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USDA Forest Service
Research Note INT-220

FOREST SERVICE
U.S. DEPARTMENT OF AGRICULTURE
INTERMOUNTAIN FOREST AND RANGE EXPERIMENT STATION
507 — 25th Street, Ogden, Utah 84401

May 1977

RESPONSE OF PONDEROSA PINE SEEDS TO LIGHT

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ABSTRACT

Rates and percentages of germination of one race of ponderosa pine seeds were increased by a 24-hour light treatment compared to seeds kept in the dark. For seeds that had undergone 0 or 7 days of stratification, red light appeared to promote germination, whereas far-red light appeared to inhibit germination. Twenty-one days of stratification essentially removed these light responses, but increased germination over seeds with less stratification.

KEYWORDS: ponderosa pine, seed germination, light, moisture, stratification

Ponderosa pine forests have been characterized as large, uneven-aged stands composed of small even-aged groups in various stages of maturity (Weaver 1943). The small groups were the result of a single year of germination in openings within the stand. Cooper (1961) also observed the almost exclusive occurrence of pine reproduction in clearings between large overstory trees or groups of trees. Similarly, in western Montana, ponderosa pine seedlings rarely exist beneath the heavy canopy cover of the more mature trees. This may be the result of limited moisture, nutrients, and sunlight. There is also the possibility that many seeds never germinate.

The ability of a ponderosa pine seed to germinate is influenced by a complex of environmental factors. Proper combinations of temperature, moisture, and oxygen are critical. In addition to and interacting with these factors, light might be important.

Although seeds of many species of trees and shrubs frequently become buried and germinate without light, others require light stimulation. *Pinus palustris* seeds germinate better in light than in darkness as do seeds of *Pseudotsuga menziesii* and *Pinus banksiana* (USDA Forest Service 1974). Most seeds must be moist at the time of exposure to light if the light is expected to influence germination. *Pinus sylvestris* is an exception because germination is increased by light exposure even when the seeds are dry (USDA Forest Service 1974).

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Germination of some species of seeds is affected not only by light quantity but also light quality. Red light may promote germination whereas far-red light may inhibit germination. Conifer seeds that respond in this manner are *Abies veitchii*, *Abies homolepis*, *Picea glehnii*, *Pinus thunbergiana*, and *P. sylvestris* (USDA Forest Service 1974). Other pines that are also influenced by light quality are *P. palustris* (McLemore and Hansborough 1970), *P. virginiana* (Toole and others 1961), *P. taeda* and *P. strobus* (Toole and others 1962), and *P. banksiana* (Orlandini and Buland 1972).

Within the seed a pigment called phytochrome is thought to absorb and interpret light energy. Hendricks and others (1956) assume the reaction is as follows:

Pigment 1 has the capacity to absorb red light and is converted to pigment 2. Pigment 2 reacts with reactant B to break dormancy (increase respiration and mitosis). However, if far-red light is absorbed by pigment 2 before germination occurs, it will be converted back to pigment 1 which reacts with reactant A to revert the seed back into a state of dormancy.

Working with *Pinus sylvestris*, Kopcewicz and Porazinski (1973a, 1973b) found that an increase in germination due to either stratification or red light treatment corresponded to a simultaneous increase in gibberellin content. Because this pine has been shown to respond to the red and far-red light treatments, red light may cause an increase in gibberellins, known to be germination promoters and a decrease in abscisic acid, known to be a germination inhibitor.

Three experiments involving the effect of light on ponderosa pine seeds were performed to determine if seed germination (1) is light dependent (photoblastic), (2) is affected by varying red and far-red light densities, and (3) follows the photoreversible phytochrome response. The experiments followed the design of Toole and others (1961) and McLemore and Hansborough (1970).

GENERAL PROCEDURES AND MATERIALS

The seeds used for these experiments were collected in the fall of 1974, 40 miles northeast of Missoula, Montana, in a Douglas-fir--pine grass habitat type. They were stored in a moistureproof container at 3°C. Before the experiments, the seeds were placed into a beaker of n-pentane, which separates viable seeds from those with empty seed coats and dried embryos.

