Responses of Seeds of Pinus Taeda & P. Strobus to Light [1]

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Seeds of loblolly pine, *Pinus taeda* L., and of white pine, *P. strobus* L., which require a long moist treatment at a low temperature (stratification) for complete germination, were chosen for this study. Seeds of the former species require 30 to 90 days at 2 to 3 C and those of the latter 30 days at 10 C or 60 days at 4 C (*27*).

The purpose of the work was to investigate responses of these seeds to red and far-red radiation, which controls germination (4) through the phytochrome pigment (13). Phytochrome exists in two interconvertible forms (4). The P_{660} form absorbs red light and is converted to the P_{730} form believed to be active biologically. The P_{730} form absorbs far red and is converted to the inactive P_{660} form. The P_{730} form also reverts in darkness to the P_{660} form (13).

Most seeds in which the action of phytochrome has been demonstrated germinate promptly and completely after one brief exposure to light (23). A few seeds in a sample of seed of Virginia pine, *P. virginiana* Mill., also germinate in response to a single brief irradiation, but most of them require a short period at a low temperature before they germinate in response to light (*26*). This change in light requirement of Virginia pine seeds after a short period at a low temperature made it important to measure similar changes in loblolly and white pine seeds, which reportedly require appreciable stratification.

Material & General Methods

Seeds of loblolly (*Pinus taeda* L.) and white pine (*P. strobus* L.) were secured from the Northeastern Forest Experiment Station and seeds of the former also from the Southern Forest Experiment Station. Seeds of loblolly, sample 1, were collected in the fall of 1955 and of white pine in the fall of 1956. They were received at Beltsville, Md., in November and October 1956, respectively. Several other samples of seeds of loblolly were studied; the history of these is given in table VIII. All samples were stored in sealed cans at −18 C after arrival at Beltsville, Md. Seeds of white pine and of loblolly, sample 1, were used in all experiments except one reported in table VIII.

The methods of planting seeds and of giving brief light exposures were the same as those for Virginia pine (26). Populations of 400 seeds in four lots of 100 each or of 200 seeds in two lots of 100 each were used for each measurement. At the level of the seeds the intensity of radiation at 580 to 695 mμ was 6,000 erg/cm²/second and at 695 to 790 mμ was 7,500 erg/cm²/second. Seeds were also exposed to unfiltered radiant energy obtained from two cool-white fluorescent tubes, which gave approximately 600 erg/cm²/second at 580 to 695 mμ.

A moist treatment of seeds at a low temperature is described in the literature as stratification or as prechilling. The former term originated from the practice by nurserymen of layering seeds in a moist medium. It is now generally used to refer more specifically to the low-temperature treatment. In this paper, holding the seeds in darkness on wetted blotters at 5 and at 15 C for periods before irradiation is referred to as stratification. The length of the imbibition period, not determined, is included in the stratification period.

Germination tests were evaluated 14 days from the beginning of irradiation. The percentages of viable seeds, of non-dark germinators that germinated in response to red light, and of promoted seeds prevented by far red from germinating were calculated as previously described (26).

All the experiments except the one reported in table VIII were repeated at least once at different times and some of them as many as three or four times. Results of these additional experiments showed the same general trends as the ones reported in the tables.

Results

► Response to Germination Temperature: Four lots of 100 seeds of each species immediately after planting were placed between folds of black cloth at 15, 20, 25, and 30 C to determine the optimum temperature for germination (table I). After the seeds had been held 24 hours in darkness on wetted blotters half the lots from each sample at each temperature were exposed 60 minutes to red light. All lots were returned, in darkness, to the original temperature. In addition, seeds were held on moist blotters 24 hours at 5, 15, and 25 C, and then after 0 and 60 minutes of

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Table I

Germination Responses of Loblolly & White Pine Seeds to Different Temperatures

Seed kind & exposure period*	% Germination of viable seeds in 14 days at indicated temperature (C) 15	20	25	30	5/25	5/25**	5/5—25	15/25	15/15—25	25/15
Loblolly pine										
0 min	1	2	6	6	9	5	1	10	1	0
60 min	0	11	35	26	43	71	1	37	4	0
White pine										
0 min	0	0	3	2	3	56	0	3	0	0
60 min	1	21	35	16	41	95	2	43	1	0

* Light treatments given after 24 hours of darkness.
** 42 days at 5 C.

