed (WITHROW *et al.* l.c.). The use of the simpler method for the establishment of the action spectrum of the germination also was more suitable for corresponding investigations on other parts of the germination process (the action spectra for the occurrence of embryo-mitosis in figure 39 and for the respiration in figures 51 and 54) and permitted a direct comparison between them (see also BLUM l.c. p. 444).

In a first experiment the action spectrum for the induction of the germination was studied. In the irradiations the same energy was used at the different wavelengths (100 mj/cm²—100 μW/cm² for 1000 sec.). Dark control seeds were left unirradiated. In the same experiment seeds were randomly sampled for investigations of the action spectrum for the occurrence of embryo-mitosis. Thus from each sample of 100 seeds, 10 seeds were sampled 24 hours after the start of the imbibition. After another 24 hours 10 new seeds were taken (cf. p. 101). Thus the germination values were based on 4×80 seeds for every wavelength.

The same procedure was used in an experiment on the action spectrum for the inhibition. In this the seeds at first were given an induction irradiation with red light (6600 Å, 100 mj/cm²) and immediately afterwards irradiated with the same energy at different wavelengths. The results from these experiments are given in figure 36 (stimulation spectrum) and figure 37 (inhibition spectrum).

The results constituting the stimulation spectrum showed that in the blue part of the spectrum the used irradiations did not influence the germination. With increased wavelength a gradually increasing effect appeared with a peak at 5500 Å (cf. corresponding peak in figures 39 B, 51 A, B and 54) but the wavelength region most effective for the stimulation was found in the red. The small differences of the germination responses in this region (used wavelengths 6080, 6200, 6300, 6600 and 6830 Å) made it impossible to reveal any narrower part with a maximal stimulation (cf. a more pro-

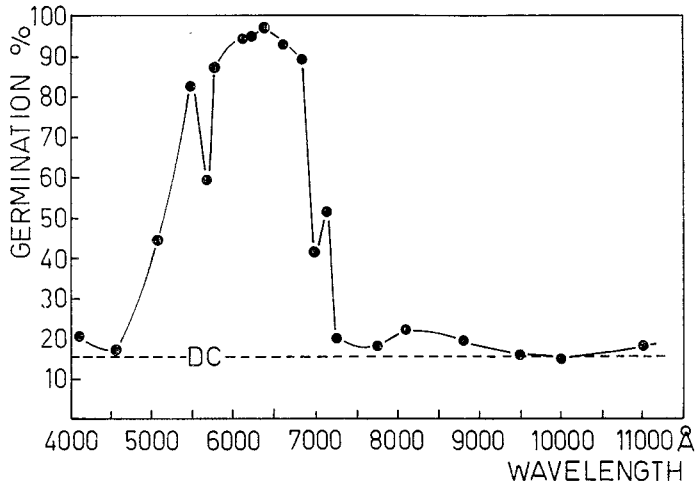

Figure 36. Stimulation spectrum for the germination of Scots pine seeds. Interference filters (cf. figure 6). Constant energy 100 mj/cm² (100 μW/cm², 1000 sec.). Imbibition as in figure 33. DC: dark control. Provenance No. 4.

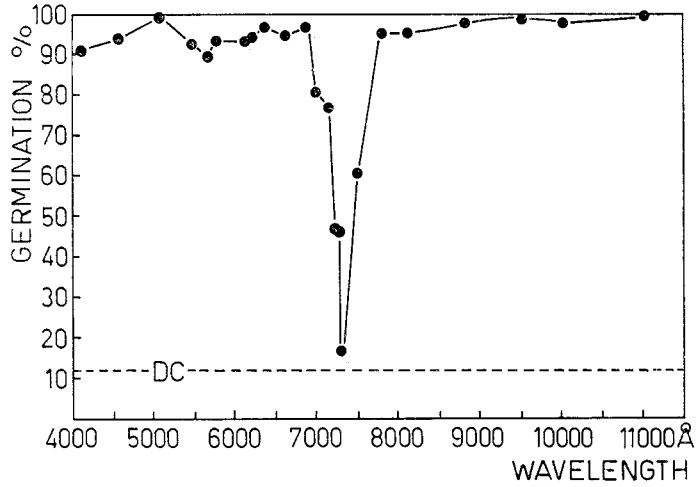

Figure 37. Inhibition spectrum for the germination of Scots pine seeds. After induction with 6600 Å, 100 mj/cm² (100 μW/cm², 1000 sec.) the same irradiations as in figure 36. DC: dark control. Provenance No. 4.

nounced effect at 6600 Å in figure 39 B). With still longer wavelengths (far-red 7000—8000 Å) the stimulation rapidly decreased with a small peak at 7125 Å (cf. corresponding peak in figures 39 B and 51 B) and finally vanished.

The results presented as an inhibition spectrum in figure 37 showed no significant inhibitions of light of wavelengths shorter than 6830 Å or longer

than 7775 Å. However, in the intervening region (6830—7775 Å) a pronounced inhibition appeared with a maximum at 7300 Å (cf. corresponding results in figures 39 D and 51 D). It can also be noticed that a weak shoulder appeared at 7125 Å (cf. also figures 39 D, 51 D and 54; in the second case with a peak shifted to 7000 Å and in the third case with a shoulder at 7125 Å for anaerobic carbon dioxide).

Under the experimental conditions employed it was possible to demonstrate that light most effective for the stimulation of the germination in Scots pine seeds was to be found in the red part of the spectrum between 6080 and 6830 Å without any more pronounced maximum. Inhibitory effects of the irradiations were to be found in the region 6830—7775 Å with a maximum at 7300 Å. Comparisons with action spectra for the occurrence of embryo-mitosis and the respiration showed correspondences.

D. Discussion

In most of the investigations on the light-controlled germination of seeds where the occurrence of red—far-red effects have been demonstrated (cf. table 11), these have not been followed by studies on the action spectra. For the pine species here studied (*Pinus silvestris* L.) the corresponding occurrence of a red—far-red reversible control of the germination (Nyman 1957, 1961) here was extended to include a study on the action spectra for the stimulation (figure 36) and the inhibition (figure 37) of the germination.

In these action spectra the germination responses after irradiations with the same energy at different wavelengths are given. This method did not permit a precise comparison with the cases in which the action spectra have been given as the energy requirements for a defined germination response at different wavelengths (*Lactuca sativa* L. Borthwick *et al.* 1952 a, Toole *et al.* 1953, Borthwick *et al.* 1954; *Lepidium virginicum* L. Toole *et al.* 1955) or as quantum responsivity (*Arabidopsis thaliana* (L.) Eastland Shropshire *et al.* 1961). Compare also the use of the last-mentioned method in studies on other red—far-red controlled processes (opening of hypocotyl hooks in *Phaseolus vulgaris* L. Withrow *et al.* 1957 a; formation of chlorophyll in leaves of *Triticum sativum* L. Virgin 1961; unfolding of leaves in the same species Virgin 1962).

However, in spite of the different methods used in the determinations of these action spectra (cf. also the action spectra for the germination of fern spores in Mohr 1956 and for the germination in *Lactuca sativa* L. in Flint and McAlister 1937 obtained with the same method as was used in this investigation) they all show a general resemblance regarding the red

and the far-red part of the spectrum. A maximal stimulation appears in red between 6000 and 7000 Å with a maximum at 6600 Å in BORTHWICK *et al.* (l.c.) and in SHROPSHIRE *et. al.* (l.c.) but without this special effect at 6600 Å for *Lepidium virginicum* L. in TOOLE *et al.* (l.c.). In both WITHROW *et al.* (l.c.), SHROPSHIRE *et al.* (l.c.) and VIRGIN (1961, 1962), however, an evident peak was obtained at this wavelength. For the Scots pine seeds in figure 36 it was not possible to demonstrate a corresponding distinct maximum, but the general resemblances with the other action spectra seems to be satisfactory. In this connection it may also be pointed out that the peak at 5500 Å in figure 36 had a correspondence in a shoulder at the same wavelength in SHROPSHIRE *et al.* (l.c.). Compare also shoulders at 5600 Å in WITHROW *et al.* (l.c.) and at 5400 Å in VIRGIN (l.c.). Another feature to observe is the peak at 7125 Å (figure 36, cf. also the shoulder at the same wavelength in figure 37), with no correspondence in SHROPSHIRE *et al.* (l.c.) and the shoulder, which WITHROW *et al.* (l.c.) and VIRGIN (1961) found at 7000 Å. Regardless of the absolute significance of these minor details of the action spectrum in figure 36, their repeated occurrence in the action spectra for the embryo-mitosis (figure 39) and the respiration (figure 51, see however figure 54) and the close correspondence in the above cited cases seem along with the general conformity between these action spectra to support the identity between them.

Concerning the action spectrum of the inhibition in figure 37 this showed the occurrence of an evident inhibition between 7000 and 8000 Å with a maximum at 7300 Å. This was also in accordance with the above-cited cases (cf. BORTHWICK and HENDRICKS 1961). However, the peaks found at 7200 and 7400 Å in the inhibition spectra of SHROPSHIRE *et al.* (l.c.) and VIRGIN (1962) and at 7100 Å in WITHROW *et al.* (l.c.) could not be detected with the method employed. In spite of these minor details the general feature of the inhibition spectrum in the far-red region of the germination of the Scots pine seeds seems to be in accordance with those of other red—far-red sensitive photomorphogenic processes.

With regard to the blue part of the spectrum (wavelengths less than 5200 Å) the few experiments did not give any sure indications of either stimulations or inhibitions. The establishment of both stimulatory and inhibitory effects with blue light (BORTHWICK *et al.* 1954, EVENARI *et al.* 1957), only inhibitory (FLINT and MCALISTER 1937) and the occurrence of a blue-red antagonism at the germination of *Lactuca sativa* L. (WAREING and BLACK 1958) together with the blue peak in the action spectrum for the photomorphogenic high-energy reaction (MOHR 1960, 1961 a, 1962) seem to confuse the general understanding of the effects in this part of the spectrum (cf. also reviews by MOHR 1961 b and EVENARI in press).

Irrespective of the results in the blue part of the spectrum, which require further studies on the Scots pine seeds, the general agreement between the action spectra for stimulation in the red and inhibition in the far-red in this species and in a number of other cases may support the idea that the same pigment system is operating in the Scots pine seeds as in other species.

A further support for this idea is given by the establishment of repeatedly reversible red—far-red effects on the germination, which are presented in figure 26 (cf. NYMAN 1961, figure 1). Thus the single reversal of the red-induced germination appearing as an inhibition in the far-red region of the action spectrum in figure 37 could be repeated for a number of times with alternating red—far-red irradiations.

Beside these qualitative similarities between the light-controlled germination of Scots pine seeds and corresponding phenomena in other species also quantitative similarities were found. The results in figures 33 and 34 suggested a logarithmic relationship between light energy and germination response for the red induction and a direct proportionality for the far-red inhibition, respectively. Such a dependence has been shown by WITHROW *et al.* (l.c.) and SHROPSHIRE *et al.* (l.c.), but the physiological background of this is obscure (MOHR 1962). After transformations of the results in figures 33 and 34 (cf. p. 70) the energies required for a 50 per cent stimulation and corresponding inhibition were estimated at 2 mj/cm² and 100 mj/cm², respectively. The corresponding values for the germination of *Lactuca sativa* L. obtained by BORTHWICK *et al.* (1954) were 2 mj/cm² and 60 mj/cm². For further comparisons with other species, see TOOLE *et al.* (1955, table VI). An estimation from the results presented by TOOLE *et al.* (1961) on the germination of *Pinus virginiana* Mill. carried out with the same method gave 35 mj/cm² in red (5800—6950 Å) and 250 mj/cm² in far-red (6950—7900 Å) for 50 per cent stimulation and inhibition of the germination, respectively. Thus this comparison shows that the energy requirements for one and the same germination response in these different species are of about the same magnitude. In all cases also the far-red energies were higher than the red ones required for the same germination response (30, 50 and 70 times for *Lactuca sativa* L., *Pinus silvestris* L. and *P. virginiana* Mill., respectively).

From the foregoing it is evident that both qualitative and quantitative responses in the light-controlled germination in *Pinus silvestris* L. showed agreement with the results from corresponding experiments on other red—far-red controlled photomorphogenic processes. Thus the establishment of phytochrome as the pigment responsible for the light absorption in such processes (cf. p. 52) makes it probable that the same pigment is operating in the light-controlled germination in the seeds of *Pinus silvestris* L.

In their studies on the germination of *Lactuca sativa* L. BORTHWICK *et al.* (1952 a), TOOLE *et al.* (1953) and BORTHWICK *et al.* (1954) demonstrated that the effect of a far-red irradiation was only slightly dependent on the energy in the red light used in the induction of the germination. Not even the temperature at the red—far-red irradiations had any influence on the effect of the irradiations. From these results they concluded that there was a red—far-red absorbing pigment in the seeds, which mediated in the light-control of the germination. This pigment was supposed to change its absorption maximum from red to far-red under an irradiation with red light and in the opposite direction under a far-red irradiation. From the absence of temperature effects on these irradiations it was supposed that this conversion was a purely photochemical reaction or a reaction in which eventual further participating reactants were in close contact with the pigment. That this red—far-red convertible pigment participated only as a regulatory mechanism for the germination through a displacement of the equilibrium between the red and the far-red absorbing form was concluded from the fact that the far-red sensitivity was only slightly dependent on the energy used in the red inductions.

In order to study whether the same relationships were valid for the Scots pine seeds, comparable experiments were carried out on the influence of the temperature and the importance of the red induction for the far-red sensitivity.

The results from the temperature experiments are given in figure 28, from which it was concluded that the temperature between + 5 and + 25° C had no influence on the effect of red and red + far-red irradiations. In a related experiment (table 12), in which the irradiations were administered with white light to the unimbibed seeds, the temperatures from — 14 to + 33° C had only a minor influence on the effects of the irradiations. Both these results were consistent with the conception that the primary light absorption caused a photochemical reaction, which contrary to common thermochemical reactions is independent of the temperature (PRECHT *et al.* 1955). These results were also consistent with the results from corresponding experiments on lettuce seeds (BORTHWICK *et al.* 1954, cf. also EVENARI 1961) and other seeds (KINCAID 1935, PAECH 1953).

However, from studies on the red—far-red controlled opening of the hooks in etiolated bean seedlings WITHROW and KLEIN (1957) and KLEIN *et al.* (1957 a) found that the reversal process was dependent on the temperature contrary to the induction with red light, which also in that case was independent of the temperature. This suggested the participation of an intervening thermochemical reaction (cf. further p. 81).

Concerning the influence of different energies on the induction of the

germination with red light on the far-red sensitivity, the results presented in figure 25 showed that the inhibition obtained with different energies in far-red light was slightly affected by the red energies used. In spite of the fact that the red irradiations for the inductions were given for 3, 30 and 300 minutes the same following far-red irradiations did not show any principally different capacities for the inhibition of the germination.

Thus both these types of experiments on the Scots pine seeds gave results which were in accordance with the results from corresponding experiments on the lettuce seeds (BORTHWICK *et al.* l.c., TOOLE *et al.* l.c.).

A further attempt to demonstrate that at least the induction process in the Scots pine seeds is a purely photochemical process was made in an experiment where red irradiations of imbibed and unimbibed seeds were given in different oxygen concentrations (table 13). These results supported the idea that the red induction was independent of oxygen. Even under anaerobic conditions the red irradiations caused a stimulated germination, which also was demonstrable in the seeds which were unimbibed under the irradiations. These results were consistent with the results obtained by BONNER (1959) with etiolated pea epicotyls and by IKUMA and THIMANN (1961) with lettuce seeds. In both these cases it was also shown that the reversibility with far-red light was independent of the presence of oxygen, which suggested that the pigment conversion in red—far-red irradiations was a purely photochemical reaction (IKUMA and THIMANN l.c.). Contrary to this WITHROW and KLEIN (1957) supposed that the induction process was dependent on the presence of oxygen from the fact that the photosensitivity of seeds is lost after puncturing of the seed coats, when access to air is provided. In accordance with this POWELL (1958) also reported that no red induction of the germination in tobacco seeds could proceed under anaerobic conditions but this was gradually resumed after transference to aerobic conditions.

From the results discussed above it seems reasonable to conclude that the reactions in the Scots pine seeds show agreement with the reactions in lettuce seeds. The absence of temperature dependence and dependence on oxygen for the red—far-red control of the germination together with the unimportance of the energy in the red inductions for the far-red sensitivity indicate that the light controls the germination through a displacement of an equilibrium between the red and far-red absorbing forms of the pigment.

In spite of the fact that this idea is in agreement with the results from the studies on the partially purified phytochrome (cf. p. 52) there are some physiological observations which are not consistent with this.

In hypocotyl hooks from etiolated seedlings of *Phaseolus vulgaris* L., in

which repeated red—far-red reversibility (KLEIN *et al.* 1956) and the same action spectra as for the germination of photosensitive seeds have been found (WITHROW *et al.* 1957, cf. also the discussion above), KLEIN *et al.* (1957 a and b) and WITHROW and KLEIN (1957) found that the red induced opening was not completely reversible with a far-red irradiation given immediately after a red one. Maximal inhibition at first appeared 1—2 hours after the red irradiation. These results were inconsistent with the theory that the reversibility should depend on a direct photochemical transformation between a red and a far-red absorbing form of the regulating pigment. Also in the red induced germination of *Amaranthus retroflexus* L. KADMAN-ZAHAVI (1957, 1960) showed that the inhibitory power of a far-red irradiation was slight immediately after the red irradiation but rapidly increased to a maximum between 2 and 16 minutes after the red irradiation. This far-red reversal also was temperature-dependent. This indicated that in these seeds the red light induced a reaction and that this was inhibited by the far-red irradiation (KADMAN-ZAHAVI 1960).

In a corresponding study on the Scots pine seeds (figure 27) a slightly increased effect of the far-red irradiations was obtained when the far-red light was given 0.5—4 hours after the red irradiation instead of immediately afterwards. A statistical evaluation, however, did not show any significance. It may also be observed that in the experiments with repeated red—far-red irradiations in the study on embryo-mitosis (table 16) and respiration (table 25), in which the irradiations followed immediately after each other, no signs of an incomplete reversal could be found. Thus no sure indications could be found in the Scots pine seeds on the existence of an intervening dark reaction between the red and far-red sensible ones. However, the experiments in figure 27 also showed that with a longer dark period between the red and the following far-red irradiation the inhibitory action of the last-mentioned gradually decreased and finally completely vanished (cf. further discussion on this point p. 88).

The results discussed above have shown the occurrence of a series of similarities between the Scots pine seeds and other seeds in their reactions to irradiations with red and far-red light. However, there are also some discrepancies. These are concerned with the relationship between the seeds' state of imbibition and the photosensitivity and with the absence of a dark reversal of the pigment in the red irradiated seeds.

It has been repeatedly denied that light-sensitive seeds can be influenced by light in unimbibed condition (cf. *e.g.* EVENARI 1956, BORTHWICK and HENDRICKS 1961). That the opposite is valid for the Scots pine seeds has

been described in the previous chapter from experiments with white light and in the present chapter in experiments with white (figure 22, table 12) and red light (figures 22, 23, 24, table 13). Also from these results it was evident that the seeds before the start of the imbibition were photosensitive in that irradiations with white and red light caused a stimulated germination. However, in spite of prolonged irradiations (up to 4 hours, figure 22) it was not possible to stimulate all the seeds to germination. The reason for this is not obvious. It was also impossible to eliminate this partial red induction of the unimbibed seeds with far-red irradiations. Even as long an irradiation as 4 hours (figure 24, no imbibition) could not reverse the induction obtained with red light. These results were contrary to the reactions in the imbibed seeds (figure 24, imbibition 6 h.), where the same irradiation with far-red light for only 15 minutes was nearly sufficient for full inhibition of the germination. Here it may be observed that after 6 hours of imbibition 50 times more energy was required in far-red than in red for the same germination response (cf. p. 72). This discrepancy may possibly be greater in the unimbibed seeds and may be the reason for the absence of the far-red reversibility in these seeds.

In some cases with photoperiodic induction of flowering (HILLMAN 1959, NAKAYAMA *et al.* 1960, BORTHWICK *et al.* 1961, PURVES 1961) a corresponding absence of a far-red reversal of a red effect has been shown. NAKAYAMA *et al.* (l.c.) attributed this to an absence of reversal in the pigment from the far-red to the red absorbing form. Other probable explanations such as that the red light did not cause any change of the pigment or that the far-red absorbing form initiated its physiological effects before the reversal was completed were rejected. Also for the absence of a far-red reversal of the red induction in the unimbibed Scots pine seeds the first explanation seems to be the most probable one. The fact that the red light caused a change of the pigment was evident from the stimulated germination after such an irradiation and also the long time required after a red irradiation before the full establishment of the red stimulation (cf. figure 27) seems to exclude the two last explanations. In connection with the results of NAKAYAMA *et al.* (l.c.) HENDRICKS (1960 b) has reported the probable existence of two forms of the far-red absorbing phytochrome, both physiologically active but only one with photoreversible capacity. If a corresponding possibility should be valid for the Scots pine seeds, the consequence seems to be that at the start of the imbibition the non-photoreversible form of the pigment should be transformed into its reversible form.

Contrary to the results on the unimbibed seeds the same far-red irradiations on the imbibed seeds caused an evident inhibition of the red-induced germination (figure 24, imbibition 6 h.). In this connection it may

be pointed out that this inhibition, which was complete after an irradiation for only 30 minutes, did not show any further changes with increased time of irradiation. In other photomorphogenic processes such longer far-red irradiations have shown effects opposite to those obtained after a shorter one. Thus SIEGELMAN and HENDRICKS (1957) and MOHR (1957) first established the occurrence of a high-energy reaction controlling the formation of anthocyanin in seedlings. MOHR (l.c.) could show that this high-energy reaction was operating side by side with the red—far-red reversible low-energy reaction in the seedlings of *Sinapis alba* L. With low intensities continuously or with higher ones for shorter times HENDRICKS *et al.* (1959) could show a far-red inhibition on the germination in seeds of *Lactuca sativa* L. (var. Great Lakes) normally not influenced by light. After such an irradiation also the low-energy red—far-red reversible reaction controlled the germination. In addition the results with far-red irradiations obtained by KADMAN-ZAHAVI (1960) in seeds of *Amaranthus retroflexus* L. suggest the co-operation of both the low- and high-energy reaction in the light-controlled germination in this species. For a review of the high-energy reaction, see MOHR (1962). For the germination in the Scots pine the present material does not indicate that also this high-energy reaction should be operating alongside the low-energy reaction in the light-control of the germination.

The results, which are presented in figure 23, show that a maximal stimulation after irradiation with a fixed energy in red was obtained after the seeds had imbibed for 12 hours. At this time the fresh weight increase was about 35 per cent (cf. figure 20). A maximal inhibition with far-red light first appeared after 3—6 hours of imbibition. At these times the corresponding imbibitions were 17 and 25 per cent, respectively. In spite of the relatively great intervals between the irradiations, which do not permit a more exact estimation of the period required for the development of full light sensitivity, these results nevertheless seem to show that the full far-red sensitivity appeared earlier than the full red sensitivity. This was in accordance with the results of EVENARI and NEUMANN (1953 a) with lettuce seeds, where, however, the corresponding times were 3 and 1 hour, respectively. In the same species (var. Grand Rapids) IKUMA and THIMANN (1960) found the maximal red sensitivity after 1.25 hour of imbibition. After this period the imbibition was 40 per cent (increase of the fresh weight). On the completed imbibition the corresponding increase was 60 per cent. Concerning the imbibition of the hypocotyls, roots and the cotyledons this was already completed before 2 hours of imbibition, thus coinciding with the maximum for the full red sensitivity. In seeds of *Pinus thunbergii* Parl. ASAKAWA and INOKUMA (1961) found a considerable stimulation with a red irradiation given 2 hours after the start of the imbibi-

tion. Maximal sensitivity appeared after 24 hours, whereupon the sensitivity decreased.