Red and far-red light were produced by modifying a method used by Poff and Norris (1967). Red light with a maximum wavelength of 650 nanometers (nm) was produced by shining a bank of nine 750-watt flood lamps through 6 cm of 1 percent cupric sulfate solution and two red filters. Far-red light with a maximum wavelength of 750 nm was produced by passing the light from nine 750-watt flood lamps through 6 cm of a ferrous ammonium sulfate solution and two far-red filters separated by the Cinemoid component from a blue filter. Light intensity was measured with an Eppley pyrliometer. The intensities of both the red and far-red light were nearly equal, reaching levels of between 4,500-5,000 erg/cm²/s.

Before and after light treatments the seed containers (petri dishes) were wrapped in foil to prevent exposure to other light. Germination took place in a growth chamber set at 25±2°C. Germination was observed at frequent intervals under a safe, green light. A seed was considered germinated when the radicle protruded about 2 mm and turned down. As seeds germinated they were removed. When the experiments were terminated, both percent germination and germination values were computed. The germination

value is a measure of both completeness and speed of germination. It is calculated by multiplying the mean daily germination rate by the highest daily germination rate (McLemore and Czabator 1961). Transformation of the germination data was not deemed necessary because of the narrow range of germination percentages within each experiment and variance homogeneity.

EXPERIMENT 1

PROCEDURES

This experiment was to determine if ponderosa pine seed germination is light dependent (photoblastic). Fifty seeds were placed in each of eight 9-cm petri dishes. Four of the dishes had sponges saturated with distilled water. The other four had no sponges or water. The petri dishes were placed in a dark chamber set at 25°C for 24 hours. This procedure allowed half of the seeds to imbibe water. Following the imbibement period, half of the imbibed seeds and half of the dry seeds were exposed to 23 hours of white light from two fluorescent tubes, and 1 hour of red light. The other seeds were kept in the dark. Following the light treatments, half of the seeds from all treatments were removed for moisture content determination by drying at 105°C for 24 hours. The remainder was placed on moist sponges in petri dishes which were wrapped in foil and placed into the germination chamber. Germination was allowed to continue for 21 days.

RESULTS

The imbibed seeds had about 5 times the moisture content of the dry seeds. Regardless of light treatment, the imbibed seeds had moisture contents which ranged from 39.8 to 43.8 percent. The range of moisture for the dry seeds was from 8.1 to 8.7 percent. The percent germination and germination values along with the results of the factorial analysis of variance tests are shown in table 1.

Both percent germination and germination values of imbibed seeds, regardless of light treatment, were greater than for dry seeds, the difference being highly significant. Light-treated seeds also had higher germination values and percentages than seeds treated with no light, but the differences were not great. Variances were homogeneous in all cases.

Table 1.--Effect of light and moisture on percentage of germination and germination value of ponderosa pine seeds

	Percent germination±S.D.		Germination values±S.D.	
	Light	Dark	Light	Dark
Imbibed	51.0±6.0	43.0±8.2	13.17±6.73	7.89±2.81
Dry	34.0±8.3	24.0±11.3	4.04±1.71	1.84±2.04

	F-Ratios (1,12 df)	
	Percent germination	Germination value
Imbibed vs. dry	¹ 17.20	¹ 14.89
Light vs. dark	² 4.30	² 3.60
Interaction	.05	.61

¹ Significantly different at the 1 percent level.

² Significantly different at the 10 percent level.

PROCEDURE

This experiment tested the effect of red and far-red radiation on ponderosa pine seed germination. Seeds were divided into three groups (each with 32 petri dishes with 25 seeds on a moist sponge per dish) and received one of the following stratification treatments: (1) stratified at $3^{\circ}\pm 1^{\circ}\text{C}$ for 21 days, (2) stratified at $3^{\circ}\pm 1^{\circ}\text{C}$ for 7 days, and (3) not stratified--imbibed water at 25°C for 24 hours. Then, one subgroup consisting of four petri dishes from each stratification treatment received one of the following light treatments:

- A. Dark control--no light
- B. Light control--continuous light
- C. 5 min red light
- D. 15 min red light
- E. 60 min red light
- F. 60 min red and 5 min far-red light
- G. 60 min red and 15 min far-red light
- H. 60 min red and 60 min far-red light

After the light exposures, all replicate petri dishes except for the light controls were wrapped in foil and placed into the germination chamber. Germination was checked at weekly intervals for 3 weeks.