Table II

Germination Responses at 25 C of Loblolly & White Pine Seeds to Red Irradiations Following Stratification at 5 C

Seed kind & period at 5 C	% Sound seeds: Germinating in darkness	Not germinating in darkness	% Non-dark germinators that responded to indicated red irradiation: ¼ min	1 min	4 min	16 min	64 min
Loblolly pine							
1 day	4	96	3	3	8	23	32
4 days	3	97	3	7	20	37	45
8 days	8	92	1	5	25	42	60
16 days	12	88	10	16	43	66	67
32 days	21	79	12	25	55	67	84
64 days	48	52	19	39	70	82	83
White pine							
2 days	9	91	5	14	25	23	32
4 days	16	84	7	10	29	38	40
8 days	29	71	15	29	54	57	59
16 days	37	63	22	42	50	62	66
32 days	52	48	24	56	69	80	81
64 days	86	14	51	57	85	85	83

Table III

Germination Responses at 25 C of Red-Light-Promoted-Seeds of Loblolly & White Pine to Far-Red Irradiations Following Stratification at 5 C

Seed kind & period at 5 C	% Sound seeds germinating after 64 min red light	% Inhibition of red-light promoted seeds after indicated far-red irradiation: ¼ min	1 min	4 min	16 min	64 min
Loblolly pine						
1 day	32	0	6	37	82	93
4 days	45	2	11	40	85	88
8 days	60	13	14	30	83	87
16 days	67	0	1	13	75	83
32 days	84	4	6	8	64	80
64 days	83	0	0	0	21	41
White pine						
2 days	32	5	16	14	57	82
4 days	40	10	11	23	67	85
8 days	59	5	0	5	41	66
16 days	66	5	0	17	36	64
32 days	81	4	5	3	25	55
64 days	83	3	1	3	10	21

red light shifted to a different temperature (designated as 5 C/25 C, 15 C/25 C, 25 C/15 C) or were placed in a germinator having a daily alternation of temperature (designated as 5 C/5 C—25 C & 15 C/15 C—25 C). The first temperature listed in a daily alternation was for 16 hours out of each 24. Also four lots were stratified 42 days at 5 C before shifting to 25 C.

The germination of seeds of both species in total darkness except that of white pine stratified 42 days at 5 C was very low. At a constant temperature, the highest germination of seeds in both species after one 60-minute irradiation with red light occurred at 25 C. Seeds held 24 hours at 5 C and 15 C and then shifted to 25 C after irradiation germinated a little better than those at constant 25 C. White pine seeds stratified 42 days prior to irradiation germinated almost 100 % but approximately 55 % of such seeds germinated in darkness. Almost twice as many loblolly seeds responded to light after stratification for 42 days as after 1 day at 5 C.

► Response to Red & Far-Red After Stratification at 5 C: Experiments were designed to measure the effect of stratification at 5 C on the responses of these seeds to irradiation. Seeds were stratified 1 to 64 days at 5 C and then were given 0 to 64 minutes of red light (table II). Identical lots were irradiated 64 minutes with red and immediately thereafter 0 to 64 minutes with far red (table III).

In both species the number of imbibed seeds germinating in total darkness increased with longer periods at 5 C (table II): the germination of loblolly was always lower than that of white pine. The number of non-dark-germinating seeds promoted by red light increased (table II) and of promoted seeds inhibited by far-red radiation decreased (table III) with longer stratification periods.

► Response to Red After Stratification at 15 C: Other work indicates that white pine (27) and loblolly (18) seeds afterripen faster at 10 C than at 5 C. Barton's results (3) indicate that delphinium seeds afterripen faster at 15 C than at lower temperatures. For any given period of stratification germination of loblolly seeds in darkness was always lower than that of white pine. Experiments to measure the effects of irradiation on germination of loblolly seeds following stratification at 15 C were designed. The seeds were stratified 1 to 64 days at 15 C and then were given 0 to 64 minutes of red light (table IV). The number of seeds that germinated in darkness or the non-dark germinators that responded to red light increased with longer periods at 15 C as at 5 C. However, the total germination was less in darkness or after irradiation when stratification was at 15 C. The results at 15 C were more variable than those at 5 C, but they still show the same general trend.

► Reversal of Red & Far-Red Action: The response of these pine seeds irradiated alternately with red and far red (table V), like that of many other kinds of light-sensitive seeds, depended on the last exposure received. If the red light was last the germination was high and if the far red was last it was low.

► Escape Rate From Control by Far Red: The number of red-light-promoted seeds that could be inhibited by far red depended on the period (table VI) between the red and far-red treatments and on the period at 5 C before the red treatment. When seeds of both species were stratified 4 days before the exposure to red, approximately 50 % of the red-light-promoted seeds were inhibited by far red 96 hours later. By contrast 44 % of the loblolly seeds stratified 70 days were inhibited by far red given immediately after red, but only 1 % by that given 96 hours after. The seeds that escaped control by far red immediately after red were probably the 40 % that germinated in total darkness following 70-day stratification.