For all these cases it was valid that the red sensitivity increased with increasing time of imbibition to a maximum, after which the sensitivity decreased with increased imbibition. In lettuce seeds BORTHWICK *et al.* (1952 a, 1954) found that the maximal sensitivity first appeared after 10—12 hours of imbibition, was unchanged for further 8 hours and then decreased. With far-red irradiations, however, they found that during the period of imbibition when the red sensitivity increased the far-red sensitivity decreased and the reverse was the case after a longer period of imbibition. This relationship between the red and far-red sensitivity was one of the first indications of the occurrence of the red—far-red reversible pigment system controlling the germination in lettuce seeds (BORTHWICK *et al.* 1952 a, cf. also TOOLE 1961). In the Scots pine seeds studied here, however, the corresponding inverse relationship between the red and the far-red sensitivity—expressed as the germination responses after irradiations with fixed energies—could not be found (cf. also EVENARI and NEUMANN l.c.). The results in figure 23 showed that with shorter periods of imbibition the red sensitivity increased. During the same time the responses to the far-red irradiations also increased, *i.e.*, the far-red sensitivity increased. Contrary to this the decreasing red sensitivity after more than 12 hours of imbibition was not reflected in any change of the germination responses after red + far-red irradiations. Because these experiments were carried out with irradiations of only one fixed energy, the results cannot conclusively be said to show the eventual absence of a corresponding change of the far-red sensitivity. In spite of this the directly correlated change of the red and far-red sensitivity of the Scots pine seeds during the first part of the imbibition also was contradictory to the results found by TOOLE *et al.* (1961) and TOOLE *et al.* (1962) in studies on the red—far-red control of the germination in *Pinus virginiana* Mill., and *P. taeda* L. *P. strobus* L., respectively, where also the inverse relationship between red and far-red sensitivity was found. However, in these investigations the imbibition was performed at + 5° C (stratification) for a relatively long period, which also resulted in an increased number of seeds germinating independent of light (cf. for the Scots pine seeds figure 32).

The same inverse relationship between red and far-red sensitivity during the imbibition in seeds of *Lepidium virginicum* L. TOOLE *et al.* (1955) proposed to be dependent on a reducing-oxidizing substance closely associated with the red—far-red reversible pigment. The establishment of physiological red—far-red effects independent of aerobiosis (BONNER 1959, IKUMA and THIMANN 1961) and the photoconversion of phytochrome also independ-

ent of reducing-oxidizing conditions and dialysis (BONNER 1960, HENDRICKS 1960 b, BORTHWICK and HENDRICKS 1961) have led to a modified explanation of the relationships between red and far-red sensitivity in imbibing seeds. Thus TOOLE *et al.* (1961) remark that there are indications which show that the inverse changes of the red—far-red sensitivities during the imbibition depend on the degree of the pigment conversion that is required for a definite germination response and not on an associated reactant.

On the assumption that the pigment conversion between the red and far-red absorbing form is a function of the energy used in the red and far-red irradiations HENDRICKS *et al.* (1956) calculated the degrees of this conversion (to the far-red absorbing form) required for corresponding germination responses in seeds of *Lactuca sativa* L. and *Lepidium virginicum* L. With the same method TOOLE *et al.* (1961) in seeds of *Pinus virginiana* Mill. found that 20 per cent of the red—far-red reversible pigment must be transformed into its far-red absorbing form in order to produce a 50 per cent stimulation of the germination.

A consequence of this seems to be that different germination responses obtained with one and the same irradiation of seeds in different stages of imbibition (with different sensitivities, cf. figure 23) are not dependent on different pigment conversions but on associated dark reactions. An independence of imbibition for the conversion of the phytochrome—indications for its participation in the light-controlled germination even in the seeds of Scots pine have earlier been discussed on page 78—also seems to be in agreement with its nature as a molecular rearrangement at a photochemical reaction (HENDRICKS 1960 b) and its reversibility even at a low temperature (— 80° C, BORTHWICK and HENDRICKS 1961).

A few further remarks based on some of the experiments with the Scots pine seeds studied here will try to point out the consequences of the assumption that the pigment conversion is independent of the state of imbibition in the seeds at the moment of the irradiations.

In figure 22 (imbibition 6 h.), it was shown that a red irradiation for 240 minutes on the seeds which before the irradiations had imbibed in darkness for 6 hours was more than a saturation dose for induction of complete germination. This irradiation caused a transformation of the pigment to its far-red absorbing form, which was sufficient to produce near 100 per cent germination. Under the assumption discussed above that this conversion of the pigment should be independent of the imbibition, the same irradiation used in figure 22 (no imbibition) on the unimbibed seeds ought to cause the identical conversion of the pigment. Directly after this irradiation the seeds were transferred to the germinator for the start of the germination process. In spite of this the germination response was

lower than in the seeds which had been given the same irradiation after 6 hours of imbibition. The "same" pigment conversion thus resulted in different germination responses depending on the conversion taking place before or after the imbibition. It may also be pointed out that the results in figure 29 suggested that a thermal dark reversal of the far-red form to the red form is a slow process in Scots pine seeds (cf. below). Finally, it may be observed that the red induction of unimbibed seeds could not be reversed with far-red under the same circumstances (cf. figure 24).

This discussion of different possible explanations of the influence of imbibition on the Scots pine seeds' sensitivity to irradiations with red and far-red light in combination with the present knowledge of the phytochrome and its probable occurrence as a regulator of the light-influenced germination in this species leads to controversial conclusions. The nature of the photoconversion in the phytochrome makes it probable that this is independent of the imbibition of the seeds, but this assumption does not seem to permit a satisfactory explanation of the present physiological observations in connection with imbibition and light sensitivity. Another reasonable explanation of these results seems to be that the pigment present in the unimbibed seeds under the imbibition, either through a direct participation of water or another reactant produced under the imbibition, is transformed into another state, which permits full conversion between its red and far-red absorbing form under a pure photochemical reaction in accordance with the results from the studies on the phytochrome.

A further discrepancy in the red—far-red mechanism between the Scots pine seeds and the lettuce seeds is suggested by the results in figure 29. These showed that it was not possible to inhibit the red induction to any higher degree by a subsequent treatment with a moderately increased temperature (+ 30—+ 35° C). In lettuce seeds Borthwick *et al.* (1954) found that corresponding treatments, *e.g.*, + 35° C for 24 hours was sufficient to decrease a red induced germination from 100 to about 20 per cent, an inhibition, which, however, could be reversed by a following red irradiation. The increased temperature caused a stimulated thermal reversal of the pigment from its physiologically active, far-red absorbing form to the inactive, red absorbing form.

However, the opposite results in Scots pine seeds (cf. figure 29) do not permit the conclusion that a thermal dark reversal of the pigment should not take place in them, because the possibility cannot be excluded here that an increased temperature stimulates the reactions initiated by the far-red absorbing form of the pigment more than it stimulates a thermal reversal of the same pigment form to its inactive form, *i.e.*, with an unchanged germination response as a consequence. The present results do not permit

any choice between these possibilities as explanations of the observed results but show an obvious difference from corresponding experiments on lettuce seeds.

Corresponding properties as in the lettuce seeds TOOLE *et al.* (1958) (see also TOOLE 1961) found in seeds of *Paulownia tomentosa* (Thunb.) Steud. A continuous irradiation for 48 hours was required for full induction of the germination in this species. This irradiation could be substituted by repeated shorter irradiations. After full induction red—far-red reversibility with short irradiations occurred, but if the seeds were kept in darkness after a far-red irradiation for 48 hours a new series with repeated irradiations was required for the induction. These results indicated that a dark reversal of the pigment from its far-red to its red absorbing form with loss of induction as a consequence occurred also in these seeds. Compare MOHR (1960 a) who has interpreted this result from a supposed new formation of red absorbing pigment from a precursor.

This is related to other cases where a photoperiodical regulation of light-controlled germination has been established (cf. review by EVENARI in press). BORTHWICK and HENDRICKS (1960) and HENDRICKS (1960 a) have pointed out that the dark reversal of the phytochrome is the general physiological basis for the photoperiodism. With direct spectrophotometric measurements it has also been possible to follow this reversal *in vivo* and to show that it proceeds more rapidly at a higher than at a lower temperature (HENDRICKS l.c.). However, in this connection it seems interesting to point out that a photoperiodical (and red—far-red) control of the germination in seeds of *Betula pubescens* Ehrh. appeared at + 15° C but at + 20° C only one irradiation was sufficient for full induction of the germination (BLACK and WAREING 1955). See also similar results by VAARTAJA (1956) and OLSON *et al.* (1959). Thus the photoperiodical regulation occurred only at the lower temperature, which seems to be explainable only by the assumption that reactions initiated by the far-red absorbing form of the pigment are more accelerated by an increased temperature than the simultaneous dark reversal of the same pigment form. Thus these results may be explained by the same assumptions as the above discussed results for Scots pine seeds, in which no directly observed, physiological reactions were found suggesting the occurrence of such a thermal dark reversal of the far-red absorbing form of the phytochrome.

From their studies on germination in lettuce seeds BORTHWICK *et al.* (1954) concluded that the germination was controlled by the red—far-red reversible pigment, either owing to the red absorbing form acting as an inhibitor or owing to the far-red absorbing form acting as a promotor.

After TOOLE *et al.* (1955) the loss of far-red reversibility within 30 minutes after a red irradiation at the photoperiodical control of flowering in *Xanthium pensylvanicum* Wallr. (cf. DOWNS 1956) shows that the far-red absorbing form of the pigment was coupled to the processes producing the biological responses after irradiations. Also calculations on the relationship between required transformation of phytochrome and a physiological response on a red irradiation show the same possibility. Thus HENDRICKS (1960 a) found that only 1/25000 of the phytochrome must be transformed to its far-red absorbing form for obtaining a measurable effect.

Estimations of the time during which the pigment must predominate in its physiologically active, far-red absorbing form for establishment of a physiological response have been done in a series of different species. This has been estimated from the time lapse between a red and a following far-red irradiation required for the loss of far-red reversibility. Such experiments on the light-controlled germination in seeds of *Amaranthus retroflexus* L. at + 37° C (KADMAN-ZAHAVI 1960) showed that 1 hour after the red irradiation the far-red reversibility was almost completely lost. Corresponding experiments on seeds of *Lactuca sativa* L. at + 25—26° C (EVENARI and NEUMANN 1953 a, IKUMA and THIMANN 1960) and in the same species at + 20° C BORTHWICK *et al.* 1954) showed complete loss of reversibility after 10 and 15 hours, respectively. After 12 hours at + 20° C the reversibility was lost in seeds of *Betula pubescens* Ehrh. (BLACK and WAREING 1955) but in *Carnegia gigantea* (Engelm.) Britt. and Rose (ALCORN and KURTZ 1959) 24 hours were required at + 30° C to produce the same effect. In seeds of *Rumex obtusifolius* L. the far-red reversibility was lost after about 30 hours at + 20° C (ISIKAWA and FUJII 1961). Contrary to this the reversibility was still complete after 24 hours in seeds of *Paulownia tomentosa* (Thunb.) Steud. (TOOLE *et al.* 1958). Seeds of *Pinus virginiana* Mill. (TOOLE *et al.* 1961), *P. taeda* L. and *P. strobus* L. (TOOLE *et al.* 1962), which, however, before the irradiations had imbibed at + 5° C for 1 and 4 days, respectively (stratification), showed only loss of reversibility to 50 per cent after 48—72 and 96 hours, respectively. This indicated a much slower progress of the changes initiated by the red irradiation than in the other investigated species. The same also seems to be valid for the seeds of *Pinus thunbergii* Parl. (ASAKAWA and INOKUMA 1961), which at + 25° C even after a time lapse of 48 hours between a red and a far-red irradiation had not lost all the far-red reversibility. In the same species and in *Picea glehnii* Mast. it was also shown that with increased initial time of imbibition before the red irradiation the far-red reversibility decreased more rapidly.

A corresponding investigation (at + 25° C) on the Scots pine seeds studied here (figure 27), however, showed that after only about 16 hours half of

the reversibility was lost. After 48 hours the far-red irradiation no longer had any inhibitory power. This indicated that the process was somewhat more rapid in this species of pine than in the above-mentioned ones but slower than in the lettuce seeds. IKUMA and THIMANN (l.c.) remarked that the appearance of full loss of reversibility in the lettuce seeds coincided with the appearance of the first signs of root protrusion. No corresponding observations could be made on the Scots pine seeds. See further in the next chapter (p. 104 and figure 42) about the relationship between loss of far-red reversibility and the appearance of the first signs of incipient growth of the Scots pine embryos.

All these results show that the far-red absorbing form of the regulating pigment (phytochrome) must predominate for a rather long time for establishing irreversible responses. The required periods are different in different species and also evidently dependent on the prevailing temperature during the dark phase between the red and the far-red irradiation (cf. BORTHWICK *et al.* l.c. and IKUMA and THIMANN l.c. and further below). The periods required for the appearance of irreversible responses at seed germination are long in comparison with corresponding periods at the photoperiodical regulation of flowering (see *e.g.* DOWNS 1956).

In figure 28 and table 12 it was shown that the effects of the irradiations were independent of the temperature (a primary photochemical reaction). On the other hand, the reactions that were initiated by this primary photoreaction reasonably must be thermochemical ones. This was studied in experiments with different temperatures during a dark phase of 48 hours between a red and a following far-red irradiation (figure 30). It was found that a decreased temperature (+ 5° C) could inhibit the red induced changes in the seeds to such an extent that a dark period of 48 hours resulted in only half of the seeds being capable of germination after a subsequent far-red irradiation.

That a lower temperature during a dark phase between a red and a following far-red irradiation increases the required time for the complete establishment of irreversible changes induced at a red irradiation is also suggested by a comparison between the results on lettuce seeds by BORTHWICK *et al.* (l.c.) and IKUMA and THIMANN (l.c.). They found that 15 hours at + 20° C and 10 hours at + 25° C, respectively, were required for complete loss of the far-red reversibility. Also DOWNS (l.c.) found that an inhibiting irradiation with red light on the induction of flowering could be reversed by a far-red irradiation after a longer time, if the temperature during the time lapse between the red and far-red irradiations was decreased.

Recent results have made it probable that the primary dark reactions controlled by the far-red absorbing form of the phytochrome are concerned

with a control of basic reactions in protein metabolism (LANDGRAF 1961). According to MOHR (1962) the phytochrome in its far-red absorbing form probably enzymatically controls basic metabolic changes, which are expressed in the different photomorphogenic effects. With the Scots pine seeds attempts have been made to elucidate somewhat the mechanism between the primary transformation of the controlling pigment and the final germination responses, the results of which are given in the Chapters V and VI.

As shown in the previous chapter a stratification increased the germination of Scots pine seeds in darkness (figure 9). These results were reinvestigated in combination with red and far-red irradiations (figure 32), where it was found that the decreased dependence of light after a stratification was correlated with an elimination of the controlling power of the red—far-red pigment system. The same fraction of the seeds which was capable of germinating without any irradiation with red light could not be inhibited by a far-red irradiation. Similar results were obtained by ASAKAWA and INOKUMA (1961) in seeds of *Pinus thunbergii* Parl. and *Picea glehnii* Mast. Also TOOLE *et al.* (1961) and TOOLE *et al.* (1962) found that in three other pine species (*Pinus virginiana* Mill., *P. strobus* L. and *P. taeda* L.) the number of seeds germinating in darkness increased after a stratification in the same time as the light-requiring seeds developed a decreased far-red sensitivity. A further interaction between stratification and red—far-red sensitivity was shown by KAHN (1960) in lettuce seeds. A dark-osmotic inhibition in this species (see also HABER 1959), which induced the participation of the red—far-red reversible system in the control of the germination, was not developed during a low-temperature treatment.

From neither of these results can a satisfying explanation be given for the interaction between stratification and red—far-red control of the germination. ASAKAWA and INOKUMA (l.c.) proposed the development of a substitutional pathway for the germination during a treatment with low temperature and TOOLE *et al.* (1962) remarked that they could not conclude whether the treatments influenced the phytochrome or other participating reactants as substrates or cofactors. For some further discussion of this problem, see page 139.

In figures 17—19 it was shown that there existed a relationship between light requirement and the presence of intact seed coats in the germination of Scots pine. These results investigated in combination with red and far-red irradiations showed (figure 31) that the decrease of light dependence after a puncturing or a removal of the seed coats was correlated with a changed ability of the far-red light to inhibit the germination. A puncturing

decreased and a removal of the seed coats eliminated the dependence on red and far-red light for the control of the germination.

These results may be compared with the results which were obtained by KLEIN and PREISS (1958), who used irradiations with deutrons on intact lettuce seeds. A control of the irradiations in such a way that all radiation stopped in the seed coats did not cause any harmful effects on the seedlings but completely eliminated the red—far-red control of the germination. It was also shown by PREISS and KLEIN (1958) that a corresponding irradiation of only the endospermal layer in the composed coverings in this species (cf. BORTHWICK and ROBBINS 1928, EVENARI and NEUMANN 1952) was required and sufficient for the elimination of the light dependence. This result was consistent with the observations made by EVENARI and NEUMANN (l.c.) from cutting experiments, in which they showed that also the innermost part (the endospermal layer) of the coverings in the lettuce seeds must be opened for a complete removal of the light dependence at the germination. The same authors (1953 a) have also shown that after a decoating a far-red irradiation could not inhibit the germination. For a discussion about the interaction between seed coat and light-dependent germination, see page 48 and 137.

Chapter V. Studies on the Occurrence of Embryo-Mitosis

A. Introduction

The effect of light on the Scots pine seeds has been estimated in the preceding chapters from the relations between the irradiations and the percentages of the seeds which had germinated, *i.e.*, which had protruding rootlets after a fixed time. The germination in general as a resumption of the growth in the embryonic plant in the seed (TOOLE *et al.* 1956 a) thus in this special case may be a result of an effect of the light on this growth process. The three main morphological parts of a growth process consist of a cell multiplication through mitosis and cell division, cell lengthening and cell differentiation (MEYER and ANDERSON 1954). Cell multiplication and cell lengthening are the principal contributors to the increase in the volume

of a growing organ. Thus these can be suspected to be the processes influenced by light that control the germination in the meaning of the directly visible growth of the embryos.

Intimately connected with this question is the time sequence between the cell multiplication and the cell lengthening at the germination. In seeds of *Pinus thunbergii* Parl. (Goo 1952, 1956) and *Prunus cerasus* L. (POLLOCK and OLNEY 1959) cell multiplication starts earlier than the lengthening of the cells. In contrast to this the cell lengthening precedes the cell multiplication in seeds of *Zea mays* L. (TOOLE 1924), *Hordeum vulgare* L. (CALDECOTT and SMITH 1952) and *Vicia faba* L. (WOLFF 1954, KLINGMÜLLER 1961). Compare also for *Phaseolus sp.* in TOOLE and TOOLE (1961). At the germination of *Lactuca sativa* L. seeds (var. Grand Rapids, EVENARI *et al.* 1957), however, the two processes are initiated at the same time, which has been confirmed by HABER and LUIPPOLD (1960 a, var. New York).

Concerning the eventual light effect in this connection EVENARI *et al.* (l.c.) remarked that in the embryos from seeds which were kept under germinating conditions in darkness—seeds of this lettuce var. Grand Rapids are light-requiring for the germination—there were no signs of cell divisions and cell lengthening. Working with the light-independent lettuce seeds var. New York, which can be made light-dependent with diverse physical and chemical treatments (HABER 1959), HABER and LUIPPOLD (1960 b) could show that the red—far-red reversible system had no controlling importance for the mitotic activity in the embryos before the directly visible germination.

Thus from this it seems pertinent to investigate the corresponding relations in the embryos from Scots pine seeds, *i.e.*, the time sequence between the cell divisions and the cell lengthening and the light effects in this connection.

B. Methods

The seeds were germinated in the same way as described previously (in Jacobsen apparatus at + 25° C in darkness). Irradiations were performed with the same equipment as before and under the same conditions. Fur further details about the irradiations, see the different experiments below.

After the seeds had been under germinating conditions for the desired time, the required number of seeds were randomly sampled among the seeds without directly visible, protruding rootlets (ungerminated seeds after HABER and LUIPPOLD 1960 a and b). Concerning exceptions from this rule, see further details about the sampling in the respective experiments. The embryos were immediately dissected and fixed in ethanol-acetic acid (3: 1) for 24 hours and then transferred to 70 per cent ethanol for storage. Before the staining the embryos were hydrolyzed for 15 minutes at room temperature with ethanol-acetic acid (3: 1) containing 10 per cent hydrochloric acid and washed with the ethanol-acetic acid

without hydrochloric acid. Then they were stained with aceto-orcein and smeared. The method was adopted from SASS (1958). With the exception of the experiment in table 17 the whole embryos were smeared in the same time. Most of the results have been given only as the fraction of the investigated embryos with one or more mitotic figures per embryo.

C. Results

1. Effect of White Light and Darkness, Red and Red + Far-Red Irradiations

In the first experiment the intention was to study whether different light conditions affected the appearance of embryo-mitosis and how this eventual effect appeared in relation to the occurrence of the first signs of the visible germination, *i.e.*, the protrusion of the rootlets.

The seeds were kept under germinating conditions in the following ways: in continuous white light (1700 lux from fluorescent tubes TL/33), in darkness, in darkness with a standard red irradiation (concerning this, see p. 57) and in darkness with a standard red and an immediately following far-red irradiation (concerning this, see p. 58). For each type of irradiation 100 seeds were used. After 24, 48 and 72 hours, respectively, from the start of the imbibition 20 seeds were randomly sampled among the seeds without signs of incipient germination from each type of experiment. The seed embryos were investigated in respect to the occurrence of mitosis. The remaining seeds after the end of the last samplings were left under germinating conditions for a total period of 10 days, after which the percentages of these seeds that had germinated were determined. The results are given in table 14.

The results showed that mitosis appeared already after 48 hours, *i.e.*, before any signs of directly visible germination. No measurements of the individual cell lengths were made but the absence of increased embryo lengths at the same time as mitosis occurred was taken as evidence for the statement that mitosis preceded the cell lengthening (cf. the corresponding statement by HABER and LUIPPOLD 1960 a). The results also showed that the type of irradiation influenced the extent of the occurrence of mitosis. Irradiation with continuous white light or a limited irradiation with red light stimulated the appearance of mitosis in comparison with that in the embryos from dark control seeds or seeds irradiated with red + far-red light. This last result suggested that the same type of red—far-red reversibility existed concerning the occurrence of mitosis as concerning the germination. A comparison between the occurrence of embryo-mitosis under the pregerminative phase (before the appearance of the protruding rootlets) of the germination process and the final germination percentages showed accordance.

Table 14. Effect of light and darkness, and red and red + far-red irradiations, respectively, on the occurrence of embryo-mitosis in Scots pine seeds. For details about the irradiations, see the text. Provenance No. 3.

Irradiation	Seeds under germinating conditions for hours	Fraction		Percentage	
		of investigated embryos with mitosis	of remaining seeds which germinated	embryos with mitosis	germinated seeds
Continuous white light	24	0/19	—	0	—
	48	20/20	—	100	—
	72	20/20	—	100	—
	240	—	34/35	—	97
Dark control	24	0/20	—	0	—
	48	5/20	—	25	—
	72	5/20	—	25	—
	240	—	7/35	—	20
Red	24	0/20	—	0	—
	48	19/20	—	95	—
	72	20/20	—	100	—
	240	—	29/34	—	85
Red + far-red	24	0/20	—	0	—
	48	1/20	—	5	—
	72	1/20	—	5	—
	240	—	5/35	—	14

2. Time Relations between Imbibition, Mitosis and Start of the Directly Visible Germination after Red and Red + Far-Red Irradiations

These experiments were performed in order to determine more closely the time sequence between the appearance of mitosis in relation to the imbibition and to the directly visible signs of an incipient germination.

