RESPONSES OF SEEDS OF PINUS VIRGINIANA TO LIGHT [1]

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The responses to light of lettuce and Lepidium seeds depend on the reversible red, far-red photoreaction (3,5). These light-sensitive seeds are rather small in comparison with seeds of several species of southern pines reported by Nelson (4) to be stimulated to germinate by light. The current experiments were conducted to determine whether seeds of *Pinus virginiana* respond to the reversible red, far-red photoreaction in the same way as lettuce and Lepidium seeds, and how the responses are modified by temperature and condition of imbibition.

MATERIAL & GENERAL METHODS

Four samples of seeds of *Pinus virginiana* Mill. were obtained from the Beltsville Experimental Forest. Seeds of sample LP-13, collected in the fall of 1955, were held at 1° C until March 1956, when they were received at Plant Industry Station and placed in a sealed can at −18°. Seeds from this sample were used in experiments during April to August 1956. Seeds of sample LP-62, collected in October 1957, were kept continuously at 1°, and those of sample LP-63, collected in October 1958, were kept at room temperature. Seeds from the last two samples were used in experiments during November 1958 to January 1959. Seeds of sample LP-64, collected in October 1958, were placed in a sealed can at −18° in November 1958. Seeds from this sample were used in experiments during April 1959.

Seeds were planted by means of a suction counter on wetted germination blotters in plastic boxes (12 cm sq), 100 seeds to each box. Immediately each box was placed between folds of black cloth on a tray at a controlled temperature. After a definite number of hours in darkness the imbibed seeds were taken to the exposure room, irradiated, again wrapped in black cloth, and returned to a controlled temperature.

The boxes containing seeds were removed from the darkness only for irradiations of definite durations. Red light was obtained by filtering the light from a bank of 2 or 18 96-inch T8 slimline cool-white fluorescent tubes through two layers of red cellophane. Far-red irradiation was obtained by filtering the light from three 300-watt internal-reflector incandescent-filament lamps through two layers of red and of blue cellophane and about 6 cm of water. A specified exposure to either light source was made at a distance of 1.1 m. At the level of the irradiated seeds the intensity in the region 5,800 to 6,950 A was 700 and 6,000 ergs/cm²/sec for 2 and 18 tubes, respectively, and in the region 6,950 to 7,900 A was 7,500 ergs/cm²/sec.

After irradiation the seeds were returned to a controlled temperature for 7 to 21 days before the germinated ones were counted. Results are given as percent germination of the sound seeds (total seeds minus empty & dead seeds); per cent germination of the sound, light-requiring seeds; or per cent inhibition of red-light-promoted seeds. Per cent germination of sound seeds expresses the results in terms of the seeds that germinate divided by the total sound seeds. Per cent germination of sound, light-requiring seeds expresses the results in terms of the sound seeds that do not germinate in the dark:

$$\frac{\text{Total germinated seeds} - \text{dark-germinated seeds}}{\text{Total sound seeds} - \text{dark-germinated seeds}} \times 100$$

Per cent inhibition refers to the red-light-promoted seeds that do not germinate after exposure to far-red:

$$\frac{\text{Total seeds germinated after red saturation} - \text{seeds germinated after far-red}}{\text{Total seeds germinated after red saturation}} \times 100$$

Transverse sections of 200 seeds from each seed lot indicated the proportions of empty, discolored, and sound seeds.

RESULTS

OPTIMUM TEMPERATURE: The optimum temperature for conducting the tests was determined. Four boxes of seeds from sample LP-13 were used for each of the nine temperature conditions shown in table I. After approximately 28 hours in darkness, two boxes of imbibed seeds from each temperature were exposed 60 minutes to red (6,000 ergs/cm²/sec)

[1] Received October 12, 1960.

TABLE I

EFFECT OF TEMPERATURE WITH* & WITHOUT LIGHT ON GERMINATION OF PINUS VIRGINIANA SEEDS (LP-13)

TEMPERATURE ° C DURING:		% GERMINATION OF SOUND SEEDS AFTER:	
		IRRADIATION WITH RED (6,000 ergs/cm²/sec)	
28-hr IMBIBITION	GERMINATION	0 min	60 min
10	10	0	0
15	15	0	0
20	20	0	8
25	25	1	62
30	30	2	8
5	25	< 1	92
10	30	2	39
20	30	< 1	20
30	20	0	18

* Irradiation after 28-hour imbib.tion.

radiation and the other two were maintained as dark controls. Seeds germinated at 10° or 15° C were counted after 21 days and those at other temperatures after 10 days. Less than 5 % of the seeds germinated in darkness at any temperature tested. Seeds exposed 60 minutes to red light germinated very well at certain temperatures, but not at others. Seeds germinated best (92 %) when imbibed at 5° and germinated at 25°.

