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The Influence of Light on Germination of Longleaf Pine Seed.

Bobbie Frank Mclemore
Louisiana State University and Agricultural & Mechanical College

THE INFLUENCE OF LIGHT ON GERMINATION OF LONGLEAF PINE SEED

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Bobbie Frank McLemore
B.S., Agricultural and Mechanical College of Texas, 1953
M.F., Louisiana State University, 1957
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ABSTRACT

The influence of light on germination of longleaf pine (Pinus palustris Mill.) seed was investigated in four separate studies, using four single-tree lots of seed for each study. All germination tests were conducted at 22.5 C.

Seed used in study no. 1 were extracted from cones in a darkroom. Half of the seed were dried and the other half left undried. Samples of these seed were exposed to irradiation from white light and red light in the 660 nanometer (nm) range, while other samples were left in darkness. Germination was subsequently tested in darkness. The only appreciable germination obtained was with nondry seed exposed to light. These seed were not imbibed, but had moisture contents of approximately 35 percent. All dry seed and nondry seed that were not exposed to light essentially failed to germinate, giving conclusive evidence that light is necessary for germination of longleaf pine seed and that they must have a high moisture level to respond to light.

The second study showed that seed extracted in a cone kiln and processed in a normal manner also have a light requirement for germination. This requirement varied greatly between individual-tree lots. Although longleaf pine seed are not thermodormant, stratification reduced the light requirement for germination of these seed. Dark-germination of seed stratified for 0, 7, 14, and 28 days at 1 C averaged 22, 29, 46, and 80 percent, respectively. Germination of all seed

in light averaged 95 percent, regardless of stratification treatment.

The third and fourth studies showed that germination of longleaf seed is controlled by red and far-red light. Red light in the region of 660 nm promoted germination while germination was inhibited by far-red light with a wavelength of 730 nm. This process was repeatedly reversible, indicating that the light requirement operates through the photoreversible phytochrome system. Irradiation periods of 1 minute were sufficient to cause significant changes in dark-germination. The degree of control exhibited by the light treatments was governed by the length of time seeds were stratified. Most of the seed stratified for 28 days germinated in darkness, regardless of the light treatment received.

One of the single-tree lots of seed showed a marked peculiarity in that short exposures to red light of 4 minutes or less promoted germination whereas longer exposures inhibited germination. Still longer exposures resulted in repromotion. Long exposures to far-red light also resulted in a slight promotion of germination in these seed. In still another lot, holding imbibed seeds in darkness at 22.5 C for prolonged periods induced dormancy that could not be overcome by exposure to light. Stratification was required to obtain complete germination of these seed subsequent to the long period of imbibition at 22.5 C. This phenomenon was not observed with the other three lots.

The series of studies conducted during the course of this investigation illustrated that germination of longleaf pine seed can be promoted by irradiation with red light and inhibited by far-red light. Control of germination can be reversed repeatedly and is

characteristic of the photoreversible reaction of phytochrome. The ability to control germination with the irradiation treatments varies greatly between single-tree lots of seed and is dependent on length of stratification.

INTRODUCTION

In the broad area concerning the relationships between light and plants, photosynthesis occupies a central position. This is obviously because of the importance of photosynthesis in the conversion of radiant energy to chemical energy. In addition to this fundamental process, light influences many other processes in plants dealing with growth and development. These processes are included under the designation of photomorphogenesis. The influence of light on germination of seeds is only one of a large number of photomorphogenic processes.

On the basis of classical concepts, seeds have been divided into three types: positively photoblastic seeds, negatively photoblastic seeds, and photo-indifferent seeds. The primary mechanism through which light influences germination of seed is through the red/far-red light system in which a photoreversible pigment termed phytochrome acts as the receptor.

The effect of light on seed germination depends on its wavelength. Generally speaking, red light in the region of 660 nanometers (nm) promotes germination and far-red light (730 nm) inhibits germination. Borthwick et al. (1952) reported a reversibility between the red and far-red photoreactions and proposed a photochemical system

including a pigment that can exist in two interconvertible forms. This pigment, called phytochrome, was purified by Butler and co-workers (1959). The connecting links between this photoreceptive pigment and the reactions leading to promotion and inhibition of germination are as yet unknown.

The photoreversibility of germination has been demonstrated for seeds of several tree species, but these species are generally dormant, requiring stratification for prompt, complete germination. Information concerning the effectiveness of this process on nondormant seed, e.g. longleaf pine (Pinus palustris Mill.), is lacking.