Table V

Germination Responses at 25 C of Stratified* Loblolly & White Pine Seeds to Alternate Red & Far-Red Irradiations

Sequence of irradiations**	% Germination of viable seeds	
	Loblolly	White pine
O (dark controls)	21	3
R	75	81
R-FR	19	16
R-FR-R	75	83
R-FR-R-FR	20	13
R-FR-R-FR-R	78	83

* Seeds stratified at 5 C for 70 and 16 days, respectively.
** Red 4 and 16 minutes, respectively, for loblolly and white pine seeds. Far red 16 and 64 minutes, respectively, for loblolly and white pine seeds.

Table IV

Germination Responses at 25 C of Loblolly Seeds to Red Irradiations Following Stratification at 15 C

Period at 15 C	% Sound seeds not germinating in darkness	% Non-dark germinators that responded to indicated irradiation				
		4 min	8 min	16 min	32 min	64 min
1 day	99	4	13	10	17	9
4 days	99	14	19	14	30	28
8 days	98	18	25	12	37	25
16 days	92	20	20	27	45	27
45 days	85	30	40	42	68	58
64 days	77	36	38	53	58	63

Table VI

Escape Rate from Control by Far Red Given Various Periods after 16-minute Red Irradiation* of Loblolly & White Pine Seeds Stratified at 5 C for 4 and 70 Days & Then Germinated at 25 C

Period of darkness between red & far red**	% Inhibition by far red of promoted seeds of		
	White pine stratified 4 days	Loblolly stratified 4 days	Loblolly stratified 70 days
0 hr	95	95	44
24 hr	79	97	49
48 hr	80	72	8
72 hr	73	62	...
96 hr	52	49	1

* Germination of viable seeds of white pine stratified 4 days and of loblolly stratified 4 and 70 days was 50, 33, and 93 %, respectively, after red and 14, 9, and 40 % in total darkness.

** Far red 32 and 64 minutes, respectively, for loblolly and white pine seeds.

Table VII

Effect of Short Daily Exposures for 5 & 12 Consecutive Days on Germination at 25 C of Loblolly Pine Seeds Stratified 3 days at 15 C

Period of exposure to red		% Non-dark germinators that responded in 14 days to irradiations for	
8:00 AM	4:00 PM	5 days	12 days
0 min	0 min	5	5
32 min	0 min	60	73
32 min	1 min	60	74
32 min	2 min	62	77
32 min	4 min	63	81
32 min	8 min	67	79
32 min	16 min	69	81
32 min	32 min	69	85

► Response to Continuous or Intermittent Light: Loblolly seeds stratified 4 days at 5 C were irradiated with continuous unfiltered light at 25 C for various periods. The seeds were held in darkness at 25 C after the light period and the numbers germinated were counted 14 days from the beginning of the light treatment. Irradiation periods were 0, 1, 3, 5, 7, 9, and 14 days and the germination percentages were 4, 42, 60, 69, 73, 81, and 80 % respectively.

Loblolly seeds stratified 3 days at 15 C were given two short daily exposures to red light (table VII) for 5 and 12 consecutive days. The germination in 14 days was higher for the 12-than the 5-day treatment. The second exposure each day induced germination of only a few more seeds than did a single daily irradiation.

► Response to Red & Far Red of Loblolly Seeds of Different Histories: Data were obtained on the germination of loblolly seeds of different origins, harvested in different years, and cured and stored differently (table VIII). The seeds were given 0 and 64 minutes of red light after 7 days' stratification at 15 C. Half the lots irradiated 64 minutes with red were then irradiated 64 minutes with far red. All samples contained seeds that responded to red and far red, but the numbers of reversibly controlled seeds varied between samples. The germination response to red of seeds from samples 4, 5, and 6 was very low. When seeds from samples 4 and 6 were stratified 28 days at 5 C, 64 minutes of red caused 58 and 84 % germination, respectively.

Discussion

Seeds of loblolly and white pine were reported to require a long stratification period for complete germination. Various workers (2, 6, 8, 9, 12, 14) have shown that light stimulates germination of seeds of several species of Pinus. One (1) reported that seeds of white pine were not remarkably affected by light. Our work shows that the effectiveness of light increases with duration of stratification at 5 C. It shows also that 5 C is a more effective stratification temperature than 15 C. With partial afterripening, seeds of these two species become similar to those of lettuce (5, 7), Lepidium (24, 25), and certain other species (16, 17, 20, 22) in their response to light through the phytochrome system. Their germination is promoted by red light and reversed by far red. The seeds become more sensitive to red light and less

Table VIII

Germination Responses of Stratified* Loblolly Pine Seeds of Different Histories to Red & Far-Red Irradiations

Sample No.	State of origin	Date of collection	Temperature of previous storage	Date received & stored at −18 C	Dark	64 min red	64 min red & 16 min far red
	History of indicated samples				% Germination after treatment as indicated		
2	North Carolina	Fall 1954	−7 C (to 1 C 4/6/56)	Apr. 25, 1956	...	87	30
3	Delaware	Fall 1955	Natural storage (to 1 C 4/10/56)	Apr. 25, 1956	52	70	18
4	Louisiana	Oct. 1956	...	Mar. 1957	1	12	0
5	Mississippi	1956	...	Mar. 1957	0	18	0
6	Mississippi	Oct. 1957	...	Jan. 29, 1958	0	16	0

* Seeds stratified at 15 C for 7 days.

sensitive to far red as the period of stratification is increased. Other workers (11, 15) reported that red light promotes the germination of pine seeds and Nyman (19) demonstrated reversibility in seeds of *P. sylvestris* L.