The studies on the imbibition were done in the following way. Before the start of the imbibition 6×50 seeds were weighed. Afterwards the seeds were placed as in the germination tests in darkness (50 seeds per germination bed). After the desired times the seeds were carefully dried between filter papers, weighed and placed back for continued imbibition. The weighings were done with the seeds in closed, dark containers. All the other manipulations were done in safelight. After an imbibition time of 6 hours all the seeds were red irradiated. Immediately after this irradiation 3×50 seeds were irradiated with far-red light. All irradiations were done under the standard conditions. After these irradiations the continued imbibition was studied as before. The results from these measurements have been given as the per cent uptake of water of the original fresh weight of the seeds. See figure 38 A.

For the studies of the embryo-mitosis 4×100 seeds were placed for imbibition in the identical way and irradiated under the same circumstances,

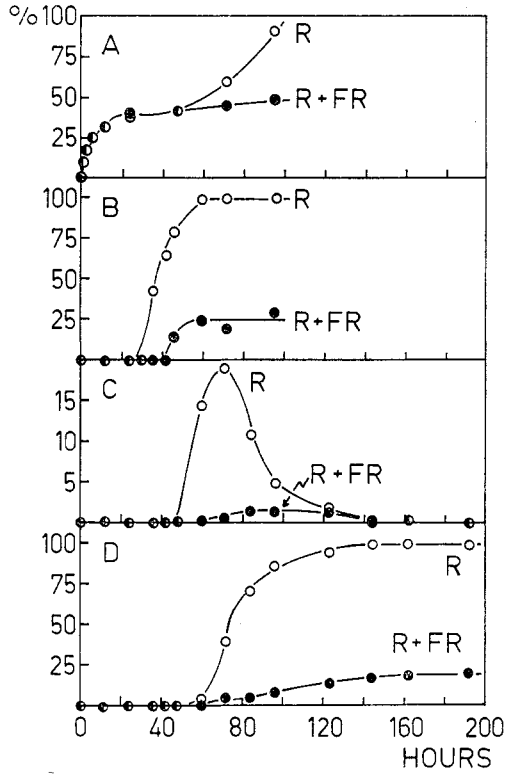

Figure 38. Effects of red (R) and red + far-red (R+FR) irradiations on the imbibition, on the appearance of mitosis in the embryos and on the directly visible germination in Scots pine seeds. A: per cent water uptake of the fresh weight. B: per cent embryos with mitosis. C: per cent seeds with opened seed coats but without protruding rootlets. D: per cent seeds with protruding rootlets. Standard irradiations (cf. figure 26). Provenance No. 3.

2×100 seeds with red light, 2×100 seeds with red + far-red light. After the end of the irradiations the seeds were kept under germinating conditions in darkness. After different periords 20 seeds were randomly sampled, the embryos dissected, fixed and investigated as described above concerning the occurrence of mitotic figures. The results from this experiment are given in table 15 and figure 38 B.

For studies on the opening of the seed coats and the started protrusion and growth of the rootlets another 4×100 seeds were red irradiated and 4×100 seeds red+far-red irradiated as above. The mean values of these experiments are presented in table 15 and in figure 38 C and D. In figure 38 D it should be observed that the percentages of all seeds with protruding rootlets are given irrespective of their rootlet lengths.

A comparison of these results showed that in the red irradiated seeds the first mitotic activity appeared after the end of the imbibition phase. At the same time as all the investigated embryos showed mitotic activity the seed coats had started to open (60 hours) and also some of the seeds

Table 15. Effect of red (R) and red + far-red (R + FR) irradiations on the occurrence of embryo-mitosis and on the directly visible germination of Scots pine seeds. Standard irradiations (cf. figure 26). Provenance No. 3.

Seeds under germinating conditions for hours	Per cent seeds after different periods of time under germinating conditions with							
	Mitosis in the embryos[1]		No rootlets, only opened seed coats[2]		Rootlets < 5 mm^2		Rootlets $\geqq 5$ mm^2	
	R	R + FR	R	R + FR	R	R + FR	R	R + FR
0	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0
24	0	0	0	0	0	0	0	0
30	0	0	0	0	0	0	0	0
36	45	0	0	0	0	0	0	0
42	65	0	0	0	0	0	0	0
48	80	15	0	0	0	0	0	0
60	100	25	14	0	6	0	0	0
72	100	15	18	1	36	1	5	0
84	100	20	11	2	48	3	24	1
96	100	30	5	2	20	5	67	3
480	—	—	—	—	—	—	100	20

[1] Determined in 20 embryos after sampling from 2 × 100 seeds for each type of irradiation.

[2] Mean values determined from 4 × 100 seeds for each type of irradiation.

had protruding rootlets. For the red + far-red irradiated seeds the results showed that the mitosis was delayed and inhibited. The percentages of seeds with mitosis before the start of the visible germination revealed the magnitude of the final germination (cf. also the results in table 16 and figure 40). Thus the irradiations influenced the extent of the mitotic activity and correlated with this the final germination. The results also confirmed that embryo-mitosis appeared before the lengthening of the embryos. In contrast to these findings the irradiations (given after 6 hours of imbibition) did not influence the fulfillment of the imbibition process (cf. figure 20). A comparison of these effects of the red and red + far-red irradiations on the different phases of the germination process seemed to show that during the period between the end of a red irradiation and the first appearance of mitosis such changes were initiated in the seeds which were followed by the embryo-mitosis and correlated with these also the directly visible germination.

However, in connection with this it can be pointed out that the appearance of the mitosis in the red irradiated seeds was to be found after the seeds had been under germinating conditions for more than 30 hours (table 15 and figure 38, cf. also corresponding results in figure 42). In contradiction to this the results in figure 39 showed a certain percentage of embryos with mitosis already after 24 hours. The reason for this discrepancy can be the

smaller number of investigated embryos and the different technique for sampling used in the first-mentioned experiments. Compare also the corresponding discrepancies in this respect after 24 hours for red + far-red irradiated seeds in tables 15 and 18. However, under a simultaneous consideration of the rapid development of mitotic activity in the time interval 24 to 48 hours in all the experiments the above-mentioned differences do not seem to have any relevance for the conclusions above.

3. Repeated Red—Far-Red Reversibility

The results in the previous experiments, which indicated a reversible effect of red—far-red irradiations on the occurrence of embryo-mitosis similar to the effects on the final germination, were here further investigated with use of a series of alternative red and far-red irradiations.

The irradiations were started after 6 hours of imbibition and performed with the standard equipment. For each combination of irradiations 2×100 seeds were used. After 48 hours from the start of the imbibition, when in the previous experiments the red irradiated seeds showed nearly complete development of the mitotic activity but no start of the directly visible germination, 2×10 seeds were randomly sampled among the seeds from each combination of irradiations. The remaining seeds were left for determination of the final germination. The embryos were investigated for the occurrence of mitosis in the usual way. However, before the staining of the embryos each individual embryo was divided into a shoot and a root portion, which were investigated separately. Also the number of mitotic figures was determined in every embryo portion. The results are given in tables 16 and 17.

In table 16 the proportions of embryos with one or more mitotic figures per whole embryo are given and compared with the final germination of the identically irradiated seeds, which were left for completion of the germination. The results showed that the occurrence of embryo-mitosis before the start of the directly visible germination was regulated by red—far-red irradiations in the same way as the final germination.

In order to illustrate further the above statement the number of mitotic figures in shoot and root portions of individual embryos were counted. The results from these investigations are given in table 17.

From this table it was evident that not only the occurrence of mitotic activity in the embryos but also the extent thereof were dependent on the type of irradiation given to the intact seeds, *viz.*, a stimulated activity after irradiations ended by a red one and inhibited after irradiations ended by a far-red one. Only far-red or darkness gave the same results. The comparisons

Table 16. Reversible effect of repeated red—far-red irradiations on the occurrence of embryo-mitosis and on the final germination in Scots pine seeds. Standard irradiations (cf. figure 26). Provenance No. 4.

Irradiation	Sample No.	Fraction		Percentage	
		of investigated embryos with mitosis after 48 hours	of remaining seeds which were germinated after 480 hours	embryos with mitosis	germinated seeds
FR	1	2/10	5/90	20	12
	2	2/10	16/90		
R	1	7/9	83/90	84	93
	2	9/9	85/90		
R + FR	1	1/10	12/90	10	14
	2	1/10	13/90		
R + FR + R	1	10/10	85/90	95	94
	2	8/9	85/90		
R + FR + R + FR	1	2/10	14/90	20	13
	2	2/10	9/90		
R + FR + R + FR + R	1	8/9	84/90	95	91
	2	9/9	79/90		
Dark control	1	1/10	11/90	10	13
	2	1/10	13/90		

between shoot and root portions did not show any fundamental difference. However, the number of cells with mitosis was somewhat higher in the shoot portions than in the root portions in embryos from red stimulated seeds.

4. Far-Red Inhibited Mitotic Activity during a Longer Germination Period

The preceding experiments showed that irradiations with only far-red or combinations with red, where the far-red irradiations were given last in a sequence, caused an inhibition of the occurrence of embryo-mitosis. However, these studies were based on studies of the embryo-mitosis during an early phase of the germination process. In this experiment it will be established whether the persisting inability of most of the seeds to germinate under prolonged stay in darkness (cf. figure 18 A) or after a far-red irradiation (cf. *e.g.* figure 26) after the fulfillment of the germination of the light-insensitive part of the seed population can be referred to a complete block of the mitotic activity.

All the seeds were irradiated with red + far-red light (standard irradiations after 6 hours of imbibition). After the seeds had been under germinating conditions for different periods 10 seeds were randomly sampled from every seed sample of 100 seeds. This sampling was done among seeds without

Table 17. Reversible effect of repeated red—far-red irradiations on the mitotic activity in shoot and root portions of embryos from Scots pine seeds after standard irradiations (cf. figure 26). Provenance No. 4.

Number of mitotic figures	Number of embryos with mitosis in shoot and root portions after irradiation with													
	R		R+FR+R		R+FR+R+FR+R		FR		R+FR		R+FR+R+FR		Dark control	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
0	3	3	2	1	2	1	18	17	18	19	16	16	18	19
1—10	1	1	—	1	1	2	2	3	—	1	3	3	2	1
11—20	—	—	1	—	—	—	—	—	—	—	1	1	—	—
21—30	—	—	—	1	1	1	—	—	1	—	—	—	—	—
31—40	1	3	—	4	1	1	—	—	—	—	—	—	—	—
41—50	2	2	—	3	—	—	—	—	—	—	—	—	—	—
51—60	1	3	1	1	2	1	—	—	—	—	—	—	—	—
61—70	3	—	2	2	—	—	—	—	—	—	—	—	—	—
71—80	—	—	3	2	—	5	—	—	—	—	—	—	—	—
81—90	1	—	1	—	3	4	—	—	—	—	—	—	—	—
91—100	1	1	1	2	—	1	—	—	—	—	—	—	—	—
101—110	1	—	1	1	—	—	—	—	1	—	—	—	—	—
111—120	1	1	1	—	—	1	—	—	—	—	—	—	—	—
121—130	—	—	1	—	1	1	—	—	—	—	—	—	—	—
131—140	1	—	2	—	2	—	—	—	—	—	—	—	—	—
141—150	—	—	—	—	2	—	—	—	—	—	—	—	—	—
151—160	1	—	—	1	2	—	—	—	—	—	—	—	—	—
161—170	1	—	3	—	—	—	—	—	—	—	—	—	—	—
171—180	1	1	—	—	—	—	—	—	—	—	—	—	—	—
181—190	—	1	—	—	—	—	—	—	—	—	—	—	—	—
191—200	—	—	—	—	—	—	—	—	—	—	—	—	—	—
201—	—	—	—	—	1	—	—	—	—	—	—	—	—	—
A	19	16[1]	19	19	18	18	20	20	20	20	20	20	20	20
B	1431	924	1715	1098	1696	1171	2	12	128	10	32	24	6	2
C	75.5	57.8	90.3	57.8	94.2	65.1	0.1	0.6	6.4	0.5	1.6	1.2	0.3	0.1
D	84.2	81.3	89.5	94.7	88.9	94.4	10.0	15.0	10.0	5.0	20.0	20.0	10.0	5.0

A: Number of investigated embryo portions.
B: Sum of all mitotic figures.
C: Mean value for the number of mitotic figures per embryo portion.
D: Percentage of embryo portions with mitosis.
[1] 3 root portions lost.

Table 18. Connection between occurrence of embryo-mitosis and final germination in red + far-red irradiated Scots pine seeds. Sampling among seeds without directly visible germination. Standard irradiations (cf. figure 26). Provenance No. 4.

Seeds under germinating conditions for hours	Sample No.	Fraction of investigated embryos with mitosis	Fraction of remaining seeds which were germinated after 10 days	Fraction of remaining seeds which were germinated after 20 days	Percentage embryos with mitosis	Percentage germinated seeds after 10 days	Percentage germinated seeds after 20 days
24	1	1/10 (-)[1]	10/90	11/90	10	14	15
	2	1/10 (+)	15/90	16/90			
48	1	2/10 (+, +++)	11/90	12/90	15	20	21
	2	1/10 (+++)	25/90	25/90			
72	1	2/9 (+++, ++)	13/90	13/90	17	16	16
	2	1/9 (+)	15/90	15/90			
96	1	2/10 (+++, ++)	11/90	11/90	20	16	17
	2	2/10 (++, -)	19/90	20/90			
120	1	1/9 (+++)	14/90	15/90	6	14	15
	2	0/10	11/90	11/90			
144	1	1/10 (++)	20/90	20/90	5	17	18
	2	0/10	11/90	12/90			
168	1	0/10	13/90	13/90	0	15	15
	2	0/10	14/90	14/90			
192	1	0/10	21/90	21/90	0	17	18
	2	0/10	10/90	11/90			
216	1	0/10	10/90	11/90	0	12	12
	2	0/10	11/90	11/90			
240	1	0/10	12/90	12/90	0	12	12
	2	0/9	9/90	9/90			
						Mean values 15.2	15.7

[1] Number of mitotic figures per embryo
\+ = 1—10
++ = 11—50
+++ = 51—

any signs of an incipient germination. At each time of sampling 2×100 new seeds were used. The remaining seeds were left for the completion of the germination. The embryos were dissected and investigated for the occurrence of mitosis. Also a classification of the number of mitosis per embryo was undertaken. The results are given in table 18.

The results showed that in the earlier samples a certain percentage of the investigated embryos had mitosis. These percentages were of the same magnitude as the final germination percentages in the actual seed samples. This occurrence of embryo-mitosis found evidently was due to the fact that

at this stage of the germination process it was impossible to sample only among the seeds which belonged to the part of the seed population sensitive to the inhibiting action of the far-red irradiation. Gradually the insensitive seeds manifested their ability to germinate in spite of the far-red irradiation and a sampling among the remaining seeds revealed a complete absence of mitotic activity in the embryos. Thus the inhibited germination after a far-red irradiation was reflected in a block of the mitotic activity, which persisted during the time of the investigation (10 days). This also probably exists for a longer time, which can be concluded from the persisting inability of the seeds to germinate under unchanged environmental conditions and the obvious correlation between the occurrence of mitotic activity and final germination under the experimental conditions used in this investigation.

5. Action Spectrum

The red—far-red reversible effects on the occurrence of embryo-mitosis described in the preceding experiments, which suggested the mediation of the same pigment system for the control of the embryo-mitosis as for the final germination, were further investigated through studies on the action spectrum for the embryo-mitosis (stimulation and inhibition spectra).

The experiments were performed in combination with the earlier described experiments on the action spectra for the germination (see p. 74). At each wavelength 4×100 seeds were irradiated at the same time. After the end of the irradiations the seeds were transferred to separate germination beds, 100 seeds in each bed. At 24 and 48 hours after the start of the imbibition 10 seeds from each sample were randomly taken, and the embryos were dissected and investigated for the occurrence of mitosis. The remaining seeds were used for the determination of the action spectrum for the germination (cf. p. 75). Thus for each wavelength and sampling time 4×10 embryos were investigated. The results are presented in figure 39.

The results in figure 39 A and C showed that only a minor fraction of the investigated embryos had mitosis after 24 hours. Owing to the variations between the replicates within the irradiations the small responses were not significant. After 24 hours the occurrence of embryo-mitosis evidently did not show any pronounced response to the type of the irradiations.

In contrast to these findings the results after 48 hours (figure 39 A and D) revealed an obvious light dependence in the occurrence of the embryo-mitosis. A maximal stimulation was produced at 6600 Å and a corresponding inhibition appeared at 7300 Å. The general trend of the action spectra showed similarities to the corresponding ones for the final germination (figures

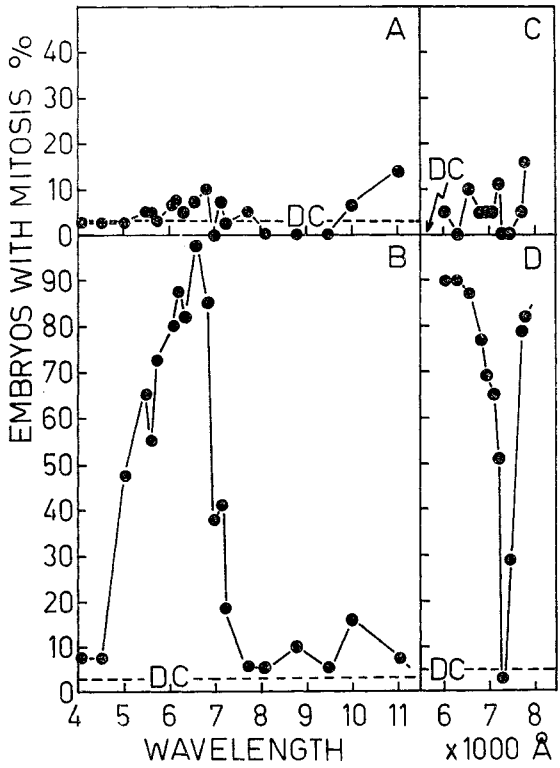

Figure 39. Action spectra for the occurrence of embryo-mitosis in Scots pine seeds. A and B: stimulation spectra measured after the seeds had been under germinating conditions for 24 and 48 hours, respectively. C and D: inhibition spectra under corresponding conditions. Irradiations as in figures 36 and 37, respectively. DC: dark control. Provenance No. 4.

36 and 37). It may also be pointed out that in the action spectra for both these processes there were peaks at 5500 Å and 7125 Å. A comparison between the results in figure 39 A, C and B, D also showed that during the time interval 24 to 48 hours (from the start of the imbibition) there was a rapid development of mitotic activity in embryos from seeds irradiated with stimulating light qualities.

From the results it was evident that the general features of the action spectra both for the stimulation and the inhibition of the embryo-mitosis in the seeds before the directly visible germination were similar to the corresponding spectra for the final germination, *i.e.*, with maximal stimulation around 6600 Å and maximal inhibition around 7300 Å. These results together with the repeated reversibility on alternating red—far-red irradiations (cf. tables 16 and 17) showed that the occurrence of embryo-mitosis was regulated by light through the mediation of the same pigment system as the final germination. The relation between the occurrence of embryo mitosis and the final germination is given in figure 40. Each individual point represents the percentage of 10 investigated embryos with mitosis

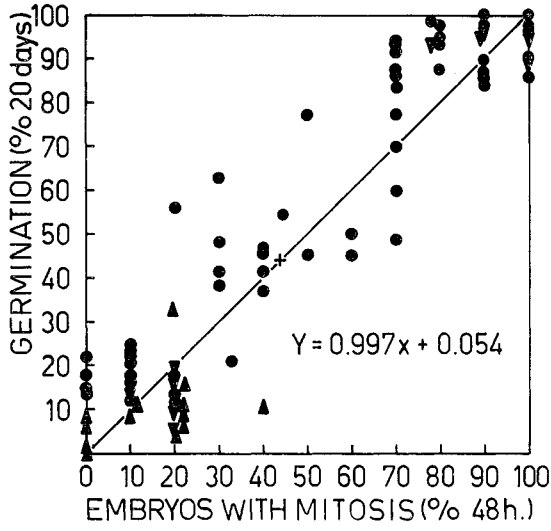

Figure 40. Relation between the percentages of embryos with mitosis after 48 hours and the germination percentages after 20 days in seeds of Scots pine. For further details, see the text. Provenance Nos. 3 and 4.

after 48 hours and the corresponding germination percentage of the remaining seeds (90) from the same seed sample after 20 days. The material is combined from the results in figures 36 and 39 B (dots and inverted triangles from repeated experiment) and from table 16 (triangles). The statistical analysis was done after BONNIER and TEDIN (1957).

Thus the appearance of embryo-mitosis before the directly visible germination and the correlation between these two processes and with the light factor seems to indicate that the final germination is regulated by light through a more direct effect on the mitosis than on the lengthening of the embryos.

6. Effect of Decoating and Red Irradiation

In earlier experiments it was shown that after a decoating the light requirement for the germination was eliminated (cf. p. 40 and figure 17 D). To investigate whether a decoating compared with a red irradiation of intact seeds produced the same effects on the appearance of the embryo-mitosis the following experiment was performed.

The red irradiation of the intact seeds was carried out after 6 hours of imbibition in darkness (standard red irradiation). The decoating was performed after the same time (2×100 seeds per treatment). After different periods 20 seeds were randomly sampled, the embryos dissected, fixed and investigated for the occurrence of mitosis. All samplings were undertaken during the time interval 24 to 48 hours after the start of the imbibition and

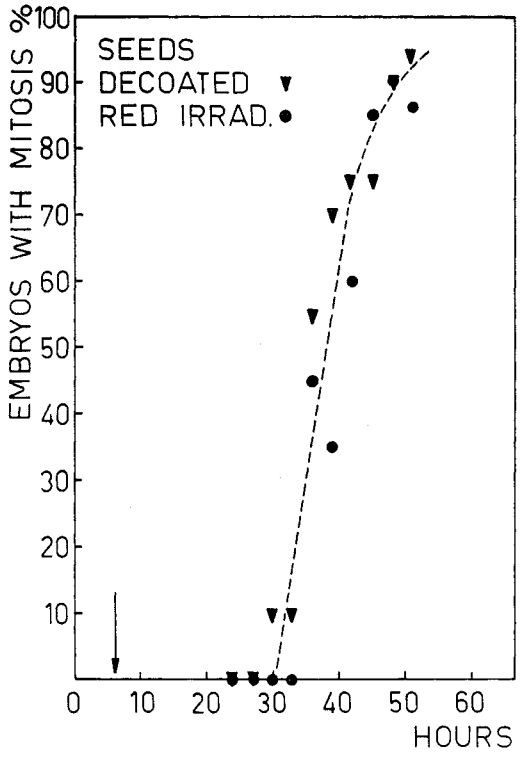

Figure 41. Effect of a red irradiation and a decoating of Scots pine seeds on the appearance of embryo-mitosis. Irradiation and decoating after 6 hours of imbibition. Standard red irradiation (cf. figure 26). Provenance No. 3.

none of the seeds had directly visible signs of incipient germination. The results are given in figure 41.

Both these treatments (indicated by the arrow in the figure) were followed by a rapid development of embryo-mitosis in the time interval 24 to 48 hours after the start of the imbibition. Thus these results showed that after a decoating the same stimulation of the mitotic activity in the embryos appeared as after a red irradiation of the intact seeds. This corresponded to the similar effects of the same treatments on the final germination (cf. figures 17 and 31). However, these results do not permit any conclusion about an eventual identity between the operating mechanisms initiating the mitotic activity after decoating or red irradiation (cf. p. 138).