In most cases, light requirements have been investigated with inbibed seeds, but Nyman (1963) claimed that germinability of Scotch pine (Pinus sylvestris L.) seeds could be controlled by irradiation when the seeds were dry. Most workers have noted that light sensitivity is usually greatly affected by prechilling or stratification. However, in actual practice, longleaf pine seeds are never stratified prior to sowing because they are nondormant and germination is always prompt when suitable environmental conditions are present.

The purpose of the studies performed during the course of this investigation was to determine the effects of light on germination of longleaf pine seed. Specifically, the studies were designed to: (1) determine the effect of light on unimbibed longleaf pine seed, (2) evaluate light- and dark-germination of longleaf seed stratified for different lengths of time, (3) determine if germination of longleaf seed can be promoted and inhibited with red and far-red light, and (4) investigate the potential for germination of longleaf pine seed to be

repeatedly promoted and inhibited by red and far-red light.

The role of light in germination of longleaf pine seed may have significant practical applications in artificial regeneration of this species. Direct-seeding of longleaf pine has gained widespread acceptance throughout the South, and repellent coatings are commonly applied to the seed for protection against bird and rodent predators. The impact that this repellent coating has on light requirements for germination is unknown. Moreover, the advent of subsurface sowing techniques has further raised the question of light requirements for germination of longleaf pine seed. Wells (1956) determined that red light penetrated farther into the soil than blue light, and far-red penetrated farther than red. He found that virtually no light passed through 5 mm of a wet silty clay and that less penetrated a 5 mm dilute suspension of silty clay than 5 mm of coarse quartz sand. In still another aspect of light requirements for germination, Federer (1966) evaluated the spectral distribution of light under forest stands. He found an energy maximum at 550 nm, a minimum of 670 to 680 nm, and a very high maximum in the near infrared at 780 nm under the shade of all forest species investigated.

Before serious attention can be given to questions raised by the factors mentioned above, the importance of light in germination of longleaf pine seed must be determined. The significance of this determination is attested by the economic importance of longleaf pine to the South. Wahlenberg (1946) presented an economic appraisal of this species, along with its silvical characteristics and recommendations for management.

Longleaf pine occurs in the Coastal Plain from Virginia to southern Florida and west to eastern Texas. It is one of the four major species of southern yellow pine. Pollination of pistillate flowers occurs in March and fertilization takes place in the spring of the following year. Hence, as in other species of the genus Pinus, cones of two ages are found on the same tree (Fig. 1). Mature cones average approximately 6 inches in length and slightly over 2 inches in diameter. A single cone may yield from 10 to over 100 filled seeds. Longleaf pine seeds are among the largest for the entire genus, averaging approximately 4,500 seeds per pound. Germination is epigeous and usually quite prompt. Unlike many of the other pines, longleaf seeds do not require a period of inbibition at low temperatures to condition them for fast, complete germination.

Definition of Terms

Technical terms used in this paper are generally confined to standard terminology commonly used by biological scientists. Some of the terms that have received the most usage in this dissertation and their definitions are listed below:

Ångstrom - A unit of measurement, equal to one hundred-millionth of a centimeter, used in measuring the length of light waves. Abbreviated in this paper as A.

Celsius - A temperature scale equal to the centigrade scale. It is abbreviated as C and the ° symbol is omitted.