Each stratification group was analyzed as a separate part of the experiment using the one-way analysis of variance and a modified Scheffé's test. Comparisons of germination between similar light treatments in different stratification groups were not attempted.

RESULTS

A number of comparisons were made to determine the effect of light and stratification on germination. Increased time of stratification caused an increase in germination: 0 days of stratification, 57.4 percent germination; stratification for 7 days, 65.0 percent germination; and 21 days of stratification, 79.4 percent germination. This situation is known to be true for a variety of species of seeds.

Table 2 shows percentage germination after 21 days within each stratification treatment. Variances were again homogeneous.

Comparisons were made as follows: light control vs. dark control; dark control vs. 5-red, 15-red, and 60-red; 60-red vs. 60-red--5-far-red, 60-red--15-far-red, 60-red--60-far-red, 15-red, and 5-red; and 60-red--5-far-red vs. 60-red--15-far-red and 60-red--60-far-red.

For all stratification periods, the light and dark controls were not significantly different after 21 days of germination. It should be noted that after 7 days of germination, the light controls had a much greater number of germinated seeds than the dark controls, but this difference diminished with time.

For 0 days of stratification, 60 min of red light increased germination over the dark controls, whereas 5 and 15 min did not. All treatments with red light only were statistically the same. Fifteen and 60 min of far-red light inhibited germination below the 60 min of red treatment, but 5 min of far-red did not. Also, 15 and 60 min of far-red light inhibited germination below that of 5 min of far-red.

Table 2.--The effect of red and far-red light, and stratification on germination of ponderosa pine seeds.
(Treatments not underscored with the same dotted line are significantly different at the 10 per-
cent level or lower.)

Days stratified	Treatment and percentape germination							
0	60R-5FR (75)	60R (65)	5R (60)	15R (59)	Light (55)	60R-60FR (51)	Dark (50)	60R-15FR (44)
								
7	60R (76)	15R (75)	5R (68)	60R-60FR (66)	60R-5FR (60)	60R-15FR (60)	Light (60)	Dark (59)
								
21	60R (81)	60R-5FR (81)	5R (80)	60R-15FR (79)	60R-60FR (78)	Light (78)	15R (77)	Dark (76)
								

For 7 days of stratification 15 and 60 min of red light increased germination significantly above the dark control, whereas 5 min of red did not. However, 60 min of red did not increase germination over 5 and 15 min of red. Both 5 and 15 min of far-red light did inhibit germination below that of the 60 min of red treatment. Sixty min of far-red did not significantly reduce germination. All treatments ending with far-red light were statistically the same.

For 21 days of stratification, no significant differences in germination occurred. Germination percentages ranged from 76 to 81 percent. This corresponds in part to the findings of Hatano and Asakawa (1964), who stated that with increased periods of stratification, germination in darkness increases, sensitivity to red light increases, and sensitivity to far-red light decreases.

EXPERIMENT 3

PROCEDURE

This experiment was performed to test the effect of alternating red and far-red light on seed germination. Four light treatments plus a dark control were used. Twenty-five seeds were placed on water-soaked sponges in petri dishes. With four dishes for each treatment, the 20 dishes were placed into a dark chamber set at 25°C, allowing the seeds to imbibe for 24 hours. After this imbibition period, the seeds were given their respective light exposures. Group A, the dark control, received no light at all. Group B received 10 min of red light. Group C received 10 min of red followed by 10 min of far-red. Group D received 10 min of red, 10 min of far-red, and 10 min of red. Group E received 10 min of red, 10 min of far-red, 10 min of red, and finally, 10 min of far-red. Following the light treatments, all the petri dishes were moved to the germination chamber. Germination was observed intermittently for 24 days. Germination percentages and germination values were computed, and means were compared by a one-way analysis of variance and a modified Scheffé's test.