7. *Relations between the Inhibitory Effect of Far-Red Irradiation and the Appearance of Embryo-Mitosis*

As shown above (figure 27) the inhibitory action of a far-red irradiation decreased with increased dark periods after a red irradiation and after 48 hours it had disappeared. These results were recalculated as the inhibi-

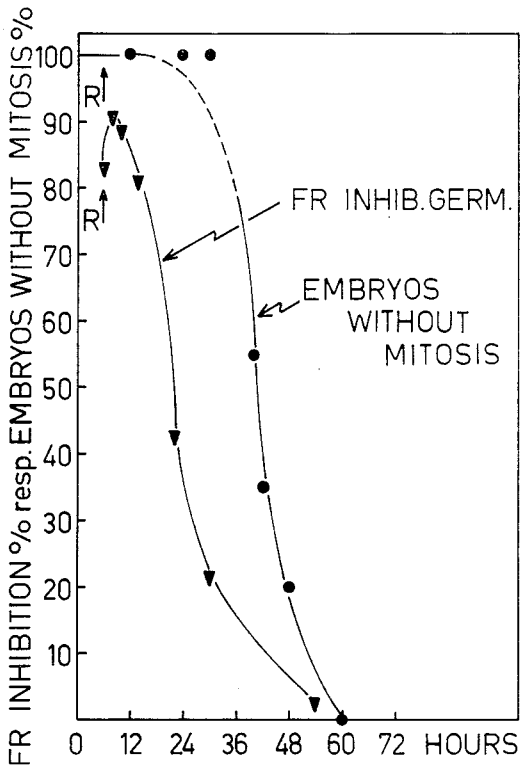

Figure 42. The disappearance of the far-red inhibiting effect on the germination of Scots pine seeds with increasing time between a red and a far-red irradiation compared with the time course of the appearance of embryo-mitosis after an identical red irradiation. Standard irradiations (cf. figure 26). For further details, see the text. Provenance No. 3.

tion per cent of the only red irradiated control (as in TOOLE *et al.* 1961) and in figure 42 combined with the also recalculated results from table 15 on the time course for the appearance of the mitosis after an identical red irradiation (given as the per cent of embryos without mitosis at different times). From this comparison it was evident that the curves did not coincide, *i.e.*, the decreasing capacity of the far-red irradiation to inhibit the final germination was not a result of the occurrence of incipient mitotic activity in the corresponding fraction of the seed embryos. Compare, for example, after 22 hours when the far-red inhibition was only 42 per cent at the same time as the mitosis was just at the point of appearing. The loss of reversibility preceded the appearance of embryo-mitosis in the corresponding per cent of the embryos. The red—far-red irradiations control the germination as long as the processes initiated by the red light have not proceeded to a too advanced point after which the whole system escapes the control of the red—far-red reversible pigment. This point is reached before the first appearance of mitosis and the following development of the embryo. These

results suggest that the red—far-red control of the germination in seeds of Scots pine is more primarily connected with metabolic processes preceding the first morphological signs of incipient growth in the embryos than with either the mitosis or the lengthening of the embryos.

D. Discussion

In the introduction to this chapter it was pointed out that the germination is equivalent to the resumption of growth in the embryos. The growth of a plant organ defined as an irreversible increase of its volume (MEYER and ANDERSON 1954) is caused by cell multiplication (mitosis and cell divisions) and cell lengthening. The first problem was the importance of these two processes for the directly visible germination in the Scots pine seeds under light conditions permitting the development of the embryos. The other one was whether it was possible to trace the controlling effect of light to any one of these participating processes or not.

From the results of the experiments where complete germination was produced by a continuous irradiation with white light or by a limited red irradiation it was evident that mitotic activity appeared in the embryos before there was any directly visible protrusion of the rootlets (cf. *e.g.* figure 38). This was in accordance with results from *Pinus thunbergii* Parl. (GOO 1952, 1956) and contrary to those described from other species investigated in this respect (cf. p. 92). However, in seeds of *Lactuca sativa* L. EVENARI *et al.* (1957) have shown that the start of lengthening of the rootlets coincided with the first appearance of mitosis. In gamma irradiated seeds of the same species HABER and LUIPPOLD (1960 a) found an inhibited appearance of mitosis, but in spite of this a directly visible germination (protruding rootlets). Compare corresponding results with wheat grains in HABER and FOARD (1962). The protrusion was only an effect of the lengthening of the embryo-cells. From such results HABER and LUIPPOLD (l.c.) have concluded that the mechanism of root protrusion in the seed germination is parallel to the mechanism of root extension in mature plants, which is caused chiefly by expansion of cells in the zone of elongation. From this it seems reasonable to conclude that the protrusion of the rootlets in the germination of the Scots pine seeds also is caused by the lengthening of the embryo-cells. However, under the conditions used for the experiments the first appearance of protruding rootlets in the Scots pine seeds could be found first after the seeds had been under germinating conditions for more than 48 hours (table 15, figure 38 C and D). At 48 hours the seeds showed no signs of beginning protrusion but a full development of the

mitotic activity in the embryos (cf. figures 38 B and 39 B). As a matter of fact, the start of the mitotic activity in embryos from seeds irradiated with a light quality stimulating the germination (protrusion of the rootlets) appeared already more than 24 hours before the first visible germination (figure 38, cf. also figure 39 A and B). In spite of the fact that the lengthening of the embryo-cells has not been studied from direct measurements of the cells but only from indirect evidence this discrepancy in time between the first appearance of embryo-mitosis and the start of the protrusion of the rootlets may permit the conclusion that in the germination of Scots pine seeds not restricted by the light factor the embryo lengthening is preceded by a mitotic activity in the embryos.

The next question was whether the light control of the germination could be traced to any specific effect on either the mitotic activity or the lengthening process of the embryos. The results in table 14, dark control and in tables 14 and 15, red + far-red, showed that under light conditions restrictive for the germination (the protrusion of the rootlets) also the occurrence of embryo-mitosis was inhibited. Table 18 presents evidence for a complete block of the mitotic activity in the light-sensitive fraction of the Scots pine seeds. In their studies on the light-requiring seeds of *Lactuca sativa* L. (var. Grand Rapids) also EVENARI *et al.* (l.c.) observed that there were no mitosis in darkness. In the same species (var. New York) HABER and LUIPPOLD (1960 a) could separate the natural coincidence between mitotic activity and protrusion of the rootlets by using a supraoptimal germination temperature, which inhibited the protrusion of the rootlets at the same time as the mitotic activity occurred. Under such conditions the same authors (1960 b) found that a continuous red irradiation stimulated but a far-red one inhibited the directly visible germination simultaneously as there was no effect on the mitotic activity in the seed embryos before the first protrusion of the rootlets. From this result they concluded that the red—far-red control of the germination in the lettuce seeds could be traced to an effect of the light on the lengthening process in the embryos.

However, the natural separation in time between the start of the mitotic activity and the lengthening of the embryos at an optimal germination temperature found in the Scots pine seeds studied here motivated a corresponding study on the pine embryos without changes of the germination conditions. The results in table 16 showed that an alternating sequence of red—far-red irradiations influenced the occurrence of embryo-mitosis before the first signs of directly detectable protrusion of the rootlets. These results corresponded to the similar effects on the final germination (cf. also NYMAN 1961 figure 1). In table 17 the same results expressed as the numbers of mitotic figures per embryo are given. Thus both the occurrence and the

intensity of the mitotic activity were regulated by the light in the same way as the final germination. The studies on the action spectra for the occurrence of embryo-mitosis in figure 39 further confirmed this. A direct comparison between the occurrence of mitotic activity before the protrusion of the rootlets and the rootlet protrusion (figure 40) also demonstrates the same light dependence of the two processes. Thus the statement made by HABER and LUIPPOLD (1960 b) (cf. also HABER 1960 and HABER and CARRIER 1961) that the definitive characteristic of dormancy in seeds is a subtle block specifically preventing the initiation of cellular expansion does not seem to be valid for the light-controlled dormancy in the Scots pine seeds studied here.

Besides the red—far-red controlled lengthening of the growing lettuce embryos (HABER and LUIPPOLD l.c.) some other photomorphogenic growth processes have been explained as effects of the light on the lengthening process of the developing plant organs. Thus KLEIN (1959) found that the red induced opening of the plumular hooks in dark-grown seedlings of *Phaseolus vulgaris* L. was only an effect on the lengthening of the cells of the concave side of the hooks. MOHR and HAUG (1962) have found that the red induced formation of plumular hook in dark-grown seedlings of *Lactuca sativa* L. depended on a stimulated cell lengthening on the ultimate outer side of the hook. In hypocotyls of *Pisum sativum* L. LOCKHART (1960) found that red light decreased the plasticity of the cell walls at the same time as an inhibition of the overall growth occurred. A red stimulated unfolding of the developing leaf in *Triticum sativum* L. by VIRGIN (1962) was shown to be connected with an increased extensibility of the cell walls and in red inhibited hypocotyls of *Sinapis alba* L. MOHR and PETERS (1960) found that this inhibition could be correlated with a decreased cell length.

Unlike this the red—far-red controlled leaf development in *Phaseolus vulgaris* L. (LIVERMAN *et al.* 1955, DOWNS 1955) probably is caused by an effect on the cell divisions (POWELL and GRIFFITH 1960, KLEIN and WANSOR 1963). MILLER (1961) has shown that the stimulated growth of a fern gametophyte by red irradiations was correlated with a stimulation of the cell divisions. Also PARKINSON (1950) (q.f. WILLIAMS *et al.* 1955) could find that the stimulating action of light on the growth of leaves in peas was caused by a stimulation of the cell divisions. From indirect evidence also WILLIAMS *et al.* (l.c.) concluded that the light effect on the growth of *Vicia faba* L. (inhibition of etiolation) must be restricted to an effect of light on the cell divisions at the apex of the stem. In dark-grown seedlings of the same species BUTLER and LANE (1959) found a decreased mitotic activity in the apical meristems but the characteristic abnormalities in the etiolated

shoot were connected with the lower parts of the shoot already present in the ungerminated seed. At the light-inhibited growth of the first internode in *Avena*-seedlings AVERY *et al.* (1937) found an inhibition of the cell divisions caused by the light. In the same material GOODWIN (1941) also found this inhibition together with a smaller inhibition of the lengthening process during longer irradiation times. THOMSON (1954, 1959) and THOMSON and MILLER (1961, 1962) combined the light regulation of the growth with a stimulation of the division-enlargement-maturation sequence and the final effect of the light thus was dependent on the events taking place in the actual plant organ at the time for the irradiation.

From the above results it seems difficult to get a general picture of the relations between the photomorphogenic effects and the individual phases of the growth process. Another indication of a close connection between the effect of red—far-red irradiations and the mechanism of the cell divisions has been shown by MOH and WITHROW (1957), WITHROW and MOH (1957) and MOH and WITHROW (1959 a and b), who have found that a far-red irradiation before an X-ray irradiation increased the occurrence of chromatide breakage. This far-red effect could be reversed by a red irradiation.

However, the loss of far-red reversibility and the time course for the appearance of mitotic activity in the Scots pine embryos (figure 42) indicate that light through the mediation of the red—far-red reversible pigment system controls processes preceding the first appearance of mitotic activity. The same conclusion can be drawn from a comparison between the results in BORTHWICK *et al.* (1954, figure 9) and in EVENARI *et al.* (1957, figure 1 B).

These results suggest that a more primary light effect can be traced in the metabolism of the seeds, *e.g.*, as it is expressed in the respiration, at a point of time earlier than the first appearance of mitotic activity. This idea will be further investigated in the next chapter.

Chapter VI. Studies on the Respiration

A. Introduction

The start of the germination process by the uptake of water (imbibition) leads to an incipient growth of the embryonic plant in a viable and not dormant seed. One condition for growth is a supply of energy. Energy is generated by the respiration and at the growth—the germination—there is a coupling of the respiration to this process. A consequence of this is that one of the earliest signs of the incipient germination ought to be an increased

respiration (TOOLE *et al.* 1956 a). In the case of light-requiring seeds, the germination of which is blocked by the absence of light, it thus seems natural that the dormancy-breaking light rather early in the germination process will have a stimulating effect on the respiration. CROCKER and BARTON (1953) also remark that whatever effect the light has on the light-sensitive seeds one may trace the light effect in the respiration of the seeds. In accordance with this some cases have been described where the respiration of light-sensitive seeds was affected by irradiations (KIPP 1929, SCHRÖPPEL 1933, LEGGATT 1948, PAECH 1953, HAGEN *et al.* 1954, LEOPOLD and GUERNSEY 1954, EVENARI *et al.* 1955). A general survey of the effect of visible light on the respiration has been given by ROSENSTOCK and RIED (1960).

From this it seemed important to investigate the relations between the respiration and the light factor in the Scots pine seeds. Also the influence of seed coat and stratification shown in the foregoing to be important for the light-controlled germination has been studied. Some of the results have been earlier reported (NYMAN 1957, 1961).

B. Methods

Before the start of the respiration measurements the seeds were placed under the same conditions as in the germination tests (at + 25° C in Jacobsen apparatuses, cf. p. 12). For details about the irradiations, see page 14 and the individual experiments.

The measurements were made with the direct method (UMBREIT *et al.* 1959) in a Warburg apparatus (Braun, Melsungen). The volumes of the flasks were 13—15 ml. In the measurements of the oxygen uptake 0.2 ml 20 per cent KOH with a filter paper wick was placed in the central well. The seeds were placed in 2.00 ml distilled water, 50 per flask or in the experiments with decoated seeds 25 per flask.

The effect of different numbers of seeds per flask and the magnitude and the rate of shaking was investigated in preliminary experiments. With 50 seeds per flask, 80 oscillations per minute and a length of oscillation of 4 cm it was shown that the diffusion of gas did not restrict the measurements. The temperature at all the measurements was + 25.00± 0.02° C. After the seeds had been transferred from the germination beds to the Warburg apparatus there was an adaptation period of 30 minutes before the start of the measurements. These were made during 4 hours with readings every half hour. All manipulations and measurements were done in safelight.

The results from the measurements have been given in µl gas NTP per half an hour and per standard quantity of seeds. As standard a definite number of seeds (50) was chosen, because both the fresh and dry weight may change during the germination process and a standard should be unchanged in the different experiments (BROWN 1942, HALVORSEN 1955, 1956). All experiments were carried out in duplicate or triplicate for each type of irradiation, time for measurement and condition of the seed coats. The seed samples were so well cleaned that no empty seeds were to be found in the individual samples. The seeds were not sterilized but no visible infection was found and the measurements in the dark control series have not shown any sign of intensified respiration due to an infection.

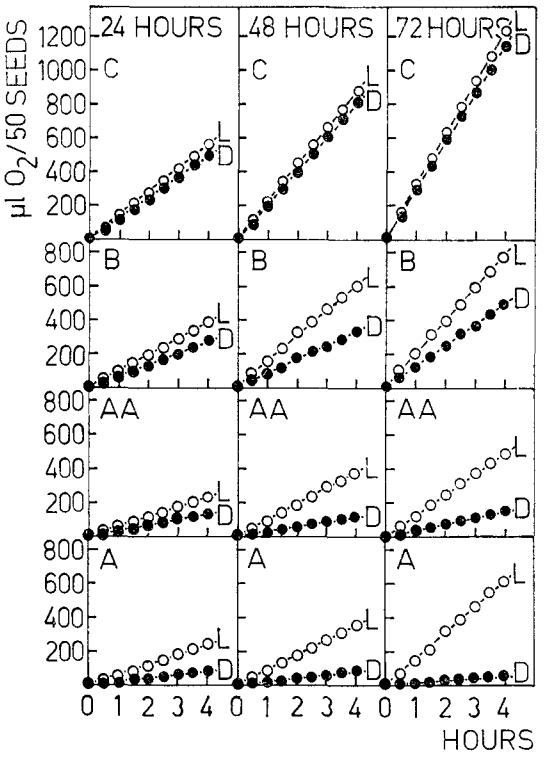

Figure 43. Oxygen uptake of Scots pine seeds after 24, 48 and 72 hours, respectively, under germinating conditions with continuous irradiation (white light, L) and darkness (D). A: intact seeds. AA: intact seeds, irradiated in unimbibed condition. B: punctured seeds. C: decoated seeds. For further details, see table 19. Provenance No. 2.

C. Results

1. Effect of White Light and Darkness on Intact Seeds

Before the start of the measurements the seeds were placed under germinating conditions for 24, 48 and 72 hours, respectively, in continuous white light (from fluorescent tubes TL/33, 1700 lux and in darkness). The total uptake of oxygen in these seeds is given in figure 43 A and the respiration rate and RQ in table 19 A.

These results showed that the respiration of the seeds in darkness was lower than that in continuous white light. Both for oxygen and carbon dioxide there was a slight decrease in the respiration after a longer time in darkness (see also the extrapolated values in figure 45). Already after 24 hours the seeds in light showed a higher respiration rate than the dark control. At this time the relative increase was 282 per cent for oxygen and

Table 19. Effect of white light, darkness and different seed coat conditions on the respiration of Scots pine seeds. Measurements started after the seeds had been under germinating conditions for 24, 48 and 72 hours, respectively, under continuous irradiation and in darkness. Irradiation as in figure 7. Provenance No. 2.

Experiment	Seed coat condition	Irradiation	Hours under germinating conditions	Respiration rate (µl gas/50 seeds/1/2 hour)		RQ
				O_2	CO_2	
A	Intact	Light	24	30.7 ± 0.6	19.8 ± 0.4	0.64 ± 0.01
			48	44.6 ± 0.4	27.1 ± 0.4	0.61 ± 0.01
			72	77.6 ± 1.4	55.8 ± 2.4	0.72 ± 0.02
		Darkness	24	10.9 ± 0.4	8.1 ± 0.4	0.74 ± 0.04
			48	9.8 ± 0.3	7.5 ± 0.3	0.77 ± 0.03
			72	7.3 ± 0.2	5.8 ± 0.4	0.77 ± 0.04
AA	Intact[1]	Light	24	29.4 ± 0.4	17.3 ± 0.4	0.59 ± 0.01
			48	47.3 ± 0.7	30.7 ± 0.7	0.67 ± 0.01
			72	62.1 ± 2.5	40.0 ± 2.4	0.64 ± 0.01
		Darkness	24	16.1 ± 0.5	11.8 ± 0.4	0.73 ± 0.05
			48	15.3 ± 0.5	8.5 ± 0.3	0.56 ± 0.03
			72	19.4 ± 0.8	12.5 ± 0.5	0.64 ± 0.02
B	Seed coat punctured	Light	24	48.8 ± 0.8	33.4 ± 0.9	0.68 ± 0.01
			48	75.5 ± 0.8	59.2 ± 1.0	0.78 ± 0.01
			72	96.6 ± 1.8	73.6 ± 3.2	0.76 ± 0.02
		Darkness	24	34.5 ± 0.9	24.7 ± 0.7	0.72 ± 0.01
			48	41.7 ± 1.3	26.9 ± 1.4	0.64 ± 0.02
			72	61.5 ± 0.7	41.6 ± 1.0	0.64 ± 0.01
C	Seed coat removed	Light	24	69.8 ± 1.3	50.4 ± 1.3	0.72 ± 0.01
			48	110.0 ± 0.7	77.7 ± 1.2	0.71 ± 0.01
			72	153.9 ± 15.3	112.0 ± 22.8	0.73 ± 0.03
		Darkness	24	61.3 ± 1.2	41.2 ± 0.9	0.67 ± 0.01
			48	100.8 ± 1.3	69.0 ± 1.8	0.68 ± 0.01
			72	142.8 ± 7.5	94.8 ± 1.2	0.66 ± 0.01

[1] Seeds irradiated in unimbibed condition (white fluorescent tubes TL/33, 6000 lux, 10 hours).

244 per cent for carbon dioxide. These elevations increased with longer time as a result of the higher respiration rate in light and the slightly decreasing respiration rate in darkness. The high respiration rate after 72 hours in light coincided with the start of the visible germination (protrusion of the rootlets). The continuous white light stimulated the respiration already before there were any visible signs of germination. No certain effects could be traced in the RQ, the values of which were in accordance with the fact that the Scots pine seeds are rich in fat (CROCKER and BARTON 1953, RÄDER-ROITZSCH 1957, THOMAS 1960). Another experiment was done with intact seeds, which were irradiated in unimbibed condition for 10 hours with white light (6000 lux) from the same light source as above.

In other respects the experiment was carried out in the same way as the preceding one. The results are given in figure 43 AA and table 19 AA.

Also here the seeds kept in darkness had a lower respiration rate, but the decrease with time, which was obtained in the preceding experiment, was substituted by a slight increase. From this it followed that the relative increase caused by the continuous irradiation was lower. For the oxygen uptake the relative increases were 183, 309 and 320 per cent after 24, 48 and 72 hours, respectively. The corresponding values in the previous experiment (without preirradiation) were 282, 455 and 1063 per cent. The relative values of the carbon dioxide output were 147, 361 and 320 per cent for the preirradiated seeds and 244, 361 and 962 per cent for the seeds in the previous experiment. It was not possible either in the preirradiated seeds to trace any certain effect on the RQ.

A comparison between the respiration rates of the preirradiated and unirradiated seeds in darkness, however, showed a significant increase ($0.001 > P$) for both oxygen and carbon dioxide. The corresponding comparison for the seeds kept in continuous white light did not show any significant difference. From these results it was evident that not only a continuous irradiation of the seeds but also a preirradiation of the unimbibed seeds could stimulate the respiration of the seeds.

2. Effect of White Light and Darkness on Seeds with Punctured Seed Coats

Before the seeds were laid to imbibition the seed coats were punctured as previously described (p. 40). Otherwise the experiment was performed in the same way as above. The results are given in figure 43 B and table 19 B.

Also in this experiment the respiration of the seeds under continuous irradiation showed a higher value than that in darkness, but at the same time the respiration rates for the seeds in both light and darkness were higher than the corresponding values for the intact seeds. From this it followed that the relative increases of the respiration caused by light were less: for oxygen uptake after 24, 48 and 72 hours, 141, 181 and 157 per cent, respectively; for carbon dioxide 135, 220 and 177 per cent. From these results it was evident that a puncturing of the seed coats stimulated the respiration at the same time as the relative stimulating effect of the light became less. The RQ did not show any principal difference between the values from seeds in light and darkness nor between them from punctured and intact seeds. This may indicate that if the seed coats have a restrictive permeability for the two gases, oxygen and carbon dioxide, the diffusion through them will be restricted to the same extent for both of the gases (cf. further p. 120).

3. Effect of White Light and Darkness on Seeds with Removed Seed Coats

The seeds used in this experiment were completely decoated before the start of the imbibition. In other details the experiment was identical with the preceding ones. The results are given in figure 43 C and table 19 C.

Here it was evident that the stimulating effect of a continuous irradiation had nearly disappeared. The relative increase of the oxygen uptake after 24, 48 and 72 hours, was 114, 109 and 108 per cent, respectively. For the rate of carbon dioxide output the corresponding values were 122, 113 and 118 per cent. At the same time as the stimulating effect of light on the respiration rate decreased the absolute rates were higher than both in the intact and punctured seeds. Not either in this experiment were the RQ values changed significantly. A decoating of the seeds stimulated the respiration more than only a puncturing at the same time as the stimulating effect of the light nearly abolished.

4. Effect of Irradiations with Red and Red + Far-Red Light on Intact Seeds

In order to investigate whether the same type of reaction could be found after irradiations with red and red + far-red light the following experiment was performed.

In accordance with the germination experiments the seeds were imbibed in darkness for 6 hours and then given standard irradiations with red and red + far-red light (cf. p. 57). The measurements were started after the seeds had been under germinating conditions for 24, 48 and 72 hours from the start of the imbibition. The results are given in figure 44 A and table 20 A.