RED, FAR-RED ENERGIES: Germination responses at 25° C of *Pinus virginiana* seeds (LP-13) held 22 hours in darkness at 5° to increasing irradiations with red (700 ergs/cm²/sec) and far-red (7,500 ergs/cm²/sec) were determined. The germinated seeds were counted 7 days after irradiation. The percentage promotion (table II) ranged from 2 % for a half minute to a maximum of 82 % for 32 minutes. After irradiation of the seeds by red for 64 minutes, 16 minutes of far-red inhibited germination of almost all the promoted seeds. Far-red for 1 minute did not inhibit germination. Measurements of the incident energies used and calculations, according to Hendricks et al (2), of the percentage pigment conversion for a given germination (fig 1) indicated that approximately 1×10^6 ergs/cm² was required for 50 % conversion of the pigment in either direction.

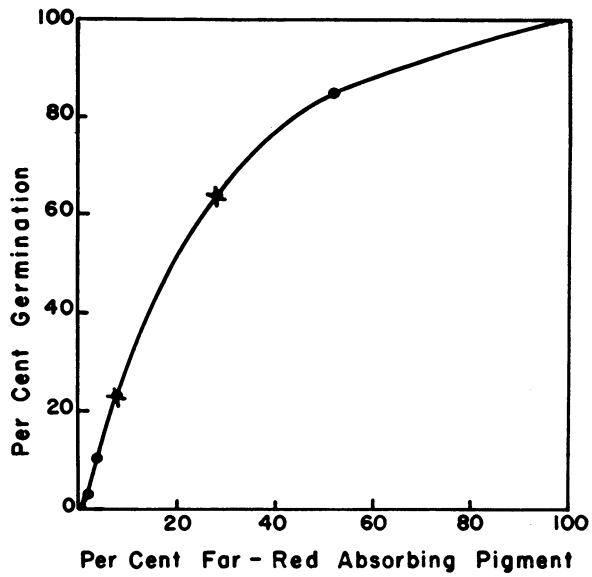

FIG. 1. Variation in the percentage germination of *Pinus virginiana* seeds (sample LP-13) with the amount of the controlling pigment in the far-red absorbing form. X indicates the two points of the experimental data chosen for calculation of the curve.

TABLE II

EFFECT OF INCREASING IRRADIATIONS OF RED (700 ergs/cm²/sec) & FAR-RED (7,500 ergs/cm²/sec) FOLLOWING A 22-HOUR IMBIBITION IN DARKNESS AT 5° C ON GERMINATION AT 25° OF PINUS VIRGINIANA SEEDS (SAMPLE LP-13)

MIN OF IRRADIATION WITH RED OR FAR-RED	% GERMINATION OF SOUND LIGHT-REQUIRING SEEDS AFTER PROMOTION BY RED*	% INHIBITION BY FAR-RED OF SEEDS PROMOTED BY 64 MIN OF RED
½	2	...
1	8	0
2	21	3
4	31	34
8	51	77
16	68	95
32	82	96
64	82	98

* Seeds germinated 1 % in total darkness.

TABLE III

EFFECT OF DURATION & TEMPERATURE OF IMBIBITION ON GERMINATION RESPONSES AT 25° C OF PINUS VIRGINIANA SEEDS (LP–64) TO INCREASING IRRADIATIONS OF RED (700 ergs/cm²/sec) & FAR-RED (7,500 ergs/cm²/sec)

MIN OF IRRADIATION WITH RED OR FAR-RED	% GERMINATION OF SOUND SEEDS AFTER IRRADIATION WITH RED WHEN IMBIBED AT:				% INHIBITION BY FAR-RED OF SEEDS PROMOTED BY 64 MIN OF RED WHEN IMBIBED AT:			
	25° C		5° C		25° C		5° C	
	1 day	16 days	1 day	16 days	1 day	16 days	1 day	16 days
¼	2	0	4	21				
½	> 1	2	3	29				
1	6	3	12	34	14	6	12	2
2	11	2	24	65	39	25	25	4
4	23	11	40	74	84	59	51	18
8	26	22	61	80	95	83	83	28
16	55	49	65	89	95	93	95	79
64	67	66	81	90	97	94	94	89