Brief exposures twice daily or continuous light for several days promoted germination of greater numbers of partially afterripened seeds than one brief exposure. The greater the number of days of continuous light or brief daily exposures the greater the number of seeds that germinated. The higher germination with repeated exposures may be due to the reconversion of the P_{660} pigment formed in darkness to the active P_{730} form (13). These results are not like those of Isikawa and Araki (14) for *P. thunbergii* Parl. and *P. densiflora* Sieb & Zucc. or of Richardson (21) for *Pseudotsuga menziesii* (Mirb.) Franco. They reported photoperiodic effects of light on germination.

During stratification, changes occurred so that some seeds germinated in total darkness. These changes in dark germination associated with stratification were also observed in seeds of *P. thunbergii* and *P. densiflora* (10). Furthermore, our results showed that stratification resulted in an increase in sensitivity of the seeds to red radiation, a decrease in sensitivity to far-red radiation, and a more rapid escape from control by far red. The increased rate of escape from control by far red of pine seeds stratified for longer periods shows that the P_{730}-activated processes are completed earlier and thus are no longer controllable by far red. Loblolly and white pine seeds require longer stratification for these changes to take place than Virginia pine (26). The design of our experiments does not permit us to conclude whether the effects of stratification are expressed through changes in the operation of the phytochrome system or through changes in other features of the germination process, such as levels of substrates or availability of cofactors.

Summary

Seeds of loblolly and white pine were tested for their responses to red and far-red radiation after they were stratified in darkness for different periods at 5 and at 15 C. After irradiation the seeds were germinated in darkness at 25 C.

A high percentage of the seeds of both species germinated in response to a single, brief exposure to red light following partial afterripening. The actions of the red and far-red radiations on the germination of the seeds were immediately and repeatedly reversible. Continuous or repeated exposures to light shortened the period of stratification necessary for complete germination.

Stratification was more effective at 5 C than at 15 C. Lengthening the period of stratification increased germination in total darkness, increased the sensitivity of the seeds to red light, and decreased the sensitivity to far red.

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Effect of Various Herbicides on Glucose Metabolism in Root Tissue of Garden Peas, Pisum sativum. I. 2,4-Dichlorophenoxyacetic Acid & Its Analogs [1, 2, 3, 4]

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Humphreys and Dugger (3) found that treatment with 2,4-dichlorophenoxyacetic acid (2,4-D) did not alter the RQ in pea root tissue which remained near 1.0. This would indicate that carbohydrate was the main substrate being oxidized in both the treated and untreated plants. They also found that the Qo_2 values for the treated plants were greater than those for the untreated, signifying a stimulation of the respiratory rate. In a later paper (4) they stated that the reducing sugars, starch and sucrose content of the untreated seedlings and short term 2,4-D-treated seedlings was the same. Working with pea root tissue Humphreys and Dugger (5) studying CO_2 production found that applying 2,4-D caused an increased metabolism of glucose-1-C^{14} via the pentose pathway which they claim was not due to a greater amount of respiratory substrate being present. In their most recent work (6, 2) utilizing 2,4-dinitrophenol (DNP) they suggest that 2,4-D and DNP which appear to have the same properties of increasing respiration via the pentose cycle do so by blocking synthetic metabolic pathways. Thus previous work utilizing analysis of respiratory CO_2 only suggests that treatment with 2,4-D, although retaining carbohydrate as the main respiratory substrate, causes a stimulation in respiration brought about by increased pentose cycle activity.

The work to be described here will be concerned with the effect of treatment with 2,4-D and its analogs not only on the absorption and conversion of glucose into CO_2 but also on the conversion of glucose into cellular residue and alcohol soluble intermediates.

Materials & Methods

The pea seeds (*Pisum Sativum* L., var Alaska) were rolled in moist paper towels, the rolls placed in beakers containing about one inch of water, and the beakers placed in a moist, dark germination chamber. The seeds were allowed to germinate for 72 hours at 20 C at which time they had developed a primary root 30 to 40 mm long. The seedlings were then selected for uniformity and placed in specially constructed plastic treatment trays with support which allowed only the root to be immersed in the treatment solution. Each tray contained 64 seedlings.

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