A comparison of these results with those from the experiments on intact seeds in continuous white light and darkness showed principally the same results. After a red irradiation the respiration rate increased; after a red + far-red irradiation there was a slight decrease with time. Already after 24 hours the red irradiated seeds showed a higher respiration rate than the red + far-red irradiated ones. The relative increases for the rate of oxygen uptake after 24, 48 and 72 hours were 147, 276 and 424 per cent, respectively. For the carbon dioxide output the corresponding values were 126, 246 and 414 per cent. A comparison of the values in tables 19 and 20 showed that the respiration rate of the red + far-red irradiated seeds was higher than in the dark control seeds in table 19. This difference may be due to the fact that the experiments were made on seeds of different provenances (No. 2 and 3, respectively). However, compare the results in table 25, which showed that the respiration rate in the red + far-red irradiated seeds was somewhat higher than in the dark control seeds.

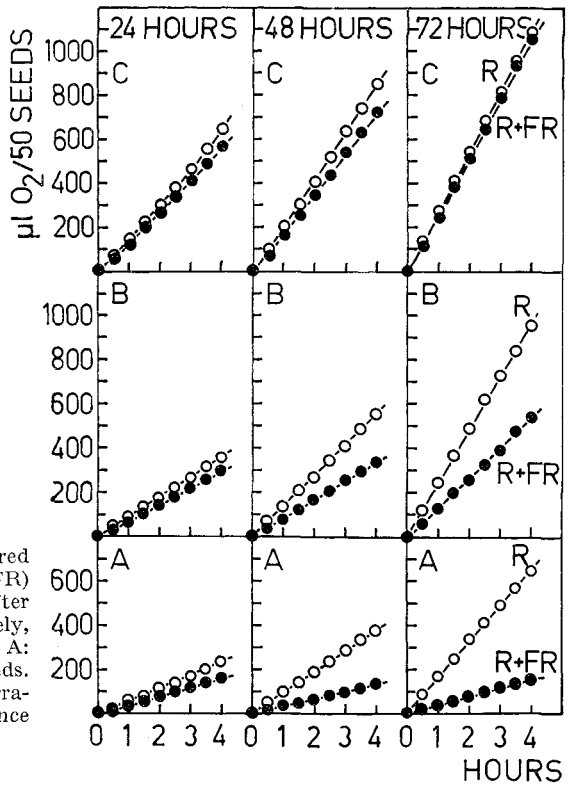

Figure 44. Oxygen uptake of red (R) and red + far-red (R + FR) irradiated Scots pine seeds after 24, 48 and 72 hours, respectively, under germinating conditions. A: intact seeds. B: punctured seeds. C: decoated seeds. Standard irradiations (cf. figure 26). Provenance No. 3.

These results showed that limited irradiation with red light stimulated the respiration. This stimulation was apparent already after the seeds had been under germinating conditions for 24 hours, *i.e.*, 17.5 hours after the end of the red irradiation (see further figure 49). The stimulating effect of this red irradiation could be nullified by an immediately following far-red irradiation. The respiration level was reversibly controlled by red—far-red irradiations, but there were no effects on the RQ values.

5. *Effect of Irradiations with Red and Red + Far-Red Light on Seeds with Punctured Seed Coats*

This experiment was done in the same manner as the previous one but the seeds were punctured. The results are given in figure 44 B and table 20 B.

For both the red and the red + far-red irradiated seeds the respiration levels were higher than for the corresponding intact seeds (cf. figure 44 A and table 20 A). The inhibiting effect of the far-red irradiation was less

Table 20. Effect of red (R), red + far-red (R + FR) irradiations and different seed coat conditions on the respiration of Scots pine seeds. Measurements started after the seeds had been under germinating conditions for 24, 48 and 72 hours, respectively. Standard irradiations (cf. figure 26). Provenance No. 3.

Experiment	Seed coat condition	Irradiation	Hours under germinating conditions	Respiration rate (μl gas/50 seeds/1/2 hour)		RQ
				O_2	CO_2	
A	Intact	R	24	29.4 ± 0.7	20.0 ± 0.8	0.70 ± 0.02
			48	47.4 ± 0.7	28.5 ± 0.8	0.60 ± 0.01
			72	80.9 ± 2.3	51.7 ± 3.2	0.63 ± 0.03
		R + FR	24	20.0 ± 0.4	15.9 ± 0.6	0.79 ± 0.02
			48	17.2 ± 0.6	11.6 ± 0.8	0.66 ± 0.03
			72	19.1 ± 0.9	12.5 ± 1.1	0.63 ± 0.03
B	Seed coat punctured	R	24	45.2 ± 0.8	31.6 ± 0.9	0.70 ± 0.01
			48	69.9 ± 1.6	48.9 ± 2.0	0.69 ± 0.01
			72	119.2 ± 2.8	94.0 ± 4.4	0.78 ± 0.02
		R + FR	24	37.2 ± 0.9	28.2 ± 1.0	0.75 ± 0.01
			48	43.0 ± 0.8	28.4 ± 0.8	0.67 ± 0.01
			72	67.6 ± 1.5	49.4 ± 1.9	0.73 ± 0.02
C	Seed coat removed	R	24	82.0 ± 1.1	59.1 ± 1.1	0.72 ± 0.01
			48	107.6 ± 0.6	70.3 ± 0.6	0.65 ± 0.01
			72	136.1 ± 2.4	96.9 ± 2.5	0.70 ± 0.08
		R + FR	24	71.2 ± 1.0	47.7 ± 0.9	0.67 ± 0.01
			48	91.6 ± 0.8	55.0 ± 0.5	0.60 ± 0.02
			72	133.3 ± 2.1	93.3 ± 2.6	0.69 ± 0.02

than in seeds with intact seed coats. The red—far-red control of the respiration was disturbed by the puncturing of the seed coats. As a consequence of this the relative increases in the respiration level caused by the red irradiation were less than in the cases with intact seed coats. After 24, 48 and 72 hours, the relative increases for the rate of oxygen uptake were 122, 163 and 176 per cent, respectively, and for carbon dioxide output 112, 172 and 190 per cent. Concerning the RQ there were no significant effects of the treatments. As in the experiment with continuous white light and darkness a puncturing of the seed coats stimulated the respiration to a higher level than after only an irradiation. The nullifying effect of far-red on the stimulating effect of red light was less after a puncturing.

6. *Effect of Irradiations with Red and Red + Far-Red Light on Seeds with Removed Seed Coats*

The seeds used in this experiment were decoated before they were placed to imbibition. In other details there were no changes. The results are given in figure 44 C and table 20 C.

After this decoating the respiration level for the seeds irrespective of the type of irradiation was higher than for both punctured and intact seeds. However, there was still a significant lower respiration ($0.001 > P$) in the far-red irradiated seeds after 24 and 48 hours. As a consequence of the high respiration in the red + far-red irradiated seeds the relative increases for the rate of oxygen uptake and carbon dioxide output were small: after 24, 48 and 72 hours, for oxygen 115, 118 and 102 per cent, for carbon dioxide 124, 128 and 104 per cent, respectively.

These results showed that the absence of seed coat influenced the capacity of light to regulate the level of the respiration. However, the nullifying ability of far-red light on the stimulation caused by red light persisted after decoating but the relative importance was small. As in the preceding experiments no effects were found on the RQ values.

A summary of the above results shows that a continuous irradiation with white light or limited red irradiation of intact seeds as well as a puncturing or decoating without irradiation were followed by a stimulated respiration. This corresponded to a stimulation of the germination after the same treatments. On the other hand, the intact and unirradiated or red+far-red irradiated seeds showed an inhibited respiration, which also corresponded to an inhibited germination under the same circumstances. However, the inhibiting effect of a far-red irradiation was decreased after disturbance of the seed coat both for the germination and the respiration. Factors stimulating the germination also stimulated the respiration. Factors inhibiting the germination also inhibited the respiration.

A further exemplifying of this relation between the germination and the respiration will be given here by a comparison of results presented earlier in figure 36 and results given further on in this chapter in figure 51 A and B. This comparison is given in figure 45, where the respiration rates of seed samples, which had been under germinating conditions for 24 and 48 hours, are correlated to the final germination percentages of other seed samples identically irradiated. There was a good correlation between the two types of values. The extrapolated values were in accordance with the observed decrease of respiration rate with increased time in darkness (see table 19 A, darkness). The conclusion from this was that a direct relationship existed between the intensity of the respiration already in an early phase of the germination process and the final visible germination.

7. Effect of Decoating at Different Times on the Far-Red Inhibited Respiration

In this experiment the far-red inhibited respiration was studied in relation to the period during which the seed coats were intact. This was done in

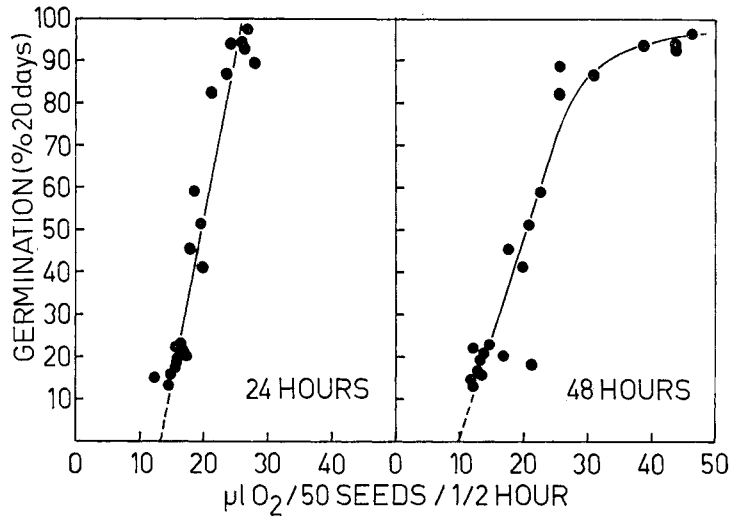

Figure 45. Relations between the respiration rates after 24 and 48 hours, respectively, and the germination percentages after 20 days in Scots pine seeds (comparison between the results in figure 36 and figure 51 A and B).

direct connection with the former experiment and some of the results presented here are repeated from table 20 (intact, R+FR; seed coat removed, R+FR). All seeds were given standard red+far-red irradiations after the seeds had imbibed in darkness for 6 hours. Four types of decoating were used: decoating before the start of the imbibition (A), immediately after the end of the irradiations (B), immediately before the start of the respiration measurements (C) and no decoating at all (D). The measurements were made after the seeds had been under germinating conditions for 7, 24, 48 and 72 hours. The results are given in figure 46 and table 21.

The results showed that the respiration rates during the measurement periods of 4 hours were constant in the different experiments. If the presence of an intact seed coat inhibits the complete imbibition and in that way restricts the respiration the stimulated respiration measured immediately after a decoating (figure 46 C) should have further increased during the measurements. In earlier results (figure 20) it was evident that the imbibition of intact and punctured seeds was the same, but the respiration rate of the punctured seeds was higher than that of the intact ones and constant during 4 hours (see figures 43 B and 44 B). However, the imbibition curve for decoated seeds in figure 20 showed that these seeds had a greater water uptake during the imbibition, which cannot be excluded as a possible cause of the greater respiration rate of these seeds in figure 46 A (see also figures 43 C and 44 C). But the stimulated respiration after a puncturing (figures

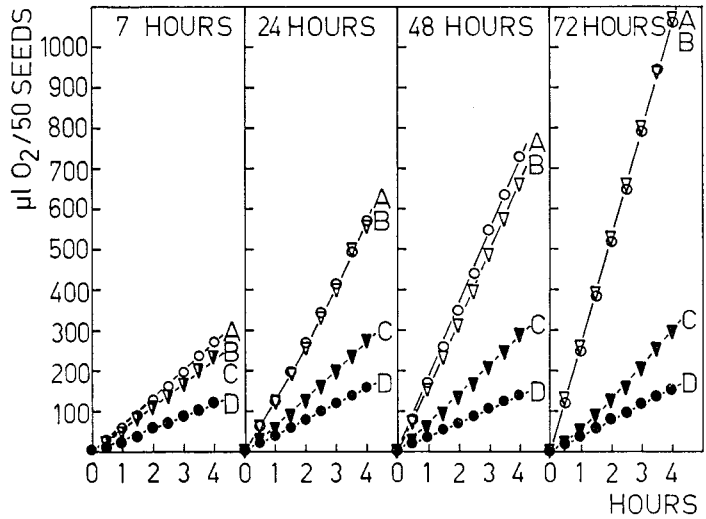

Figure 46. Oxygen uptake of red + far-red irradiated Scots pine seeds at different times after decoating. A: decoating before the start of the imbibition. B: decoating immediately after the end of the irradiations (after the seeds had been under germinating conditions for 7 hours). C: decoating immediately before the measurements. D: no decoating. Measurements started at the indicated times after the start of the imbibition. For further details, see table 21. Provenance No. 3.

Table 21. Effect of decoating at different times on the far-red inhibited respiration in Scots pine seeds. Standard red + far-red irradiations (cf. figure 26) after the seeds had imbibed in darkness for 6 hours. Treatments A—D as in figure 46. Provenance No. 3.

Measurements started after the seeds had been under germinating conditions for hours	Treatment	Seeds in decoated condition before measurements for hours	Respiration rate (µl gas/50 seeds/1/2 hour)		RQ
			O_2	CO_2	
7	A	7	33.6 ± 0.4	27.6 ± 0.4	0.82 ± 0.02
	B, C	0	28.6 ± 0.5	23.0 ± 0.5	0.80 ± 0.05
	D	—	14.7 ± 0.6	9.5 ± 0.6	0.65 ± 0.01
24	A	24	71.2 ± 1.0	47.7 ± 0.9	0.67 ± 0.01
	B	17	68.8 ± 0.7	45.3 ± 0.8	0.66 ± 0.04
	C	0	34.1 ± 0.5	23.5 ± 0.5	0.70 ± 0.02
	D	—	20.0 ± 0.4	15.9 ± 0.6	0.79 ± 0.02
48	A	48	91.3 ± 0.8	55.0 ± 0.5	0.60 ± 0.02
	B	41	82.9 ± 0.9	50.5 ± 1.0	0.61 ± 0.02
	C	0	35.9 ± 0.5	24.6 ± 0.3	0.69 ± 0.01
	D	—	17.2 ± 0.6	11.6 ± 0.8	0.66 ± 0.03
72	A	72	133.3 ± 2.1	93.3 ± 2.6	0.69 ± 0.02
	B	65	134.5 ± 1.9	89.8 ± 4.6	0.65 ± 0.06
	C	0	37.3 ± 1.3	22.9 ± 1.0	0.62 ± 0.03
	D	—	19.1 ± 1.3	12.5 ± 1.1	0.63 ± 0.03

43 B, 44 B) or a decoating immediately before the respiration measurements (figure 46 C) do not suggest that an increased water uptake during the measurements should be the reason for the increased respiration in these cases. It seemed more probable that the influence of the seed coat on the respiration rate was mediated by another factor than the water factor.

A comparison of the respiration rate in the intact seeds (figure 46 D) and in the seeds decoated immediately before the measurements (figure 46 C, table 21) showed that the respiration rate was immediately changed to a higher and constant level after the decoating. This increase was approximately the same independent of the time when these decoatings were undertaken. The relative increases for oxygen were after 7, 24, 48 and 72 hours 194, 171, 209 and 195 per cent, respectively. For carbon dioxide the corresponding values were 242, 148, 212 and 183 per cent. From these results it was evident that the inhibited respiration after a far-red irradiation to a certain extent was immediately overcome after a decoating.

In another part of this experiment the decoating was performed immediately after the end of the irradiations, *i.e.*, after the seeds had been under germinating conditions for 7 hours (figure 46 B). The measurements on these seeds then were made after the seeds had been in decoated conditions for different periods of time. These results were compared with the corresponding values for the seeds which had been decoated since the start of the imbibition, *i.e.*, had been without seed coats during the irradiations. The results showed that the presence of an intact seed coat during the irradiations was of small importance for the development of the stimulated respiration. The longer the seeds were in decoated condition the higher the respiration rates were. The RQ values (table 21) did not show any significant deviations in the different experiments.

8. Effect of Different Oxygen Concentrations on Red Irradiated and Unirradiated Seeds

In order to test whether the stimulated respiration obtained after a disturbance of the seed coat could be explained by the assumption that the intact seed coat had too low a permeability for oxygen the following experiment was done.

The seeds imbibed in darkness for 6 hours. Half of the samples were then red irradiated (standard irradiation). The other samples were left unirradiated. After the seeds had been under germinating conditions for 24 hours the measurements were started after a period of adaptation, during which the vessels were flushed with different gas mixtures (about

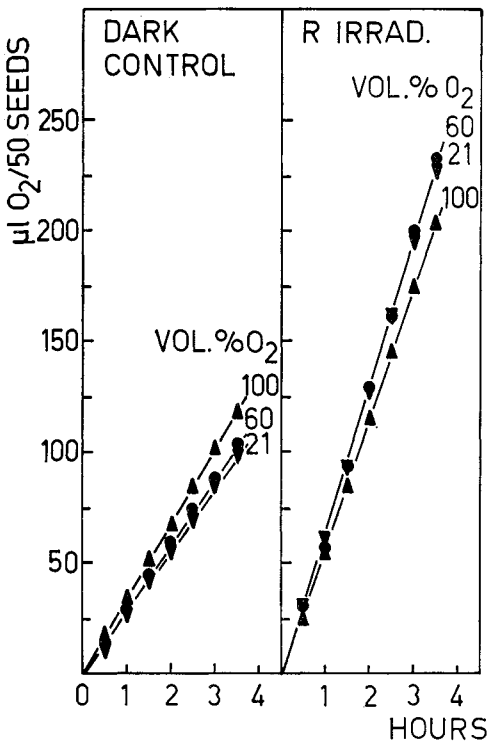

Figure 47. Oxygen uptake of red irradiated and unirradiated Scots pine seeds at different oxygen concentrations. For further details, see table 22. Provenance No. 4.

4 l) of nitrogen and oxygen prepared as previously described (p. 44). Ordinary air was used as a control (21 vol. per cent oxygen). The other oxygen concentrations used were 60 and 100 vol. per cent. The results are given in figure 47 and table 22.

In the unirradiated seeds the respiration rate increased with increasing oxygen concentration. For the oxygen uptake a significant increase (0.001

Table 22. Effect of the oxygen concentration on the respiration in Scots pine seeds. Measurements started after the seeds had been under germinating conditions for 24 hours. Standard red irradiation (cf. figure 26). Dark control unirradiated. Provenance No. 4.

Irradiation	Per cent oxygen volume	Respiration rate (µl gas/50 seeds/1/2 hour)		RQ
		O_2	CO_2	
Dark control	21	14.1 ± 0.4	9.3 ± 0.1	0.67 ± 0.02
	60	14.8 ± 0.3	11.6 ± 0.6	0.78 ± 0.03
	100	16.9 ± 0.2	12.4 ± 0.3	0.73 ± 0.02
Red irradiation	21	33.4 ± 0.9	22.5 ± 1.0	0.67 ± 0.02
	60	33.7 ± 1.3	23.0 ± 1.3	0.69 ± 0.02
	100	29.5 ± 0.4	16.3 ± 0.5	0.55 ± 0.01

$> P$), however, could be observed only in a comparison between the results in 60 and 100 vol. per cent oxygen. For the output of carbon dioxide the same significant effect was found only between the results in air and 100 vol. per cent. The relative increases (of the rate in air) for the oxygen uptake were for 60 and 100 vol. per cent 105 and 120 per cent, respectively. For carbon dioxide the corresponding values were 125 and 133 per cent.

For the red irradiated seeds there were no significant increases but the respiration rate in 100 vol. per cent showed a decrease. The relative values of the respiration rates (of the rate in air) for oxygen were in 60 and 100 vol. per cent 101 and 88 per cent, respectively. For carbon dioxide the values were 102 and 72 per cent (in the last case a significant decrease). Disregarding the results of the last case with an inhibition after an increasing of the oxygen concentration, the reason for which is not obvious (cf. in the discussion p. 138 about oxygen poisoning), the effects of the higher oxygen concentrations were small in comparison between the respiration rates in air of red irradiated and unirradiated seeds. In this case the relative increase was 237 per cent for oxygen and 242 per cent for carbon dioxide, which showed that the stimulation of the respiration rate caused by higher oxygen concentrations was small compared with the stimulation produced in ordinary air by a red irradiation. The small absolute increases of the respiration rate with increased oxygen concentration in darkness combined with the impossibility of increasing the germination under the same conditions (figure 21, in darkness) seem to indicate that the inhibiting effect in darkness of intact seed coats on respiration and germination was not dependent on an insufficient permeability of the intact seed coats for oxygen. Compare also the similar RQ values in intact and decoated seeds (tables 19 A and C, 20 A and C) indicating the same relation between the carbon dioxide output and the oxygen uptake in intact and decoated seeds. These also suggest that the seed coat effect was not connected with a corresponding insufficient permeability of the intact seed coats for carbon dioxide (see further p. 136).

9. Effect of Stratification on Unirradiated Seeds

In order to investigate whether a stratification could stimulate the respiration in the same way as the other factors (red light, decoating) also stimulating the germination, the following experiment was done with intact seeds in darkness.

The seeds were stratified in the same way as earlier described (p. 28 and p. 69). The control seeds were stored for 1 week under identical conditions but in a dry state. The stratified seeds were treated for 1 and 2 weeks,

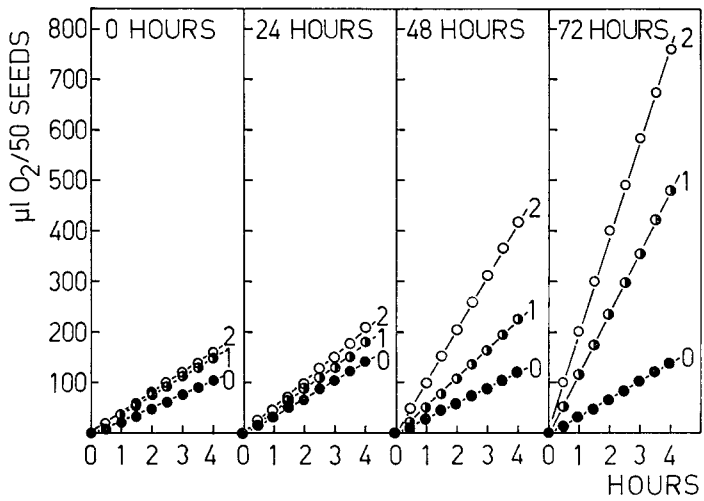

Figure 48. Effect of stratification on the oxygen uptake of unirradiated Scots pine seeds. Seeds stratified during one and two weeks, respectively (1 resp. 2, stratification as in figure 32). Control seeds unstratified (0). Measurements started after the seeds had been under germinating conditions in darkness during the indicated periods. See also table 23. Provenance No. 3.

whereupon the respiration measurements were directly started with some of the seed samples (figure 48; 0 hours). The other seeds were placed under germinating conditions for 24, 48 and 72 hours, before the measurements were started (figure 48; 24, 48 and 72 hours). No irradiations were given. The complete results are given in table 23.

Table 23. Effect of stratification on the respiration of unirradiated Scots pine seeds. Provenance No. 3.

Stratification (+ 3—4° C) weeks	Under germinating conditions (+ 25° C) hours	Respiration rate (µl gas/50 seeds/1/2 hour)		RQ
		O_2	CO_2	
2	0	20.1 ± 0.4	16.1 ± 0.5	0.80 ± 0.01
	24	25.7 ± 0.5	15.5 ± 0.7	0.60 ± 0.02
	48	52.5 ± 0.6	31.9 ± 0.5	0.61 ± 0.01
	72	95.2 ± 2.0	65.9 ± 1.7	0.69 ± 0.01
1	0	18.7 ± 0.5	15.1 ± 0.4	0.82 ± 0.03
	24	21.9 ± 0.6	15.3 ± 0.4	0.70 ± 0.02
	48	28.5 ± 1.3	14.9 ± 1.6	0.50 ± 0.06
	72	46.0 ± 2.0	34.8 ± 3.1	0.60 ± 0.03
0	0	13.3 ± 0.4	6.8 ± 0.7	0.53 ± 0.05
	24	17.9 ± 0.3	13.8 ± 0.5	0.77 ± 0.02
	48	15.1 ± 0.4	9.8 ± 0.4	0.65 ± 0.02
	72	17.6 ± 0.8	9.9 ± 1.1	0.53 ± 0.04

The results showed that the stratified seeds had a stimulated respiration in comparison with the unstratified ones. The longer the seeds had been stratified and under germinating conditions the greater the respiration rate. Contrary to this the unstratified seeds had about the same respiration rate regardless of whether the seeds had been under germinating conditions for 24 or 72 hours. From these results it was evident that a stratification caused a stimulated respiration in the intact and unirradiated seeds. In the RQ values no effects of the stratification were found. These results may be compared with the stimulated germination in darkness after a stratification previously described (cf. figures 9 and 32).