IMBIBITION TEMPERATURE: The temperature during imbibition controlled the total number of seeds that germinated in response to a saturating light stimulus. Seeds imbibed in darkness at 5° C or 25° and held at these temperatures for 1 or 16 days were given increasing irradiations with red (700 ergs/cm²/sec) and far-red (7,500 ergs/cm²/sec) and then placed in darkness at 25°. After 7 days the germinated seeds were counted (table III). A 64-minute exposure promoted 80 to 90 % of the seeds imbibed at 5° but about 65 % of those imbibed at 25°. Seeds imbibed at 25° were very easy to inhibit. Seeds held for the longer period at 5° were easier to promote and more difficult to inhibit than those held for only one day at the lower temperature.

IMBIBITION PERIOD: Lengthening the imbibition period in darkness at 5° C from 1 to 16 days increased sensitivity of seeds to red light. Seeds held at 5° for 1, 2, 4, 8, and 16 days were given increasing irradiations with red (6,000 ergs/cm²/sec) and far-red (7,500 ergs/cm²/sec) and then placed in darkness at 25°. Germination in total darkness was only slightly higher with the longer periods of imbibition for seeds of sample LP-62, but was markedly higher for those of sample LP-63 (table IV). Thus the population of seeds in the latter sample on which one could test light effects was small for the longest period of imbibition (table V).

The energy of red light required for a given germination decreased as the period at 5° C increased (table V). As the seeds changed during dark imbibition so that they required less red light for promotion, they also changed so that they required more far-red radiant energy for inhibition (tables V & VI).

RED, FAR-RED REVERSIBILITY: The action of the red and the far-red radiation on the germination of pine seeds was immediately and repeatedly reversible (table VII). Twelve lots of 100 seeds were planted and held 96 hours in darkness at 5° C. Two lots were kept as dark controls and the remaining ones were exposed to red (6,000 ergs/cm²/sec) for 8 minutes. Two of these lots were immediately put under black cloth and the remaining eight were exposed 16 minutes to far-red (7,500 ergs/cm²/sec) and two of these were placed under black cloth. Alternating treatments with red and far-red, with removal of two lots to darkness after each operation, were continued until all lots were used. The last two lots thus received three red treatments alternated with two far-red treatments. The germinated seeds were counted 7 days after irradiation. When the exposure to red was last, approximately 90 % of the seeds germinated. When far-red was last the promoted seeds and even some of the dark germinators failed to germinate. Almost identical results were obtained on two samples.

RATE OF GERMINATION PROCESSES: When light-requiring seeds are exposed to red light the pigment is converted to the far-red-absorbing form, the active form of the pigment. With the pigment in this form the seeds progress towards germination. The ability to prevent the promoted seeds from germinating by far-red radiant energy depends on the interval of time

TABLE IV

EFFECT OF PERIOD OF IMBIBITION AT 5° C ON SUBSEQUENT GERMINATION OF PINUS VIRGINIANA SEEDS IN TOTAL DARKNESS AT 25°

SAMPLE NO.	% GERMINATION IN DARKNESS OF SOUND SEEDS AFTER INDICATED PERIODS OF IMBIBITION				
	1 day	2 days	4 days	8 days	16 days
LP–62	4	11	12	7	16
LP–63	4	9	27	42	66

TABLE V

EFFECT OF INCREASING IRRADIATIONS OF RED AFTER VARIOUS PERIODS OF IMBIBITION AT 5° C ON GERMINATION AT 25° OF PINUS VIRGINIANA SEEDS

SAMPLE NO. & IMBIBITION PERIOD	% SOUND SEEDS REQUIRING LIGHT FOR GERMINATION	% LIGHT-REQUIRING SEEDS GERMINATING AFTER IRRADIATION (6,000 ergs/cm²/sec) FOR:					
		1 min	2 min	4 min	8 min	16 min	32 min
LP-62:							
1 day	96	46	60	79	82	78	77
2 days	89	54	62	73	83	73	86
4 days	88	67	84	89	90	92	91
8 days	93	75	77	83	96	91	95
16 days	84	83	81	86	89	89	90
LP-63:							
1 day	96	43	44	57	69	70	67
2 days	91	60	69	68	88	82	89
4 days	73	81	83	91	94	93	94
8 days	58	87	87	94	91	96	95
16 days	34	86	88	98	88	91	92