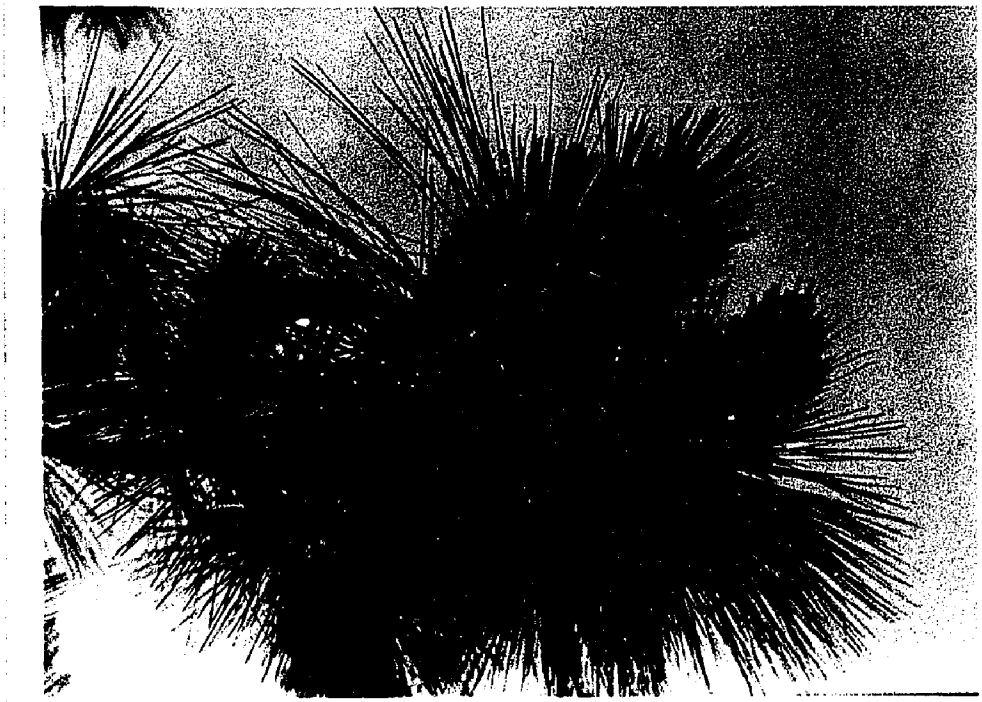


Figure 1. Typical branch of longleaf pine showing mature cones and 1-year-old conelets.

Erg - A unit of energy equal to the amount of work done by one dyne acting through a distance of one centimeter. As used in this paper, it indicates the amount of energy received from a source of radiation and is given in terms of ergs per square centimeter per second or ergs/cm^2/sec.

Lux - A unit of illumination equal to one lumen per square meter or the illumination of a surface uniformly one meter distant from a point source of one international candle. One foot-candle is equal to 10.8 luxes.

Millimicron - A unit of length used for measuring light waves. It is equal to one thousandth of a micron, one millionth of a millimeter, ten Ångstroms, or one nanometer. It is abbreviated in this report as mμ.

Nanometer - A unit of length used for measuring light waves. It is equal to 10^{-9} meters or one millimicron, and is abbreviated as nm.

Photoblastic - A term used to denote the effect of light on germination of seeds. Positively photoblastic indicates that light is necessary for germination; negatively photoblastic indicates that light inhibits germination; and photo-indifferent indicates that seeds germinate equally well in light or in darkness.

Phytochrome - A blue, protein, pigment that occurs in minute amounts in plant material. It is a photoreceptor for light energy and is a photoreversible pigment that can be changed from one form to another by red and far-red light.

Significant - This word has a statistical connotation and when used in this paper it is considered as being statistically significant, either at the .05 or .01 level.

REVIEW OF LITERATURE

Historical Background

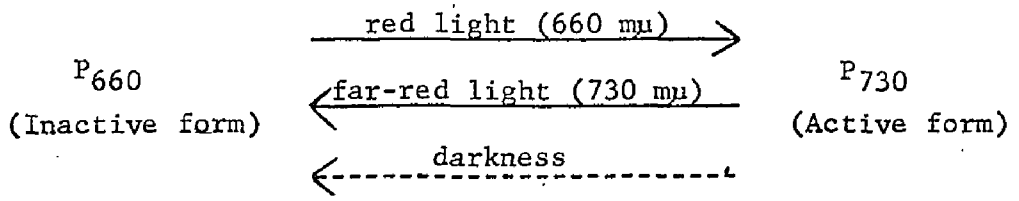
In two separate reviews of light effects on germination of seed, Crocker (1936) and Evenari (1956) reported that Caspary (1860) was the first botanist to observe the beneficial influence of light on seed germination. He noted that seeds of Bulliarda aquatica germinated well in full sunlight, but poorly in diffuse light. The same two reviewers also noted that it was not until 1881 that Stebler investigated the effect of light on germination in a systematic manner. Using the achenes of different grasses, he proved that the grains of a number of grass species germinated much better in light than in darkness. Evenari (1956) also reported that Heinricher (1903), working with seeds of Acanthostachys strobilacea, was the first to note inhibition of germination caused by light. Since that time, numerous publications have reported either beneficial or adverse influences of light on seed germination. Crocker (1936) presented a detailed review of research on light effects up to 1936. In summary, he noted that light favored germination of Viscum album L. and other Loranthaceae (mistletoe) and epiphytes, all Gesneriaceae, many grasses, Ranunculus sceleratus L. (cursed crowfoot), Lythrum salicaria L., Lythrum hyssopifolia L. (loosestrifes), and various species of Oenothera (evening primroses) and Epilobium (cotton-weeds), while light inhibited germination of several species of Phacelia and other Hydrophyllaceae,

three species of Nigella (fennel-flowers) and several species of Allium (onions). Twelve years later, Crocker (1948) reported that of 964 species investigated, germination of seeds from 672 were favored by light and 258 were inhibited. Light had no effect on the remaining 34 species.