In connection with the decoating effect and the water factor previously described (section 7 above) it may be pointed out that the unstratified (unimbibed) seeds (figure 48; 0 hours, 0) did not show any obvious increase of the respiration rate during the period of the measurements (4 hours), thus suggesting that changes of the respiration rate with imbibition (for this cf. figure 20) were slow and small compared with the effects of decoating *per se*.

10. Development of Stimulated Respiration after Irradiation

In earlier experiments (figures 43, 44) a significant stimulation of the respiration was found after 24 hours in continuous white light and 17.5 hours after a limited red irradiation. In order to investigate how soon after an irradiation the first stimulation could be found the following experiments were carried out.

In a first experiment the respiration rate was measured in seeds imbibed in darkness for 24 hours. The measurements at first were done in darkness for 2 hours. At the end of this period an incandescent lamp (200 W) placed 30 cm above the water level in the Warburg apparatus and giving an irradiance of 3000 lux just above the water level, was switched on. The measurements were continued under these circumstances for 8 hours. After this period the Warburg vessels with the seeds were placed in a damp chamber under white fluorescent tubes (1700 lux, + 25° C) for 14 hours and then the measurements were started again and continued for another 2 hours. The mean values of the respiration rate and RQ values for different periods are given in table 24.

This continuous irradiation gave only a weak and slow increase of the respiration. At first after 8 to 10 hours a significant higher uptake of oxygen could be detected and after the last period of irradiation from fluorescent tubes a higher respiration for both oxygen and carbon dioxide could be shown. The respiration rate after this time was about the same as in table

Table 24. Respiration of Scots pine seeds at different times after the start of an irradiation. Provenance No. 2.

Irradiation	Time from the start of the measurements hours	Respiration rate (µl gas/50 seeds/1/2 hour)		RQ
		O_2	CO_2	
Dark-phase	0—2[1]	14.1 ± 0.3	10.3 ± 0.4	0.73 ± 0.02
Light-phase	2—4[2]	14.2 ± 0.6	9.4 ± 0.7	0.65 ± 0.03
	5—7	17.1 ± 0.8	12.5 ± 0.7	0.73 ± 0.03
	8—10	19.6 ± 0.6	12.6 ± 0.8	0.64 ± 0.04
	24—26	26.9 ± 1.0	16.1 ± 1.2	0.59 ± 0.02

[1] Before the start of the measurements the seeds had imbibed in darkness for 24 hours.
[2] Irradiation during the measurements with an incandescent lamp (200 W), which gave 3 000 lux just above the water level in the Warburg apparatus. After the measurements during 10 hours the vessels with the seeds were kept in a damp chamber in white light (fluorescent tubes TL/33, 1700 lux) at + 25° C until the start of the new measurements after 24 hours.

19 A (24 hours). From this experiment, in which the seeds were irradiated when placed under water in the Warburg vessels, it seemed that the development of the stimulated respiration caused by white light was a slow process. No certain effects could be detected in RQ.

In a second experiment the seeds were given a standard red irradiation after 6 hours of imbibition and lay on the germination beds in the Jacobsen apparatus till immediately before the start of the measurements. These were started at different times after the red irradiation. As previously there was an adaptation period (30 min.) before each series of measurements. These were made during 4 hours and the values, which are given in figure 49, are the mean values from such series. The dark control seeds were treated in the same way but were not irradiated.

The rate of the oxygen uptake measured during the period 0 to 4 hours after the end of the red irradiation was in the dark control and the irradiated seeds 15.7 ± 0.6 and 17.2 ± 0.8 µl/50 seeds/½ hour, respectively. For the period started 6 hours after the red irradiation the corresponding values were 14.8 ● 0.6 and 21.3 ± 0.7 µl/50 seeds/½ hour. The difference between the two last values was significant ($0.001 > P$). This difference increased with longer time. From these results it was evident that the stimulation of the respiration caused by a limited red irradiation increased slowly and was significant after more than 6 hours after the irradiation. The slight decrease in the respiration rate of the dark control seeds agreed with the results on intact seeds in darkness (cf. figure 43 A). An irradiation with light stimulating the germination caused a stimulated respiration before any directly visible signs of germination. A comparison of these results with those in Chapter V concerning the appearance of embryo-mitosis after an identical red irradia-

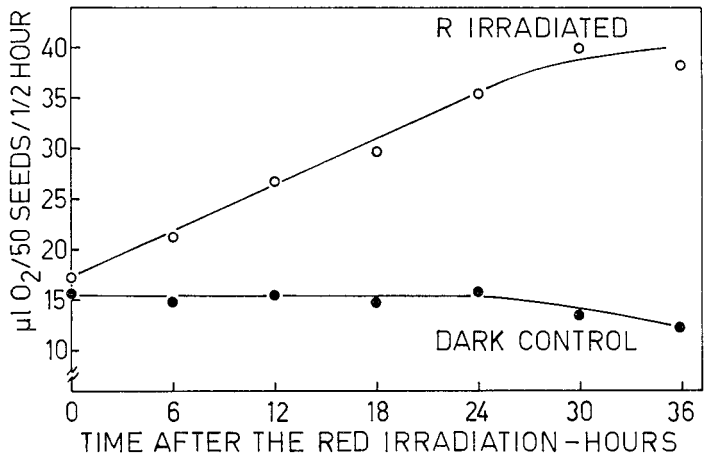

Figure 49. Rate of oxygen uptake in red irradiated Scots pine seeds at different times after the irradiation. Standard irradiation (cf. figure 26). Provenance No. 4.

tion (figures 38, 41, 42) showed that the red stimulated respiration appeared *before* the red stimulated mitotic activity. Because the mitosis was found before the directly visible germination, these three parts of the germination process in the Scots pine seeds all stimulated by red light could be arranged in the following time sequence: stimulated respiration, appearance of mitotic activity and a stimulated, directly visible germination.

However, in order to establish whether the respiration was correlated to the two other processes not only through a red sensitivity but also through the same type of repeated red—far-red reversibility and action spectrum the following experiments were undertaken.

11. Repeated Red—Far-Red Reversibility

The results in figure 44 A and table 20 A showed that a stimulating red irradiation could be nullified by an immediately following far-red irradiation. In this experiment the effect of a longer series with alternating red—far-red irradiations was investigated.

The seeds were given standard red and far-red irradiations, which followed immediately after each other. The irradiations were started after the seeds had imbibed in darkness for 6 hours. After the end of the last irradiation the seeds were placed back into the germinator. The measurements were started after the seeds had been under germinating conditions for 48 hours (from the start of the imbibition). The results are given in figure 50 and table 25.

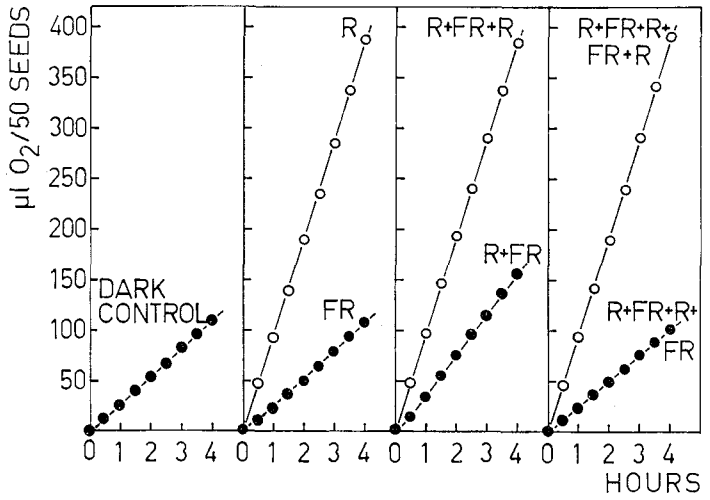

Figure 50. Reversible effect of repeated red—far-red irradiations on the oxygen uptake of Scots pine seeds. For further details, see table 25. Provenance No. 3.

The results showed that the uptake of oxygen was stimulated after a red irradiation but this stimulation was eliminated by a following irradiation with far-red. There was a repeated reversibility. The nature of the last irradiation determined whether a stimulation would appear or not. The dark control seeds showed the same magnitude of their oxygen uptake as the seeds irradiated only with far-red light or with far-red as the last light quality in a series of repeated irradiations. Concerning the output of carbon dioxide corresponding results were obtained. In the RQ values no effects could be found. A comparison of the results for R and R+FR irradiated

Table 25. Reversible effect of repeated red—far-red irradiations on the respiration of Scots pine seeds. Measurements started after the seeds had been under germinating conditions for 48 hours. Standard irradiations (cf. figure 26). Provenance No. 3.

Irradiation	Respiration rate (µl gas/50 seeds/1/2 hour)		RQ
	O_2	CO_2	
FR	13.4 ± 0.4	11.0 ± 0.2	0.83 ± 0.02
R	48.9 ± 0.6	31.0 ± 0.8	0.63 ± 0.01
R + FR	19.6 ± 1.1	14.2 ± 1.4	0.70 ± 0.04
R + FR + R	48.1 ± 0.7	32.3 ± 1.0	0.67 ± 0.02
R + FR + R + FR	12.6 ± 0.4	10.1 ± 0.5	0.81 ± 0.03
R + FR + R + FR + R	48.8 ± 0.6	30.3 ± 0.9	0.63 ± 0.01
R + FR + R + FR + R + FR	15.9 ± 0.4	10.5 ± 0.7	0.66 ± 0.01
Dark control	13.5 ± 0.4	9.9 ± 0.3	0.73 ± 0.02

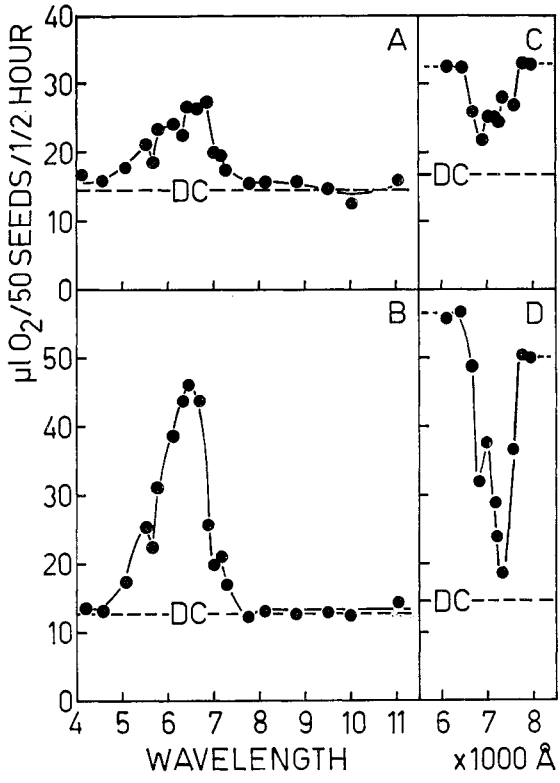

Figure 51. Action spectra for the rate of oxygen uptake in Scots pine seeds. A and B: stimulation spectra measured after the seeds had been under germinating conditions for 24 and 48 hours, respectively. C and D: inhibition spectra under the same conditions. Irradiations as in figure 36 and 37, respectively. DC: dark control. Provenance No. 4.

seeds in table 50 with the corresponding results in table 20 A (48 hours) showed good accordance. The same repeated red—far-red reversibility could be found in the respiration as in mitosis and germination (table 16, figure 26, see also NYMAN 1961, figure 1).

12. Action Spectrum

The studies on the action spectra for stimulation and inhibition of the respiration were performed in the same way as the corresponding ones for the germination (p. 74) and the occurrence of mitosis (p. 101). The seeds were transferred to the Warburg vessels after they had been under germinating conditions for 24 and 48 hours. After the same adaptation period as before the measurements were started and performed during 4 hours with readings every half hour. In all the experiments the total uptake of oxygen was directly proportional to the time. For each time (24 and 48 hours) and irradiation 2×50 seeds were used. The results are given in figure 51, where every point is the mean value of 16 measurements.

Already after 24 hours an action spectrum for the stimulation could be found, which had the same principal features as the one after 48 hours. The maximal respiration rate after 24 hours was found in the spectral region 6350—6830 Å (figure 51 A) but after 48 hours the three largest values were obtained after irradiations with 6200—6600 Å (figure 51 B). It may also be observed that this action spectrum showed two additional peaks, one at 5500 Å and the other at 7125 Å, which also could be traced after 24 hours (figure 51 A). The same peaks were found in the action spectra for the germination in figure 36, and for the mitosis in figure 39 B.

The action spectrum for the inhibition after 24 hours was irregular (figure 51 C) but after 48 hours a pronounced inhibition maximum was found at 7300 Å and with a peak at 6975 Å (figure 51 D). Compare the shoulder at 7125 Å in figure 37 and the maximal inhibition at 7300 Å also for the germination (figure 37) and the occurrence of embryo-mitosis (figure 39 D). In spite of certain discrepancies it seemed evident that also the action spectra for the respiration were the same as for the germination and the occurrence of mitosis.

13. Effect of Irradiations with Red and Red + Far-Red Light on Anaerobic Respiration

The results hitherto presented have shown the influence of light on the aerobic respiration. The purpose of this experiment was to investigate whether a stimulated output of anaerobic carbon dioxide also could be found after a red irradiation and whether such a stimulation could be abolished by a far-red irradiation. In order to compare aerobic and anaerobic respiration also the aerobic output of carbon dioxide was studied.

The experiments were done in the same way as in a preceding one, in which the effects of red and red + far-red irradiations were studied (cf. p. 114 and table 20 A). The measurements were started after the seeds had been under germinating conditions for 24, 48 and 72 hours. For each time of measurement, type of irradiation and gas 2×50 seeds were used. During the adaptation period immediately preceding the start of the measurements the vessels used for studies on the anaerobic production of carbon dioxide were flushed with nitrogen (about 4 l). The nitrogen was obtained from a commercial steel container (AGA, maximal content of oxygen 0.10 vol. per cent). The results for anaerobic and aerobic carbon dioxide are given in figure 52 and 53, respectively. See also table 26.

Concerning the values for the output of aerobic carbon dioxide these were comparable to the results obtained in an earlier experiment (cf. table 20 A). From a comparison between the results in figures 52 and 53 it was

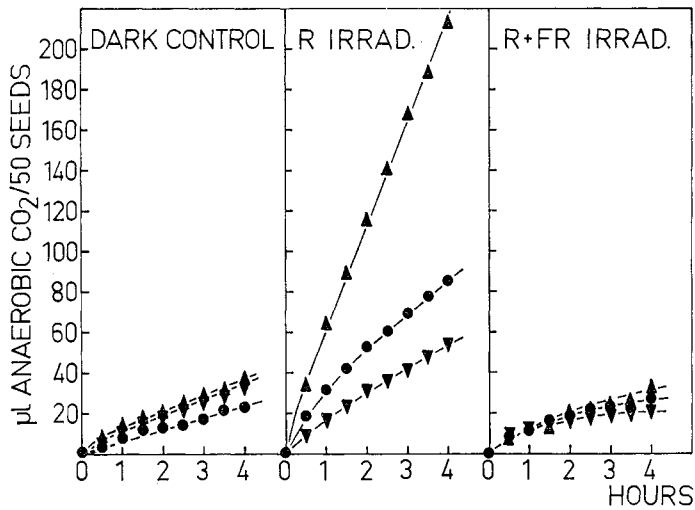

Figure 52. Carbon dioxide output of red and red + far-red irradiated Scots pine seeds under anaerobic conditions after 24 (inverted triangles), 48 (dots) and 72 (triangles) hours under germinating conditions. Dark control unirradiated. Standard irradiations (cf. figure 26). See also table 26. Provenance No. 4.

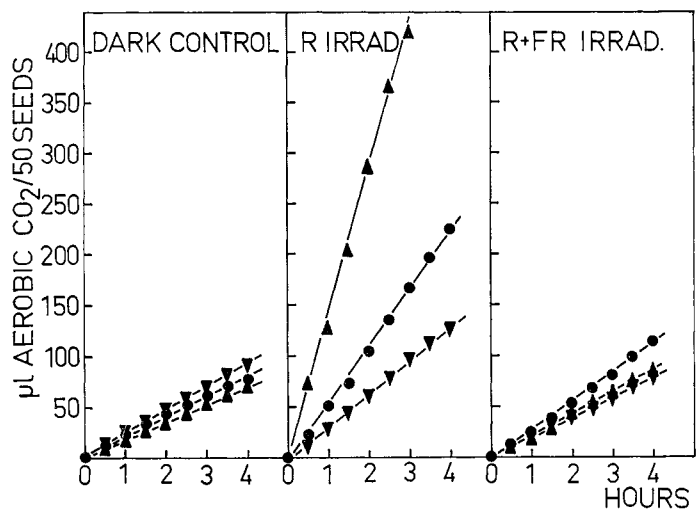

Figure 53. Carbon dioxide output of red and red + far-red irradiated Scots pine seeds under aerobic conditions after 24 (inverted triangles), 48 (dots) and 72 (triangles) hours under germinating conditions. Dark control unirradiated. Standard irradiations (cf. figure 26). See also table 26. Provenance No. 4.

evident that the output of anaerobic carbon dioxide was influenced by red and far-red irradiations in the same way as the aerobic output. Already after 24 hours there was a significantly higher output of anaerobic carbon dioxide from the red irradiated seeds than from the unirradiated and the

Table 26. Effect of red (R) and red + far-red (R + FR) irradiations on aerobic and anaerobic respiration in Scots pine seeds. Measurements started after the seeds had been under germinating conditions for 24, 48 and 72 hours, respectively. Standard irradiations (cf. figure 26). Provenance No. 4.

Irradiation	Hours under germinating conditions	Respiration rate (µl gas/50 seeds/1/2 hour)			Ratio $CO_2^{N_2}/CO_2^{air}$	RQ
		O_2^{air}	CO_2^{air}	$CO_2^{N_2}$		
Dark control	24	15.9 ± 0.3	10.6 ± 0.4	4.2 ± 0.5	0.40 ± 0.04	0.67 ± 0.02
	48	13.1 ± 0.5	9.6 ± 0.7	2.5 ± 0.4	0.26 ± 0.06	0.73 ± 0.04
	72	14.8 ± 0.5	8.7 ± 0.4	4.9 ± 0.6	0.56 ± 0.10	0.59 ± 0.03
R	24	28.0 ± 1.0	17.5 ± 1.1	6.7 ± 0.5	0.38 ± 0.05	0.63 ± 0.03
	48	43.1 ± 1.7	27.6 ± 2.2	12.0 ± 1.2	0.43 ± 0.06	0.62 ± 0.03
	72	98.4 ± 2.2	72.9 ± 3.0	26.8 ± 1.0	0.37 ± 0.04	0.75 ± 0.03
R+FR	24	16.3 ± 0.2	10.1 ± 0.3	2.6 ± 0.8	0.26 ± 0.11	0.62 ± 0.01
	48	18.3 ± 0.8	14.3 ± 0.9	4.0 ± 0.7	0.28 ± 0.07	0.78 ± 0.03
	72	19.6 ± 1.1	10.8 ± 0.7	4.5 ± 0.8	0.42 ± 0.11	0.59 ± 0.03

red+far-red irradiated ones ($0.001 > P$). The ratios between the anaerobic and the aerobic values are also given in table 26. In spite of some variation the mean value 0.37 ± 0.03 indicated a resemblance to the theoretical value 0.33 for a respiration without a Pasteur effect (see further p. 143). These results showed that the red—far-red reversible effect on the aerobic respiration was found also in the anaerobic respiration as this was reflected in the output of carbon dioxide in an atmosphere of nitrogen.

14. Action Spectrum—Comparison between Aerobic and Anaerobic Respiration

In earlier experiments (section 12 above) the action spectra for the stimulation and inhibition of the respiration were investigated. Only the oxygen uptake was studied because the reversibility experiments (table 25) did not show any effects on the RQ values. However, the preceding experiment, which showed a red—far-red effect also on the output of anaerobic carbon dioxide, made it pertinent to establish whether the action spectrum for the anaerobic respiration was the same as that for the aerobic one. In order to permit a direct comparison between them the action spectrum also for the aerobic output of carbon dioxide was studied.

Only the stimulation spectra were investigated here. The measurements were made on seeds which had been under germinating conditions for 48 hours. The seeds were placed in a similar plexiglass frame as used in earlier

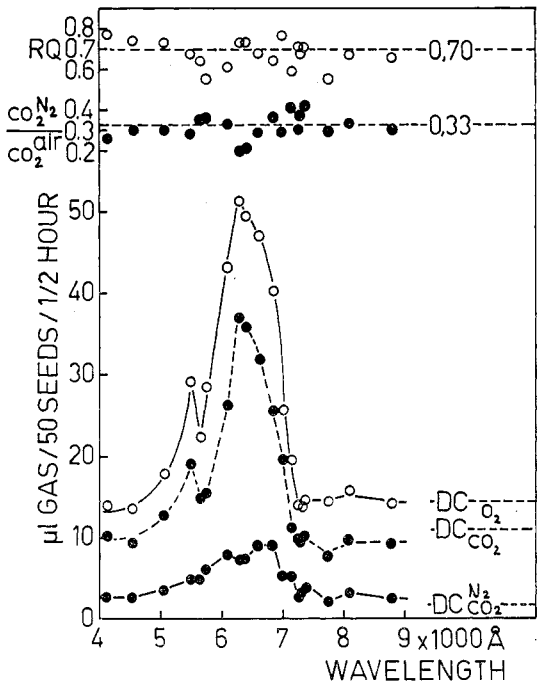

Figure 54. Action spectra for the stimulation of oxygen uptake and of aerobic and anaerobic carbon dioxide output in Scots pine seeds. Oxygen: unfilled circles. Aerobic carbon dioxide: filled circles with dashed line. Anaerobic carbon dioxide: filled circles. Irradiations as in figure 36. DC: dark controls. Measurements started after the seeds had been under germinating conditions for 48 hours. Provenance No. 4.

experiments but with six compartments, each one for 50 seeds. Thus 6×50 seeds were irradiated at the same time, 2×50 were used for the measurements of the oxygen uptake, 2×50 for the carbon dioxide output in air and 2×50 for the carbon dioxide in nitrogen. The irradiations were the same as in preceding experiments on action spectra. Flushing of the vessels was performed as described above. All measurements made during 4 hours with readings every half hour showed constant respiration rates except for the first readings in the anaerobic measurements (after 30 min.), which often were greater and were excluded from the calculations of the mean values. The results are given in figure 54.

The action spectrum for the oxygen uptake in principal features was the same as the one earlier found (cf. figure 51 B). The greatest stimulations were also obtained here in the region 6200—6600 Å but with a maximum at 6200 Å. However, the differences between the three values in this region were not significant. The same was valid for the corresponding values for the aerobic output of carbon dioxide. Thus with the method used here it was not possible to establish any wavelength with a maximal effect in this spectral region. See the results in figure 36 for the germination and in figure 39 B for the occurrence of embryo-mitosis, see also Borthwick *et al.* (1954,

figure 6). The peak at 5500 Å earlier observed (figures 36, 39, 51) was present but no peak or shoulder could be found at 7000—7100 Å. The action spectrum for the aerobic output of carbon dioxide showed consistent features, which also was reflected in the RQ values not being dependent of the wavelength. Their mean value was 0.67 ± 0.02, which was not significantly different from the average value 0.7 for seeds rich in fats (THOMAS 1960). These results confirmed the earlier findings that the light quality, which in a profound way modified the intensity of the respiration, had no effects on the RQ values.