between the two irradiations. The period that the pigment must be kept in the far-red-absorbing form before the processes leading to germination can no longer be stopped by far-red was measured. Seeds of *Pinus virginiana* held in darkness at 5° C for 1 or 20 days were exposed 16 minutes to red (6,000 ergs/cm²/sec). These promoted seeds were held in darkness at 25° for 0, 8, 16, 24, 48, 72, 96, and 120 hours and then given 16 minutes of far-red (7,500 ergs/cm²/sec). The seeds were returned to darkness after the far-red treatments and the germinated seeds counted after 9 days. Inhibition of 50 % of the promoted seeds in lots imbibed 1 day resulted from far-red treatments given 48 to 72 hours after the red but for lots imbibed 20 days the corresponding periods were 8 to 16 hours (table VIII).

DISCUSSION

The germination response of *Pinus virginiana* seeds to light is basically the same as that of other seed kinds (3, 5). The photoreaction is controlled by the red, far-red pigment system. It is immediately and repeatedly reversible. And, like other seeds, *Pinus virginiana* seeds become more sensitive to red when they become less sensitive to far-red and vice versa.

TABLE VI

EFFECT OF INCREASING FAR-RED IRRADIATIONS AFTER VARIOUS PERIODS OF IMBIBITION AT 5° C ON GERMINATION AT 25° OF RED LIGHT PROMOTED* PINUS VIRGINIANA SEEDS

SAMPLE NO. & IMBIBITION PERIOD	% SOUND SEEDS PROMOTED BY RED LIGHT	% INHIBITION OF RED LIGHT PROMOTED SEEDS AFTER FAR-RED IRRADIATIONS (7,500 ergs/cm²/sec) FOR:				
		2 min	4 min	8 min	16 min	32 min
LP-62:						
1 day	77	7	29	81	94	94
2 days	86	12	43	83	91	93
4 days	91	8	10	19	82	83
8 days	95	10	13	50	80	83
16 days	90	1	3	23	63	75
LP-63:						
1 day	67	36	75	93	99	98
2 days	89	26	62	91	98	99
4 days	94	8	33	83	90	97
8 days	95	0	10	55	90	92
16 days	92	0	2	32	60	68

* 32 minutes red (6000 ergs/cm²/sec) after days of imbibition and prior to far-red radiations.

TABLE VII

EFFECT OF ALTERNATE RED & FAR-RED IRRADIATIONS ON GERMINATION AT 25° C OF PINUS VIRGINIANA SEEDS IMBIBED 96 HOURS AT 5° IN DARKNESS

CHARACTER OF IRRADIATIONS*	% GERMINATION OF VIABLE SEEDS IN	
	LP–62	LP–63
O (dark control)	4	14
R	92	89
R + FR	4	2
R + FR + R	94	93
R + FR + R + FR	3	1
R + FR + R + FR + R	93	92

* Red, 6,000 ergs/cm²/sec, 8 minutes; far red, 7,500 ergs/cm²/sec, 16 minutes.

Pinus virginiana seeds required ten times the energy needed by Lepidium seeds for 50 % conversion of the far-red-absorbing form of the pigment to the red-absorbing form. And they required about five times the energy for 50 % conversion in the opposite direction. The greater incident energy requirement of *Pinus virginiana* seeds probably results from the greater thickness of their seed coats, which consequently transmit less energy than seeds of Lepidium.

Pine seeds become more sensitive to red and less sensitive to far-red radiant energy as the imbibition period at 5° C is increased from 1 to 16 days. The light requirement of many seeds for promotion of germination to a given percentage often varies as a function of temperature or duration of imbibition. The far-red energy for a given percentage inhibition almost invariably changes in the opposite direction. Hitherto, the explanation for the greater sensitivity to red of these pine seeds would have been that the longer periods were effective in changing a reactant for conversion of the pigment into a more oxidized form. A more likely explanation comes from recent studies on the extracted pigment. These studies (1) show that the percentage conversion depends on the energy of irradiation. Moreover, dialysis of the pigment solution or the addition of oxidizing or reducing agents does not inactivate the photoreaction. Thus indications are that the inverse change of sensitivities depends on the degree of pigment conversion required for a given germination rather than an associated reactant.

The germination processes proceed at a faster rate for seeds promoted with red light after a 20-day imbibition period at 5° C than for seeds promoted after a 1-day imbibition period. A longer time can elapse between red and far-red radiations for seeds held 1 day at 5° than for those held 20 days and still prevent 50 % of the promoted seeds from germinating. The pigment was already converted to the germinating form in all these seeds. The difference, therefore, in rate of germination is due to some factor other than photoreaction. Also the fact that the number of seeds that germinated in total darkness increased with longer periods at 5° showed that changes (afterripening) occurred in these seeds while they were held at the low temperatures.