Up until this point, work had been primarily concerned with germination of seeds in white light and in darkness. Flint and McAlister (1935) were pioneers in the study of the effect of different wave lengths of visible light on germination. In 1935, they announced the discovery of a band in the region of 7600 Angstroms (A) which inhibited the germination of light-sensitive lettuce (Latuca sativa L.) seed far more than similar inhibitory influences previously noted in the region of 4200 to 5200 A. Later, these same two workers (1937) reported that radiation ranging from about 5200 to 7000 A promoted germination of lettuce seed, with the longer wave lengths being by far the most effective. They determined that the critical wave length of radiation promoting germination within the most effective range was approximately 6700 A.

It remained for a group of researchers with the Agricultural Research Service at Beltsville, Maryland, to explain Flint and McAlister's findings by demonstrating that the photoreaction controlling seed germination is reversible. In studying the control of flowering by photoperiod, Borthwick, Parker, and Hendricks (1950) found the action spectrum in the red region for seed germination to be essentially identical with that for initiation of flowering in both long- and short-day plants. With short-day plants, they found that the action of red light in interrupting the dark period could be overcome by subsequent

exposure to far-red irradiation (wave lengths longer than 7000 A). E. H. Toole, a seed scientist working in the same building with Borthwick, used the same equipment that was being used to study different lengths of light waves on flowering to test a sample of lettuce seed. This led to the highly significant contribution of Borthwick and his co-workers (1952) toward explaining the effects of light on germination. They found that germination of a sample of Grand Rapids lettuce seeds that had been imbibed for 4 to 16 hours could be promoted by red radiation in the region of 5800 to 6800 A. The same seed could then be inhibited by exposure to radiation with wave lengths longer than 7000 A. (Germination tests were subsequently conducted in darkness.) Later, Borthwick et al. (1954) determined that maximum promotion was obtained at 6600 A while maximum inhibition occurred at 7300 A. They proposed that a photoreversible pigment (later termed phytochrome) was involved. The pigment was later extracted and upon purification showed the same photoreversible response in vitro with absorption peaks at 6600 and 7300 A (Butler et al. 1959, Butler 1964, and Butler, Lane, and Siegelman 1964). Borthwick and his colleagues reasoned that absorption of radiation in the red region (6600 A) caused a photochemical reaction that changed the pigment into the far-red (7300 A) absorbing or active form, while absorption in the far-red region changed it into the red absorbing or inactive form. They also noted a shift to the red absorbing form in darkness. E. H. Toole (1961) used the following formula to illustrate this process:



Throughout the remaining portion of this paper, the inactive form of phytochrome or P_{660} in the above formula will be referred to as PR, while the active form or P_{730} will be referred to as PFR.

General Review of Light Effects

Since the initial breakthrough discussed above, red and far-red light has been demonstrated to control many phenomena other than flowering and germination (Borthwick 1961 and 1965). Piringer and Heinze (1954) showed that it regulated the development of a yellow cuticle in ripening tomato fruits; Klein, Withrow, and Elstad (1956) detected its influence in leaf expansion and straightening of the hypocotyl hooks of seedlings emerging from soil; Downs, Hendricks, and Borthwick (1957) and Borthwick, Hendricks, and Parker (1951) showed that it controlled stem elongation; and Downs and Siegelman (1963) demonstrated its regulation of the production of anthocyanin pigments. More recently, Surrey (1967) reported that the enzyme lipoxidase was controlled by light. Under continuous irradiation, he found that the rate of enzyme disappearance from cotyledons of squash (Cucurbita melopepo Alef.) was accelerated by red and retarded by far-red light. Acceleration of enzyme disappearance in seedlings that received an initial exposure to red light was reversed repeatedly by far-red light.

In view of the findings mentioned here, it is evident that the phytochrome system influences many phenomena in plants other than germination.

In retrospect, it is not surprising that seeds respond to this photoreversible reaction since Furuya and Hillman (1964), Briggs and Siegelman (1965) and Koukkari and Hillman (1966) have all reported that the highest levels of phytochrome are generally found in meristematic tissues.

Wareing and Black (1958a) reported a suppression of germination of Nemophila insignis Dougl. (or Nemophila menziessii Hook. & Arn.) seeds in the blue portion of the spectrum, from 4000 to 5000 A. Hendricks, Toole, and Borthwick (1959) referred to this as a high-energy photorequirement where inhibition of germination is controlled not only by the reversible change of the pigment forms, but also by their continued excitation. Negbi and Koller (1964) reported that germination of seeds of smilo grass (Oryzopsis miliacea Asch. & Schw.) is inhibited by continuous light and promoted by a short-irradiation. After running spectral and kinetic analyses they found that the promotive effects of short irradiations were caused by the promotive PFR form of phytochrome. The inhibitory effects of continuous irradiation with white light were traced to the blue and far-red portion of the spectrum. Continuous irradiation in these regions prevented subsequent germination in darkness, while continuous irradiation with white light prevented germination only as long as it was applied, and caused promotion if followed by darkness. Negbi and Koller suggested an additional pigment system

participates in the photocontrol of germination in this species, namely, the high-energy blue-far-red system.