The measurements on the anaerobic output of carbon dioxide also gave a corresponding action spectrum. The greatest stimulation even in this case was found in the red part of the spectrum with maximum values at 6600 and 6830 Å, which, however, did not exactly correspond to the results from the measurements of the aerobic carbon dioxide. See, however, the indications of shoulders at 5500 and 7125 Å together with the ratios between the anaerobic and aerobic carbon dioxide output, which in spite of some variation made clearer the similarities between the action spectra for the aerobic and anaerobic output of carbon dioxide. The mean value for this ratio (0.30 ± 0.01) was not significantly different from the theoretical value 0.33 for a respiratory system operating without Pasteur effect (cf. TURNER 1960, see also in the discussion p. 143). These results together with those from the preceding experiment (section 13) support the assumption that the anaerobic part of the respiration in the Scots pine seeds is regulated by light through the mediation of the same pigment as the aerobic respiration.

D. Discussion

In the studies on the effect of continuous white light the respiration rates were only determined at three different times during the early phase of the germination process: after 24 hours when the imbibition was complete, after 48 hours when the seeds were near the moment for the opening of the seed coats and after 72 hours when the protrusion of the rootlets had started (see also figure 38). The results showed that the light caused a stimulation of the respiration which was apparent already after 24 hours. This effect in the respiration before the start of the visible germination corresponded to the results received in the light-requiring seeds of *Nicotiana tabacum* L. by KIPP (1929) and SCHRÖPPEL (1933). These authors, who studied the time course of the respiration in detail, found that there was a maximum in darkness after 10—12 hours. After this time the respiration rate decreased but increased on an irradiation with white light. This light stimulation

appeared before the visible germination. A further increase of the respiration was found at that moment when the visible germination started.

Besides this similarity in the appearance of light-stimulated respiration in seeds of tobacco and Scots pine both of them showed a decrease of the respiration rate under prolonged stay in darkness (see table 19 A, darkness, and the extrapolated values in figure 45). Also for the light-requiring seeds of *Lactuca sativa* L. (var. Grand Rapids) EVENARI *et al.* (1958) showed a decrease of the respiration rate under corresponding conditions. This could not be correlated with any changes in the contents of fats or sugars.

However, the decrease of the respiration rate found in the Scots pine seeds in darkness was substituted by an increase, if the seeds were preirradiated with white light before the start of the imbibition (see table 19 AA). This corresponded to the stimulated germination in darkness, which could be produced by a similar preirradiation (cf. Chapter III).

Concerning the RQ values no certain differences could be found between the light and dark experiments possibly indicating that light caused a qualitative change of the respiratory metabolism. In the tobacco seeds KIPP (l.c.), however, found a small decrease of RQ in darkness.

In the experiments with red and far-red irradiations, it was shown that a limited irradiation with red light stimulated the respiration in the same way as a continuous irradiation with white light. This effect, which could be nullified by a following far-red irradiation, was valid both for oxygen and carbon dioxide (table 20) and was also repeatedly reversible (table 25). EVENARI *et al.* (1955) studied the respiration in seeds of *Lactuca sativa* L. (var. Grand Rapids), where they found a stimulation after a limited irradiation with red light. However, this stimulation was significant only until the protrusion of the rootlets in a comparison with far-red irradiated seeds or dark control seeds. No effects on the RQ values were found. After a dry storage a red irradiation stimulated only the output of carbon dioxide. Also the respiration rates in darkness and after a far-red irradiation were different. The respiration rate of red + far-red irradiated seeds was intermediate between that of red and of only far-red irradiated ones. From these results EVENARI *et al.* (l.c.) concluded that red light eliminated a respirational block which could be re-established by a far-red irradiation. Also HAGEN *et al.* (1954) have described respiratory responses in lettuce seeds after red and far-red irradiations, correlated with changes in the red—far-red sensitivity with respect to the germination. A correlation between a light effect on the germination and on the respiration has also been demonstrated by LEGGATT (1948). With blue light both the germination and the respiration were inhibited as a result of "carbon dioxide zymasis" induced by the blue light.

A corresponding relation between germination and respiration has been found in Scots pine seeds. Irradiations stimulating the germination stimulated the respiration, inhibiting the germination also inhibited the respiration (cf. the action spectra in figures 36, 37 and 51). In this connection it may be observed that LEOPOLD and GUERNSEY (1954) have described stimulatory effects of red light on the respiration in plants whose development was stimulated by red light, inhibitory effects where red light was inhibiting and reversal of the red effects by far-red irradiations. Lettuce seeds (var. Grand Rapids) were also included in this study and showed principally the same responses as found by EVENARI *et al.* (l.c.). However, concerning their magnitude and duration quite different results were obtained (see further p. 141).

In accordance with the stimulating effect of a puncturing or a decoating on the protrusion of the rootlets (the germination) and the occurrence of mitosis (figure 17 and 41, respectively) there was also a stimulation of the respiration after the same treatments (tables 19, 20, figures 43, 44). It was found that after a puncturing the respiration was stimulated to the same level in darkness as in intact seeds in continuous white light. A complete removal of the seed coats gave a higher respiration level. Also in combination with red—far-red irradiations the puncturing and decoating stimulated the respiration. The more the seed coats were removed, the less the relative light control of the respiration. However, a far-red inhibiting effect was still left even after the complete removal of the seed coats (table 20 C). This seems to indicate that the red—far-red reversible pigment system was not located in the seed coats but that the controlling effect of this pigment system on the respiration level was dependent on a cooperation with the intact seed coat. It may be observed that the respiration rate of the seeds in darkness after a complete removal of the coats was about 10 times higher than in the intact seeds (table 19, 48 hours). The corresponding higher respiration level of red irradiated, intact seeds was about 3 times higher than for the unirradiated intact ones (table 25, 48 hours). That only a decoating resulted in a higher respiration level than a red irradiation of intact lettuce seeds, var. Grand Rapids, was also shown by EVENARI *et al.* (l.c.). Interesting to notice is the fact that the decoated lettuce seeds of this variety are not light-requiring (EVENARI and NEUMANN 1952) and that the intact, light-requiring seeds of the lettuce sort Progress had the same respiration level as the decoated seeds of the variety Grand Rapids (EVENARI *et al.* l.c., LEVARI 1953).

A further demonstration of the relation between the far-red inhibited respiration and the seed coat is given in table 21 and figure 46. The inhibition

by far-red light was evident only as long as the seed coats were intact. Immediately after the decoating the respiration rate increased. The stimulation was of the same magnitude independent of the total period the seeds had been under germinating conditions. On the other hand, the seeds which were decoated from the start of the imbibition but irradiated in an identical way had a higher respiration level. This increased during the germination in the same way as for the seeds which were decoated immediately after the irradiation. This showed that the presence of an intact seed coat under the irradiation had no importance. Instead the presence of an intact seed coat *after* the irradiation was important for the inhibition of the respiration.

These results show a general resemblance between the conditions in lettuce and Scots pine seeds. However, the decreased capacity of the red—far-red reversible pigment system for controlling the respiration level and at the same time its incapacity for controlling the germination in decoated seeds can be compared with the statement made by BORTHWICK and HENDRICKS (1960) that this pigment system controls a "bottleneck" in the metabolism, which possibly is closely associated with reactions of acyl coenzyme A. In explaining the above results it seems more probable that either the pigment system does not control a "bottleneck" or that the seeds have alternative pathways in their metabolism dependent on the condition of their seed coats.

The above effects of the seed coats on the respiration level possibly could be explained under the assumption that the seed coats have too low a permeability for oxygen and carbon dioxide. That the presence of an intact seed coat can influence the respiration of germinating seeds has been reported for different species (SHULL 1914, HARRINGTON and HITE 1923, SIERP 1925, STÅLFELT 1926, FRIETINGER 1927, STILES and LEACH 1932, BROWN 1942, 1943, LARMOUR *et al.* 1944, KOZLOWSKI and GENTILE 1959, cf. also NYMAN 1957 and reviews by STILES 1960 and CARR 1961). In these papers a higher respiration rate has been found in decoated seeds than in intact ones. In some of the cases it has also been shown that the respiration increased in higher oxygen concentrations and that the increases were higher in intact than in decoated seeds (SHULL l.c., FRIETINGER l.c., STÅLFELT l.c., KOZLOWSKI and GENTILE l.c.). These results were interpreted so that the seed coat was a partial hindrance for the exchange of gases. The basis for such a conclusion was that there was a greater increase of the respiration rate in the intact than in the decoated seeds in an increased oxygen concentration. However, in similar experiments on Scots pine seeds (table 22, figure 47) the effects of higher oxygen concentrations were investigated only on intact seeds. The absolute values of the increased respiration under such conditions (in darkness) were small and

made it improbable that these increases should be significantly greater than in decoated seeds under the same conditions, *i.e.*, the results, which should be required for establishing the seed coats as a hindrance for the gas exchange. This indirect demonstration of the unlikelihood for the intact seed coats being a hindrance for the free permeation of oxygen and carbon dioxide was further supported by the inability of higher oxygen concentrations to increase the germination in darkness (figure 21). In cases where a higher oxygen concentration has been able to stimulate dormant seeds to germination this has been ascribed to too low a permeability of the intact seed coats for oxygen (CORRENS 1906, CROCKER 1906, BECKER 1912, SHULL 1911, 1914, THORNTON 1935; concerning light-sensitive seeds, cf. review by EVENARI 1956). Here it also can be observed that the oxygen effects obtained by SHULL (l.c.) and THORNTON (l.c.) on seeds of *Xanthium spp.* have been found to depend on a reduction of germination inhibitors (WAREING and FODA 1956, 1957). Compare similar results from studies on the light-sensitive seeds of *Betula spp.* (BLACK 1956, BLACK and WAREING 1959), which indicate a relationship between oxygen and the breakdown of inhibitors (cf. also the discussion to Chapter III, p. 49 and below p. 138).

From the discussion above it follows that the stimulating effect of irradiation with red light could not be explained under the assumption that the irradiation should increase the permeability of the seed coats. There was no significantly greater stimulation of the respiration with increased oxygen concentration (see table 22) in the unirradiated than in the red irradiated seeds. Here it may be pointed out that even at a decreased oxygen concentration (lower than in air) under a continuous irradiation with white light there was 60 per cent germination (figure 21, 10 vol. per cent oxygen, in light).

To explain the results from studies on the light-requiring achenes of *Chloris ciliata* SW. GASSNER (1911 a and b) developed the hypothesis that light stimulated the germination in this species by preventing the formation of a layer in the coats, which should restrict their gas permeability (cf. also PAECH 1953). These *Chloris*-achenes lost their light dependence after dehulling, which also has been described for decoated seeds of many other species (cf. reviews by EVENARI 1956 and in press). Also in other cases light effects on permeability have been described, see for example BRAUNER and BRAUNER (1940) and a review by STÅLFELT (1956). In spite of this it does not seem probable that the seed coat effect in the Scots pine seeds can be explained along this line. Neither the red—far-red effects on the respiration of decoated seeds (table 20 C), nor the nature of the mediating pigment system (phytochrome, cf. p. 52) support the idea that the light

effect should be localized in the seed coat (schlerenchymatic tissue, LAKON 1911, SCHNARF 1937).

The results in table 22 also showed a slight decrease of respiration rate in the red irradiated seeds in pure oxygen. The reason for this is not obvious but may indicate the presence of an oxygen poisoning, which, however, could not be found in the germination experiments (figure 21, 100 vol. per cent oxygen, in light). However, MACK (1930) has described that the output of carbon dioxide from wheat seedlings decreased in oxygen concentrations above 90 vol. per cent. In other investigations harmful effects of pure oxygen on the respiration have only been obtained at pressures above 1 atmosphere (see BEEVERS 1961 and review by STILES 1960 a) but inhibiting effects on growth have been found in pure oxygen without overpressure (cf. *e.g.* ELIASSON 1958 and a review by CARR 1961).

Finally it may be observed that the RQ values for the Scots pine seeds did not give any indications of the intact seed coats being a selective hindrance for either oxygen or carbon dioxide. See, however, BROWN (1940), who found a greater permeability for oxygen than for carbon dioxide in seed coats of *Cucurbita pepo* L.

Instead of the intact seed coats in Scots pine being a hindrance for the gas exchange a possible explanation of the seed coat effect may be that the opening or removal of the coats permit the escape of a volatile inhibitor (cf. p. 49), *i.e.*, in accordance with the explanation given by LATIES (1957) for the observed stimulation of respiration in potatoe tissue with increased surface to volume ratio of this tissue (see also STILES 1960 a). However, both decoating and irradiations as factors eliminating a respiratory block in the Scots pine seeds and simultaneously also a block for the germination do not necessarily indicate that both these factors influence the same mechanism.

This last conclusion may also be valid for the effect of stratification on the respiration of intact and unirradiated seeds (figure 48, table 23). In these seeds a stimulated respiration appeared in the same way as after a puncturing or removal of the seed coats (figure 43 B and C) or a red irradiation of the intact seeds (figure 44 A). Thus all these three types of treatments, which caused a stimulated germination (figures 9, 17 and *e.g.* 26), also caused a stimulated respiration. It may also be observed that the decreased importance of far-red light after puncturing or decoating for controlling the level of the respiration (table 20) and the germination (figure 31) can be compared with a corresponding inability in stratified seeds to control the germination (figure 32).

The effect of a stratification has been studied in many seeds with different

types of dormancy (cf. reviews by CROCKER 1948, CROCKER and BARTON 1953, BARTON 1961, VEGIS 1961). Besides studies on the germination *per se* also many investigations have been devoted to biochemical transformations during the stratification process (see *e.g.* BARTON l.c., VEGIS l.c.) from which the dormancy-breaking effect has been explained in different ways. Thus STONE (1957, 1958) postulated the formation of an intermediate compound "I" from the substrate in seeds of *Pinus jeffreyi* Murr. during the stratification. This compound would afterwards be directly utilized in the growth process. A relationship between stratification and respiration has also been demonstrated in different materials. In seeds of apple HARRINGTON (1923) and VISSER (1954, 1956) showed that the respiration increased after a stratification. The same was found by POLLOCK and OLNEY (1959) in seeds of *Prunus cerasus* L. and by POLLOCK (1960) in a cold treatment of buds of *Acer saccharinum* L. POLLOCK (l.c.) attributed the stimulatory effect of the stratification on the respiration to an increased coupling between energy-releasing and energy-utilizing processes as one of alternative explanations. Similar effects on the respiration have been described by STANLEY (1958) in stratified seeds of *Pinus lambertiana* Dougl. In the same species STANLEY (1957) and STANLEY and CONN (1957) showed that mitochondria isolated from endosperm and germinating seedlings could oxidize acids in the TCA cycle. However, this capacity decreased with longer time of germination as well as with longer time of stratification (STANLEY and CONN l.c.).

From the results of stratification in seeds of Scots pine discussed above it may be concluded that the similar effects of red irradiation, seed coat disturbance and stratification, which gave comparable results in respect to the investigated physiological processes, are not necessarily expressions of the same primary action of the three different treatments. It may be equally probable that they act primarily on different steps in the metabolism but produce the same effect on the respiration and on the germination. As soon as one of these factors is able to stimulate the respiration to a critical level or above the other two lose their importance for controlling the respiration level and also the germination.

The observations that a continuous irradiation with white light after 24 hours and a limited irradiation with red light after 17.5 hours induced a higher respiration rate (figure 43 and 44, respectively) were followed by experiments in which the development of this stimulation was studied. With white light (table 24) it was found that the stimulation developed slowly, probably depending on the experimental conditions. However, in a repeated experiment with a red irradiation (figure 49) a significant stimula-

tion was found 6 hours after the irradiation, and an increased respiration appeared already in the measurements, which were started immediately after the end of the irradiation. Comparable results were found by KIPP (1929) and SCHRÖPPEL (1933) in seeds of *Nicotiana tabacum* L. They observed an immediate change of the declining respiration rate in the dark to a constant and then gradually increasing tendency after the start of an irradiation with white light. With red light on seeds of *Lactuca sativa* L. EVENARI *et al.* (1955) could observe a significantly higher respiration already one hour after the irradiation. In the same material (var. Grand Rapids) LEOPOLD and GUERNSEY (1954) found the corresponding stimulation after half an hour to one hour. Concerning the rate with which the respiration was changed after a stimulating irradiation in these different materials, it seems as if the Scots pine seeds were somewhat more sluggish in their responses. A general agreement seems to exist in these cases, *viz.*, that a type of irradiation inducing a stimulation of the development (the germination) also caused a stimulated respiration rather soon after the start of the irradiation and that this stimulation in all these cases could be observed *before* the stimulated protrusion of the rootlets, *i.e.*, the germination in a more general sense.

In the preceding chapter it was shown (figure 42) that between the decreasing inhibitory capacity of far-red light and the occurrence of embryos without mitosis with increasing time after a red irradiation there was a mean difference in time of about 20 hours. This time difference indicated that processes preceding the appearance of mitosis eliminated the inhibitory capacity of the far-red irradiation. These results compared with the development of the respiration in seeds which were given an identical red irradiation (figure 49) showed that the decreasing capacity of far-red light to inhibit the following protrusion of the rootlets coincided with the development of the red stimulated respiration. In this time interval the determinative metabolic changes caused by the red—far-red reversible pigment system probably can be found. The same comparison (figures 42, 49) also showed that the significantly higher respiration rate, which was observed 6 hours after the red irradiation, *i.e.*, after the seeds had been under germinating conditions for 12.5 hours, appeared at a time when any evidence of mitotic activity could not yet be observed (figure 38). The red induced stimulation of the respiration preceded the appearance of the mitosis, which in its turn preceded the protrusion of the rootlets.

The appearance of these processes—induced by light in the same way—on the time scale indicated a causal connection between them. A later appearing phenomenon on the time scale can hardly be the cause of the appearance of an earlier one. For this reason it seems probable that the

stimulation of the germination evoked by red light in the Scots pine seeds is more primarily correlated with the respiratory metabolism than with the two growth processes, mitosis and cell lengthening. HABER and LUIPPOLD (1960 b) concluded from their studies on lettuce seeds (var. Grand Rapids) that the dormancy-breaking effect of the red—far-red system is located in the cell lengthening process. See also the red—far-red effect on the etiolation phenomenon as an effect on the cell lengthening earlier discussed (p. 108 and DOWNS *et al.* 1957 and DOWNS and CATHEY 1960).

A further comparison of interest is the magnitudes of the red stimulated respiration in the Scots pine seeds studied here and in the lettuce seeds used by EVENARI *et al.* (l.c.) and by LEOPOLD and GUERNSEY (l.c.). At that moment, when the first significantly higher respiration rate was observed in the Scots pine seeds, *i.e.*, after 6 hours from the end of the red irradiation, the stimulated oxygen uptake was 44 per cent (figure 49). In the lettuce seeds (EVENARI *et al.* l.c.) the mean stimulation during the period before the appearance of the rootlets was about 10 per cent. Contrary to this LEOPOLD and GUERNSEY (l.c.) obtained a stimulation of 660 per cent. From a series of compiled observations on the effect of visible light on the respiration in different types of plant material ROSENSTOCK and RIED (1960) calculated the mean value 29 per cent, irrespective of stimulation or inhibition. They also remarked that the results described by LEOPOLD and GUERNSEY (l.c.) were quite extraordinary.

Concerning the permanency of the red stimulation there are divergent findings. The stimulation found by LEOPOLD and GUERNSEY (l.c.) persisted only for one and a half hours after which the respiration rate became about the same as in the dark control seeds. The red effect shown by EVENARI *et al.* (l.c.) persisted until the start of the visible germination in spite of the fact that the difference between the germination in the red and the unirradiated seeds was about 60 per cent. Compare this with the results from the Scots pine, where there was a steadily increasing difference between the red and red + far-red irradiated seeds in spite of the fact that the rootlets had started to protrude also in the red + far-red irradiated seeds after 72 hours (table 20 A). Also in the experiments with white light (table 19 A) the same results were found. It can be remarked that an irradiation with white light for a limited period used by KIPP (l.c.) and SCHRÖPPEL (l.c.) caused a stimulation of the respiration rate, which persisted and was accelerated when the protrusion of the rootlets started.

Contrary to the statement made by HABER and LUIPPOLD (1960 b) that the breaking of dormancy in seeds is not generally related to gross metabolic alterations before the start of the growth in the embryos, the above discussed effects of red—far-red irradiations on seeds of Scots pine and lettuce showed

a regulating effect by light already in an early phase of the germination process. EVENARI *et al.* (l.c.) have pointed out that the red—far-red system in freshly harvested lettuce seeds regulates a respirational block. Evidence for this was also obtained in the Scots pine seeds through the occurrence of repeatedly reversible effects of red—far-red irradiations on the respiration (figure 50, table 25), *i.e.*, the corresponding results, which have been found for the occurrence of embryo-mitosis (tables 16, 17) and for the final germination (figure 26).

A further confirmation of the relationship between the effect of light on the respiration, on the occurrence of mitosis and on the directly visible germination was obtained from a study on the action spectrum of the respiration (figure 51) and a comparison of this with the corresponding spectra for the mitosis (figure 39) and the germination (figures 36, 37).

As earlier pointed out (p. 77 and 129) both the general features of the action spectra for stimulation and inhibition of the respiration as well as certain minor details agreed with the corresponding ones for mitosis and germination. See the results in figures 40 and 45, which also support their identity. Even after 24 hours (figure 51 A and C) action spectra with distinct features could be observed, a case which was not possible at the same time for the occurrence of mitosis (figure 39 A and C). This also supports the above discussed (p. 140) relation in time between the appearance of stimulated respiration and mitotic activity.

In spite of the fact that the action spectra neither showed a definitive maximal stimulation exactly at the wavelength 6600 Å nor a definitive maximum for inhibition exactly at 7350 Å (cf. BORTHWICK *et al.* 1954), the general features showed mutually a good resemblance and also agreement with action spectra for other photomorphogenic processes in different types of plants:

germination in seeds of *Lactuca sativa* L. (BORTHWICK *et al.* 1952 a, 1954), of *Lepidium virginicum* L. (TOOLE *et al.* 1955), of *Arabidopsis thaliana* (L.) Eastland (SHROPSHIRE *et al.* 1961), germination in spores of *Dryopteris filix-mas* (L.) Schott (MOHR 1956), induction of flowering in short- and long-day plants (PARKER *et al.* 1946, BORTHWICK *et al.* 1952 b, and BORTHWICK *et al.* 1948, PARKER *et al.* 1950, respectively), elongation of internodes (GOODWIN and OWENS 1948, BORTHWICK *et al.* 1951, PARKER *et al.* 1949), development of leaves (PARKER *et al.* 1949, DOWNS 1955, VIRGIN 1962), disappearance of the plumular hook in *Phaseolus vulgaris* L. (WITHROW *et al.* 1957), development of an orange colour in fruits of *Solanum lycopersicum* L. (PIRINGER and HEINZE 1954), formation of anthocyanin in seedlings of *Brassica oleracea* L. (SIEGELMAN and HENDRICKS 1957), of *Sorghum vulgare* Pers. (DOWNS and SIEGELMAN 1963) and in apple skin (SIEGELMAN and HENDRICKS 1958 a), chlorophyll formation in leaves of *Triticum sativum* L. (VIRGIN 1961), phosphate metabolism in seeds of *Lactuca sativa* L. (SURREY and GORDON 1962) and movements of chloroplasts in *Mougeotia sp.* (HAUPT 1959).