The interaction of red light treatment with period of imbibition at a low temperature (stratification) permits shortening of the stratification period required to induce high germination.

The four seed lots, collected in different years and stored differently, showed differences in the degree of response to light and temperature. Because the samples varied by more than one characteristic, it was not possible to relate the responses to effects of age or storage conditions.

TABLE VIII

RATE OF ESCAPE FROM CONTROL BY FAR-RED RADIANT ENERGY OF PROMOTED SEEDS OF 2 SAMPLES OF PINUS VIRGINIANA IMBIBED AT 5° C FOR 1 OR 20 DAYS & GERMINATED AT 25°

HOURS OF DARKNESS BETWEEN RED & FAR-RED*	% INHIBITION OF RED LIGHT PROMOTED SEEDS IMBIBED FOR:			
	1-DAY		20-DAYS	
	LP–62	LP–63	LP–62	LP–63
0	90	99	69	61
8	91	99	89	68
16	80	95	73	33
24	73	91	37	11
48	43	65	8	0
72	13	30	4	< 1
96	5	0	...	...
120	2	0	...	...

* Red, 6,000 ergs/cm²/sec, 16 minutes; far-red, 7,500 ergs/cm²/sec, 16 minutes.

SUMMARY

Seeds of *Pinus virginiana* Mill. imbibed at different temperatures for various periods were tested for their response to red and far-red radiant energies.

Very little germination occurred in darkness at any temperature tested unless the seeds were held at 5° C for a period prior to placing them at the germinating temperature. With light, an imbibition temperature of 5° followed by a germination temperature of 25° was most favorable.

Germination of the seeds was promoted by red and inhibited by far-red radiant energy. About 1×10^6 ergs/cm² of energy was needed for conversion of 50 % of the pigment by red or far-red radiant energy.

The maximum germination of seeds in response to red light was greater if they were imbibed at 5° C than at 25°. Lengthening the period of imbibition at 5° increased germination in total darkness—very slightly for one sample and markedly for another. Lengthening the period of imbibition at 5° increased

the sensitivity of both lots of seeds to promotion by red.

The action of the red and far-red radiant energies on the germination of the seeds was immediately and repeatedly reversible.

After seeds were imbibed for 20 days before exposure to red light the processes of germination progressed much faster than in those seeds imbibed 1 day. Thus far-red could affect 1-day-imbibed seeds for longer periods after exposure to red than 20-day-imbibed seeds.

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UPTAKE OF MAGNESIUM & ITS INTERACTION WITH CALCIUM IN EXCISED BARLEY ROOTS [1, 2]

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Very often Ca and Mg are considered as similar ions with respect to their absorption by higher plants. No doubt, this attitude is based on the behavior of the ions in greenhouse and field studies, as well as upon their chemical similarities. For instance, Collander (3) concluded from a greenhouse study that, in general, Ca and Mg were absorbed in about equal amounts. Overstreet and Jacobson (20) point out in their review that Ca and Mg are slowly absorbed ions compared to the monovalent cations.

Almost no work has been done on the absorption of Mg by higher plants under controlled laboratory conditions. Handley and Overstreet (7) report that Ca and Mg have little influence on the respiration of excised barley roots, presumably because they are slowly absorbed. However, the absorption of Mg was not actually determined in their study.

Although Ca and Mg are generally grouped together as being slowly absorbed ions, there is little or no direct experimental evidence to warrant this conclusion. Most, if not all, of the Ca uptake by excised barley roots in the so-called physiological pH range was found to be non-metabolic (18). Recently, Conway and Beary (4) and Rothstein, et al (23) have reported Mg to be rapidly absorbed by yeast from single salt solution. In light of the latter findings, the uptake of Mg by excised barley roots was investigated.

Materials & Methods

All of the experiments were conducted using 6-day-old excised barley roots of the variety Tennessee Winter, 1956 crop. The method of culturing the root material was essentially that of Jacobson, et al (13). The excised roots were washed several times in distilled water prior to their use. After washing, the roots were centrifuged at 65 × g for 5 minutes. Representative samples of roots were weighed out and placed in bottles containing the desired salt solutions kept at constant temperature with suitable aeration. All experiments were conducted at 25° C unless otherwise indicated. The pH of the solutions

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