RESULTS

Percentage of germination and germination values of seeds exposed to the five different light treatments were as follows:

<i>Treatment</i>	<i>Percent germination $\pm$ S.D.</i>	<i>Germination values $\pm$ S.D.</i>
A--Dark control	20.0 $\pm$ 6.5	0.90 $\pm$ 0.47
B--10-R	19.0 $\pm$ 3.8	1.18 $\pm$ 0.63
C--10-R, 10-FR	18.0 $\pm$ 5.2	.95 $\pm$ 0.45
D--10-R, 10-FR	39.0 $\pm$ 8.4	4.17 $\pm$ 1.20
10-R		
E--10-R, 10-FR, 10-R, 10-FR	23.0 $\pm$ 8.4	1.61 $\pm$ 1.30

The percent germination and germination value of treatment D, which ended with 10 min of red light, were significantly greater than the other four treatments at the 1 per cent level. The other four were statistically the same.

SUMMARY AND DISCUSSION

The results of experiment 1 revealed that germination is improved greatly with increasing seed moisture content. Light increased germination slightly but not highly significantly. Evenari (1956) stated that at the thermal optimum of germination, photosensitivity is at a minimum. Therefore, the further from this optimum temperature, the more pronounced will be the effect of light on photoblastic seeds. USDA Forest Service (1974) reported that the optimum temperature for ponderosa pine seed germination is between 75° and 86°F. Germination temperatures in these experiments ranged from 77° to 81°F, which was certainly in the optimum range. This may account for the lack of significant increase in germination with light.

The results of experiment 2 indicated that the phytochrome system may be working in ponderosa pine seeds. In most cases, 60 min of red light increased germination over that of the dark controls. Also, various amounts of far-red following a red light treatment often decreased germination below that of red light only. These results held for 0 and 7 days of stratification but not for 21 days. In all cases, the light controls did not have a higher number of germinants than the dark controls. This may have been the result of the type of light source used. A 300-watt incandescent light bulb was placed 75 cm from the seeds in the light control treatment. Withrow and Withrow (1956) reported that an incandescent light bulb puts out more energy as the wavelength increases, indicating that there is more far-red energy than red energy from this type of light source. Also, the effects of red and far-red light may have been enhanced even more had this experiment been conducted at temperatures other than the germination optimum.

Stratification time was found to be an important factor. As the cool, moist storage time increased so did germination. Therefore, just as Toole and others (1961) and McLemore and Hansborough (1970) reported, increasing stratification time breaks seed dormancy, as light apparently does.

Experiment 3 showed that the potential for the photoreversible reaction exists in ponderosa pine seeds. Two treatments of red light separated by one treatment of far-red light, increased germination significantly over the other treatments. Apparently one treatment of red light was not of sufficient intensity to cause any reaction within the seeds, as was one treatment of far-red light.

A few authors have reported the results of light quantity and quality measurements in the shade of various tree species. Atzet and Wareing (1970) reported that under certain conifer canopies the energy percentage of far-red light is increased significantly compared to direct sunlight. Other wavelength energies either decrease or do not change. Vezina and Boulter (1966) revealed that under a *Pinus resinosa* canopy, the amount of red light energy is only 14 percent compared to 23 percent in direct sunlight. The amount of far-red light increased from 18 percent in direct sunlight to 24 percent under the canopy. In both *Pinus resinosa* and *Pinus strobus* stands, a minimum energy level was found in the 670-680 nm range and a very high maximum was found in the 740-750 nm range (Federer and Tanner 1966). Therefore, we might assume that increased amount of far-red light over red light occurs under many conifer canopies.

Light conditions under forest canopies do not appear to be favorable for the breakage of seed dormancy. Cool, moist conditions can, however, prepare seeds for germination regardless of light. But, under dense canopies, snow may not accumulate and often melts early in the spring. This situation may not allow seeds the cool, moist stratification which would occur under a deep layer of snow. Rather, it subjects them to varying moisture and temperature situations which may not be proper for dormancy breakage. Therefore, under these temperature, moisture, and light conditions, many seeds of the Northern Rocky Mountain variety may not germinate. This could be an adaptation to reduce intraspecific competition for ponderosa pine, which in this region occurs in areas of low rainfall.

It must be emphasized that these results can only be applied to western Montana ponderosa pine and perhaps only the specific race from which the seeds were taken. Great differences in germination responses of ponderosa pine seeds from varying geographic areas have been found (Callahan 1962). Genetically fixed temperature responses and perhaps light and moisture responses are certainly not the same for all trees or all populations of a species.

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