However, the relation between respiration, growth and light quality in the Scots pine seeds shown and discussed above was contrary to the corresponding results on seeds of *Cucurbita pepo* L. ROSENSTOCK (1951) has shown that the respiration of these seeds was stimulated by wavelengths longer than 7000 Å. Also at 4300 Å and at wavelengths shorter than 3000 Å stimulations appeared. A comparison of these results with the spectral sensitivity of the germination in the same species studied by MEISCHKE (1936) showed that the wavelengths, which stimulated the respiration, did not stimulate the germination and the reverse (ROSENSTOCK l.c.). But the light influence on the respiration described by MONTFORT and ROSENSTOCK (1950) and ROSENSTOCK (l.c.) in later papers (ROSENSTOCK 1955 a, b and c) could be explained from changes in the temperature associated with the irradiations. From a thorough discussion of these and similar results ROSENSTOCK and RIED (1960) also concluded that in no case has it been definitely proved that a direct photochemical action on the respiratory system is the cause of the effect of visible light on the respiration ("Lichtatmung"). Instead it seems more probable that light can influence the respiratory substrate or start developmental processes with stimulated respiration as a consequence. In such a case the effect may persist after the end of the irradiations, *i.e.*, in accordance with the results here found in the Scots pine seeds.

The studies on the red—far-red reversible effect and the action spectrum for the respiration in air were further extended to include a corresponding investigation concerning the anaerobic respiration. The results from the experiments on the red—far-red reversibility (figure 52, table 26) showed that the same reversibility could be found in the anaerobic as in the aerobic respiration (figure 53). A comparison of the stimulation spectra for the anaerobic and aerobic output of carbon dioxide (figure 54) showed correspondence, and therefore the controlling action of the red—far-red reversible pigment system evidently also includes the anaerobic phase of the respiration. In this connection it can be observed that SCHRÖPPEL (1933) found a stimulation of anaerobic carbon dioxide output with light in seeds of *Nicotiana tabacum* L.

In table 26 and figure 54 the ratios between the rate of anaerobic and aerobic output of carbon dioxide are given (I/N). In spite of certain variations the mean values from these two types of experiments showed a magnitude of these ratios not far from the theoretical value 0.33, which is valid for a respiration mechanism operating without a Pasteur effect (cf. THOMAS 1961). However, for a certain conclusion about the absence of such an effect BLACKMAN's extrapolation method must be used combined with chemical analysis on the appearance of anaerobic metabolites in the cases, where

the values of these ratios (less than unity) suggest the absence of this effect (see TURNER 1960, BEEVERS 1961, THOMAS 1961). Disregarding this, there was no correlation between the ratios anaerobic/aerobic carbon dioxide and the light quality indicating the occurrence of the same stimulation spectra for both these types of respiration. No studies were done on the inhibition spectrum. However, the existence of a red—far-red reversibility and the conformity between the stimulation spectra for the aerobic and the anaerobic respiration may suggest the occurrence of a corresponding inhibition spectrum also for the anaerobic respiration.

The occurrence of a higher output of carbon dioxide during the initial phase of anaerobiosis (cf. THOMAS 1961) also was observed in the Scots pine seeds (figure 52). In the calculations of the I/N values (cf. above) from these experiments in table 26 all measurements were included and probably caused some of the variations in these values. In the experiments, from which the action spectrum of the anaerobic respiration (figure 54) was combined, the corresponding observations were made. However, in the calculations in this case the first and higher values from each series of measurements were excluded. In consideration of the fact that the rates of the carbon dioxide output were constant during later measurements this calculation method is equivalent to the extrapolation method.

Besides the I/N values the RQ values did not show any evident dependence on the light quality. Compare the action spectra for the oxygen uptake and output of aerobic carbon dioxide.

In the discussion above (see p. 141) it was concluded that the light control of the germination was dependent on a more primary action of light on the respiration than on the growth processes in the embryos. That also the anaerobic respiration was regulated by light through the mediation of the same red—far-red reversible pigment system possibly can limit the problem to a more restricted part of the respiratory mechanism through which the light controls the respiration and the following development of the embryos.

The question is whether the action of light, *i.e.*, the action of the pigment system, can be considered to be more intimately connected with the anaerobic than with the aerobic phase of the respiration or the reverse or with both of them.

Against the assumption that the primary action is connected only with the aerobic phase is the fact that the light effect was quite evident even in the anaerobic experiments.

On the other hand, the assumption that the action of light is connected only with the anaerobic phase of the respiration corresponds well to the light effects on the anaerobic respiration. From this it also follows that the corresponding effects of light shall be found on the aerobic respiration,

because a factor affecting the anaerobic part always seems to be connected with a corresponding effect on the aerobic respiration (one of the general evidences for the common-path theory, cf. JAMES 1953). This is evidently in accordance with the obtained results. However, these results cannot exclude other types of explanations, *e.g.*, that light influences a process which is followed by a general increase of the respiration rate.

The germination is equivalent to a growth process and owing to this a process requiring energy (endergonic process). The chemical energy stored in fats and sugars in the endosperm is made available during the respiration for diverse endergonic reactions. The principal substance for this energy transfer is adenosine triphosphate (ATP) (cf. *e.g.* BEEVERS 1961). The start of the germination involves a coupling of the respiration to the growth of the embryos (TOOLE *et al.* 1956 a), which thus also involves the participation of the ATP-system. The control of diverse developmental processes, among which the germination is one, by the red—far-red reversible pigment system, can be supposed to be connected with an effect of the pigment on the turnover of this high-energy phosphate. In accordance with this GORDON and SURREY (1958, 1960) found a stimulating effect with red light and a reversible effect with far-red light on the oxidative phosphorylation in mitochondria from *Avena*-coleoptiles, but they also remarked (1960) that the red stimulation on the formation of ATP might be only a consequence of a stimulated oxidation of substrate. Working with coleoptiles and mesocotyles of *Avena* and seedlings of *Phaseolus vulgaris* L. SISLER and KLEIN (1960, 1961) could not find any effect of red—far-red irradiations on the formation of ATP but supposed that the light effect on the morphogenesis of these species could be connected with a direction of the ATP in a specific utilization process. In contradiction to these results SURREY (1961) has preliminarily reported and SURREY and GORDON (1962) have described the same red—far-red effects and action spectrum as for the germination on the phosphate metabolism in seeds of *Lactuca sativa* L. (var. Grand Rapids). However, these effects were correlated with the appearance of the visible germination, *i.e.*, the incipient growth of the embryos. In experiments with 2,4-dinitrophenol (DNP), known as an effective uncoupler of the oxidative phosphorylation (cf. *e.g.* LATIES 1957, BEEVERS l.c.), KLEIN (1956) (q.f. EVENARI 1957), HABER and TOLBERT (1959) and KLEIN (1961) have shown the existence of an oxidative phosphorylation in lettuce seeds (var. Grand Rapids). However, EVENARI (l.c.) has shown in germination experiments with the same material that the inhibition of the germination caused by DNP was independent of the light factor. Contrary to this HABER (1959) could make light-independent lettuce seeds (var. New York) dependent on light in the presence of DNP. These inconsistent results together with

the same light influence on both the aerobic and the anaerobic respiration in the Scots pine seeds seem difficult to explain under the assumption that the red—far-red pigment system should influence only the mechanism of the oxidative phosphorylation and as a result thereof controls the respiration rate (cf. LATIES l.c.).

On the other hand, the assumption that the red—far-red light should influence the respiration rate, not directly through an effect on the mechanism for the formation of high-energy phosphate but through a process which utilize such phosphates, *e.g.*, formation of protein in connection with the growth process (BEEVERS l.c.) the rate of turnover for the ATP system should change with an increased or decreased respiration rate as a consequence. Confer in this connection the relations between protein metabolism and irradiations described by LANDGRAF (1961).

The assumption discussed above that the red—far-red pigment system should either influence the oxidative phosphorylation or stimulate the utilization of ATP and as direct consequences of this affect the uptake of oxygen is not necessarily the only possible explanations for the red—far-red effects on the respiration rate. Thus GORDON and SURREY (1960) remarked that the red—far-red effects on the ATP content in *Avena*-coleoptiles could be only a consequence of a stimulated oxidation of substrate, which may indicate that the light action is concerned with enzymes engaged in substrate transformations during the respiration process. The results showing red—far-red effects on the activity of TPN triosephosphate dehydrogenase described by MARCUS (1959, 1960) seem to support such an idea. See also the different development in light and darkness of co-factors for the same enzyme described by HAGEMAN and ARNON (1955). TURNER (1960) points out the importance of this enzyme for the rate regulation of the glycolysis. These results thus should be well comparable with the red—far-red effects on the rate of the anaerobic respiration here described in the Scots pine seeds and in accordance with the common-path theory should also be able to explain the corresponding results on the aerobic respiration (cf. above p. 145).

A reasonable way for the operation of the red—far-red system supposed by BORTHWICK and HENDRICKS (1960) is deduced from the influence of this pigment system on the development of anthocyanins (SIEGELMAN and HENDRICKS 1957, 1958 a and b, MOHR 1957), indicating the influence of the pigment on a reaction closely associated with acyl coenzyme A compounds, which beside the formation of anthocyanins are also closely allied with fat transformations and the TCA cycle. Acetyl coenzyme A as a controlling substance for the respiration rate has been pointed out by KREBS (1957). A postulated red—far-red effect linked to this substance thus might be correlated with the observed light effects on the respiration rate.

However, in this connection it can be observed that HABER and TOLBERT (1959) with lettuce seeds (var. Grand Rapids) could not find any difference in the fixation of C^{14} from $NaHC^{14}O_3$ in seeds irradiated with continuous white or far-red light. This indicated that the operation of the TCA cycle was independent of the red—far-red system and further rejected the hypothesis that the light action should be connected with the formation of the high-energy phosphates linked to the operation of the TCA cycle.

The different possibilities for explaining the light responses of the respiration discussed above may at least be summarized in a negative conclusion, *viz.*, that the action of the red—far-red system evidently does not act through an effect on the mechanism for the oxidative phosphorylation. The evidence for this conclusion is the following: the red—far-red effects on the anaerobic respiration here described for the Scots pine seeds, the divergent results in direct experiments on the oxidative phosphorylation and the absence of light effect on the operation of the TCA cycle.

Concerning other possibilities for the seat of action of the red—far-red pigment system the similarities between the red—far-red effects on the activity of the TPN triosephosphate dehydrogenase (MARCUS l.c.) and the corresponding results on the anaerobic respiration of the Scots pine seeds seem to constitute an interesting point for further research. Mark also in this connection the presence of sucrose as a requisite for the red—far-red control of the growth of a fern gametophyte (MILLER and MILLER 1961) and of segments from etiolated pea stems (BERTSCH and HILLMAN 1961).

That white light should influence the activities of different enzymes in seeds and in that way control the germination has been pointed out in many cases. For example, TIETZ (1953) has described a light-stimulated lipase activity in seeds of *Oenothera biennis* L., the germination of which is stimulated by light and a light-inhibited lipase activity in seeds of *Nigella damascena* L., the germination of which is inhibited by light. Compare also reviews by EVENARI (1956 and in press). Beside this light effects have been described on diverse types of enzymes (cf. reviews by WEINTRAUB 1944 and ROSENSTOCK and RIED 1960). Studies on such enzymes combined with red—far-red irradiations may possibly lead to a more thorough understanding of the actual seat of action for the red—far-red reversible pigment system. However, at the same time a satisfactory explanation on this point ought to be able to clarify why this pigment system can be without importance in light-sensitive seeds after decoating. This is probably connected with the existence of alternative pathways in the metabolism of seeds (see reviews by EVENARI 1961 and in press).

Chapter VII. Summary

From studies on the germination in continuous white light and in darkness it was found that the light dependence was not correlated with the origin of the seeds. An unchanged light dependence was sustained for about three years when the seeds were stored in darkness (+ 3—4° C). Storage of the imbibed seeds under the same conditions (stratification), however, for less than one month eliminated the light dependence in most of the seeds. Instead of a continuous irradiation during all the germination process a complete germination could be induced by a limited irradiation given to the seeds after an optimal period of imbibition (6—12 hours). Even the unimbibed seeds were sensitive to an irradiation. This could also be demonstrated in seeds of different provenances. But there were not any relationships between the germination responses and the origin of the seeds or the actual water content of the seeds. A comparison between the responses in a laboratory and a field experiment showed that the differences between the responses of the irradiated and the control seeds were smaller under field conditions depending on an increased emergence of the unirradiated seeds than in the laboratory tests. The stimulated germination after an irradiation of the unimbibed seeds could even be detected after a storage of the seeds. In spite of a decreased effect with increasing period of storage there was a significant effect after 17 months. Attempts to correlate the occurrence of the light dependence with the morphological ripeness of the seeds were made with an X-ray technique, but they could not reveal any connection between the development of the embryos and the light effects on the germination. Instead the presence of an intact seed coat was determinative for the occurrence of the light-controlled germination. The inhibited germination of intact seeds in darkness could not be explained from the assumption that the intact and unirradiated seed coats should constitute an obstacle for the imbibition process or the exchange of oxygen and carbon dioxide (see below). Attempts to demonstrate the occurrence of a water-soluble inhibitor connected with the light effects were unsuccessful. It has been proposed that the seed coat effect may possibly be correlated with the escape of a volatile inhibitor.

Studies on the effects of irradiations with coloured light have shown that the wavelengths most effective for promotion of the germination were to be found in red (around 6600 Å) and that the red promotion could be reversed by light of longer wavelengths with maximal effects in far-red (around 7300 Å). There was a repeatedly reversible red—far-red mechanism controlling the germination, *i.e.*, indicating the existence of phytochrome

as the regulating pigment. This was also supported by the magnitudes of the required energies in red and far-red and the absence of temperature dependence for the effects of the red and far-red irradiations. Further details as the far-red sensitivity independent of the magnitude of the red induction and the absence of an oxygen effect at the red irradiations were also in conformity with the present knowledge of the nature of phytochrome. However, it was found that a red irradiation of unimbibed seeds could induce germination and that this induction could not be reversed by a far-red irradiation, that there were no evidences for a dark reversal of the far-red absorbing form of the pigment and that the increased sensitivity to a red irradiation with increasing imbibition appeared at the same time as the sensitivity to a far-red irradiation increased. With an intervening dark period of 48 hours between a red and a far-red irradiation at $+25°$ C the reversibility was completely lost. With a lower temperature during this dark phase the loss of reversibility was retarded. The increased ability of the seeds to germinate in darkness after a stratification or removal of the seed coats was connected with a corresponding inability of far-red irradiations to inhibit the germination.

Studies on the occurrence of mitotic activity in the seed embryos have shown that such an activity appeared before the lengthening process and was regulated by light in the same way as the visible germination (protrusion of the rootlets). The inhibited germination in darkness or after irradiation with inhibiting light qualities was reflected in a block of the mitotic activity. A comparison between the loss of far-red inhibition with increased time after a red irradiation (for the visible germination, see above) and the appearance of mitotic activity showed that the two phenomena did not coincide, *i.e.*, the loss of reversibility preceded the appearance of mitosis. This suggested a more primary effect of light on the metabolism of the seeds, through which subsequent growth of the embryos (mitosis and cell lengthening) was manifested as a directly visible germination.

The respiration as an expression of the metabolism was studied in relation to the quality of the irradiations. Both the occurrence of a repeated red—far-red reversibility and the action spectra showed conformity with the corresponding ones for the mitosis and the visible germination. All these three processes were correlated phenomena and different displays of the same primary physiological effect of the irradiations. However, the appearance of these three light-regulated processes on the time scale suggests a causal connection between them. A red induced stimulation of the respiration before the appearance of mitotic activity showed that the irradiation had a more primary effect on the respiration than on the other processes. A comparison between the red—far-red effects and the action spectra for

the aerobic and the anaerobic respiration showed conformity. These results have been discussed in relation to the probable seat in the respiratory mechanism, where the red—far-red reversible pigment system (phytochrome) controls the respiration and the following visible germination of Scots pine seeds.

Concomitant with the light effects on the respiration also the seed coat effect has been studied under the assumption of the seed coat as an obstacle for the gas exchange during the respiration. However, the results could not be evaluated from this point of view. The occurrence of both light and seed coat as controlling factors in the germination of Scots pine seeds could not be integrated into a common picture. In spite of the same responses of red irradiations and decoating on the respiration and the subsequent growth of the embryos, this is probably not caused by a common action mechanism but can be compared with the existence of alternative metabolic pathways in seeds.

Acknowledgements

To my teachers, Professors Gottfrid Stålfelt and Torsten Hemberg, former and present head of the Institute of Physiological Botany, the University of Stockholm, I wish to express my sincere thanks for valuable discussions, impulses and encouragement. I am also very much indebted to my chief, Professor Erik Björkman, head of the Department of Forest Botany, the Royal College of Forestry, Stockholm, for his kindness in placing the facilities of his department at my disposal and for his help in other respects, as well as to Professor Lennart Nordström, head of the Department of Silviculture, the Royal College of Forestry, Stockholm, for his initiative in the performance of this investigation and for help and advice. For many valuable discussions, advice and criticism I owe a great debt of gratitude to Professor Hemming Virgin, head of the Department of Plant Physiology, the University of Göteborg, to Docent Auseklis Vegis, the Institute of Physiological Botany, the University of Uppsala, and to Docent Göran Stenlid, the Institute of Plant Physiology, the Royal Agricultural College, Uppsala. I am also very much indebted to my colleagues in the Department of Forest Botany for valuable discussions, to Docent Einar Huss, the Department of Reforestation, the Royal College of Forestry, Stockholm, for placing seed materials at my disposal, to Professor Åke Gustafsson and Dr Milan Simak for the possibility of using the X-ray equipment at the Department of Forest Genetics, the Royal College of Forestry, Stockholm, to Mr Per Herou, M. Forestry, for cooperation in the performance of the provenance experiments and to Mrs Kerstin Lindström and Miss Anne-Marie Persson for skilful technical assistance. The English text has been revised by Mrs Gail Åkerman and Mr Carl Follin, B. Hort., to whom I also wish to express my sincere thanks. The investigation has been supported by a grant from the Swedish Forest Research Foundation.

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Sammanfattning

Studier över frögroningen hos tall med särskild hänsyn till ljusfaktorn

Groningsexperiment i kontinuerligt vitt ljus och mörker visade, att den ljusstimulerade groningen hos frön av tall (*Pinus silvestris* L.) inte hade något samband med fröets ursprung. Ett oförändrat ljusberoende kvarstod under ca tre år, om fröna lagrades i mörker (+ 3—4° C). Lagring av de svällda fröna under samma förhållanden (stratifiering) avlägsnade emellertid efter mindre än en månad ljusberoendet hos de flesta fröna. I stället för en kontinuerlig belysning under hela groningsprocessen kunde en fullständig groning induceras genom en begränsad belysning av fröna efter en optimal svällningsperiod (6—12 timmar). Även de osvällda fröna var känsliga för belysning. Detta visades också på frön av olika härkomst, men det förelåg inget samband mellan groningssvaren och fröets ursprung eller vattenhalten i fröna vid belysningen. En jämförelse mellan resultaten i ett laboratorie- och ett fältförsök visade, att skillnaderna mellan groningssvaren hos de belysta fröna och kontrollerna var mindre under fältförhållandena beroende på en ökad uppkomst av de obelysta fröna i fältförsöket. Den stimulerade groningen efter en belysning av de osvällda fröna återfanns även efter lagring så att trots en avtagande effekt med ökad lagringstid en signifikativ effekt ännu fanns efter 17 månader. Försök att sammanställa ljusberoendet med frönas morfologiska mognadsgrad gjordes med hjälp av röntgenfotografering men kunde inte visa något samband mellan embryoutvecklingen och ljuseffekterna på groningen. Däremot var närvaron av ett intakt fröskal av betydelse för ljuset som groningskontrollerande faktor. Den hämmade groningen hos intakta frön i mörker kunde inte förklaras med antagandet att fröskalet skulle utgöra ett hinder för svällningsprocessen eller för syrgas- respektive koldioxidpermeabiliteten (se nedan). Försök att sammanställa ljusberoendet med förekomsten av någon vattenlöslig groningshämmare i fröna gav även negativt resultat. Det har antagits, att fröskalseffekten kan stå i samband med avgivandet av en flyktig groningshämmare.

Belysningsförsök med färgat ljus visade, att det röda våglängdsområdet (omkring 6 600 Å) gav den största stimulationen av groningen och att en sådan kunde hävas med ljus av längre våglängd med maximal effekt i infrarött (mörkrött, omkring 7 300 Å). Både dessa aktionsspektra och de upprepbart reversibla röd-infrarödeffekterna tyder på förekomsten av fytokrom som det reglerande pigmentet. Också storleksordningarna av de erforderliga ljusenergierna i rött och infrarött, belysningseffekternas temperaturoberoende, infrarödkänslighetens oberoende av storleksordningen hos den groningsinducerande rödbelysningen samt frånvaron av en syrgaseffekt vid rödbelysningen var i överensstämmelse härmed. Emellertid befanns det, att den groningsinduktion, som erhölls genom rödbelysning av osvällda frön, inte kunde hävas genom en följande infrarödbelysning. Ej heller erhölls några resultat, som tydde på förekomsten av en mörkeromvandling av den infrarödabsorberande formen av pigmentet. Vidare förekom en samtidig ökning av både röd- och infrarödkänsligheten med begynnande svällning. Med en mörkerperiod av 48 timmar mellan en röd- och en infrarödbelysning vid + 25° C gick hela reversibiliteten förlorad, men med en lägre temperatur under denna mörkerfas blev minskningen mindre. Den ökade förmågan hos fröna att gro i mörker efter en stratifiering eller ett avlägsnande

av fröskalet stod i samband med en motsvarande oförmåga hos en infrarödbelysning att hämma groningen.

Studier på förekomsten av mitosaktivitet i fröembryona visade, att en sådan aktivitet uppträdde före sträckningsprocessen och reglerades av ljus på samma sätt som den synliga groningen (utskjutandet av groddarna). Den hämmade groningen i mörker eller efter en infrarödbelysning återspeglades i en blockering av mitosaktiviteten. En jämförelse mellan förlusten av infrarödhämningen med ökad tid efter en rödbelysning och uppträdandet av mitosaktivitet visade, att de två fenomenen inte sammanföll, d. v. s. förlusten av reversibiliteten föregick uppträdandet av mitoser. Detta antydde en mer primär effekt av ljuset på frönas metabolism.

Andningen som ett uttryck för metabolismen studerades i förhållande till belysningskvaliteten. Både förekomsten av en upprepad röd-infrarödreversibilitet och aktionsspektra för andningen visade överensstämmelse med motsvarande för mitoserna och den synliga groningen. Dessa tre processer var korrelerade fenomen och olika uttryck för samma primära fysiologiska effekt av belysningarna. Uppträdandet på tidsskalan tyder emellertid på ett orsakssamband mellan dem. En rödinducerad stimulation av andningen före uppträdandet av mitosaktiviteten visade, att belysningen hade en mer primär effekt på andningen än på de andra processerna. Både röd-infrarödeffekterna och aktionsspektra för den aeroba och den anaeroba andningen överensstämde. Dessa resultat har diskuterats i relation till den troliga plats i andningsmekanismen, där det röd-infrarödreversibla pigmentsystemet (fytokromet) skulle kontrollera andningen och därigenom den följande, direkt synliga groningen hos fröna.

Samtidigt med ljuseffekterna på andningen studerades fröskalseffekten under antagandet att fröskalet skulle utgöra ett hinder för frönas gasutbyte vid andningen. Emellertid kunde inte de erhållna resultaten tolkas från denna utgångspunkt. Förekomsten av både ljus och fröskal som kontrollerande faktorer vid tallfrönas groning kunde inte sammanföras till en gemensam bild. Trots att samma svar erhölls efter rödbelysning och skalning i såväl andningen som den efterföljande tillväxten av embryona orsakas detta troligen inte av en gemensam verkningsmekanism utan kan jämföras med förekomsten av alternativa metaboliska processer i frön.