The present paper deals with the low-energy photomorphogenic reaction or phytochrome system referred to by Hendricks et al. (1959). which deals with the reversible photoreaction at 6600 and 7300 A.

It is emphasized at this point that the reversible photoreaction described by the PR-PFR mechanism is not as simple and straightforward as might appear at first glance. Like most other biological reactions, a number of interrelated factors may come into play in the germination process. When a seed absorbs water and the protoplasm is rehydrated, a number of changes take place. Pollock and Toole (1961) and E. H. Toole (1961) have pointed out that these changes do not necessarily follow a linear sequence, but may follow any one of several pathways. They noted that seed dormancy may be thought of as resulting from a block, or blocks, somewhere in these pathways. The multiplicity of pathways and the occurrence of various blocks are shown by the interaction of light and temperature. E. H Toole et al. (1956), Koller et al. (1962) and Evenari (1965) have presented comprehensive reviews of the literature concerning the knowledge of light and temperature interactions in seed germination.

Although the temperature coefficient for the action of light on seed germination is approximately 1, according to Borthwick (1965), i.e., the energy required to induce 50 percent germination is the same regardless of whether the seeds are irradiated at 10 or 40 C, the temperature at which the seeds are held during the imbibition process or during germination may have a pronounced impact.

As noted earlier, when seeds are held imbibed in the darkness there is a gradual shift from the PFR to the PR form of phytochrome. E. H. Toole et al. (1953) noted that this change is hastened by an increase in temperature. They also pointed out that the total amount of light energy required to promote germination varied with different kinds of seeds and for different lots of the same species.

E. H. Toole (1959) and E. H. Toole et al. (1955a, 1955b, and 1957) have done extensive research concerning the interactions of light and temperature on germination of peppergrass (Lepidium virginicum L.) seeds. These seed will not germinate in the dark if kept at 15 or 25 degrees Celsius (C), or at alternations of these two temperatures. Toole and his colleagues found that a single shift of temperature from 15 to 25 C at the time of irradiation with red light greatly increased germination, but a shift from 25 to 10 C had no effect on germination. They also reported that both total germination and the sensitivity of the photoreaction could be increased by holding the seeds at 35 C for 24 hours in darkness preceding a germination temperature of 20 C. The same workers also noted that germination of Lepidium seed could be increased by an alternating temperature of 15 - 25 C in conjunction with irradiation treatments. They concluded that the germination process is controlled at different points by several factors and that the photoreaction, while possibly present in all seeds, is not obligatory for germination of all seeds. The photoreaction controls the levels of PR and PFR which are also under control by other reactions subject to influence by temperature.

Mancinelli, Borthwick, and Hendricks (1966) and Mancinelli, Yaniv, and Smith (1967) found that germination of tomato (Lycopersicon esculentum Mill.) seed was inhibited by far-red light and repromoted by red. At temperatures of 17 to 20 C, a single exposure to far-red irradiation was sufficient to inhibit germination, but at higher temperatures they found the same exposure less effective in the inhibition of germination of tomato seeds.

Recently, Klein, Edwards, and Shropshire (1967) in working with beans (Phaseolus vulgaris L.) reported that PR disappeared rapidly in the dark at 25 C, and was not detectable after 6 hours. They found no indication that the PR reverted to PFR. At 4 C, the PR did not disappear to any measurable extent and was nearly totally reversible to PFR.

Germination of seeds of the scorpion weed (Phacelia tanacetifolia Benth.) is inhibited by light. Chen and Thimann (1965) found that after one or two days' exposure to light, germination of seed of this species was prompt upon transfer to darkness, but with longer exposures secondary dormancy was incurred and the seeds would not germinate in darkness. They also noted that low temperatures allowed germination in light provided its intensity was not too high. Above 30 C, no germination occurred. They reported that removal of the seed coat or rupturing it at the radicle end resulted in complete germination in full light.

Fujii and Yokohama (1965), E. H. Toole (1959 and 1961), and other workers have noted the beneficial influence of removing the seed coat, or pricking holes in it, on germination. This does not imply that the light acts on anything contained in the seed coat, but rather that another one of the pathways mentioned earlier may be involved. Delouche and Bass (1954), working with western wheatgrass (Agropyron smithii L.), found that samples consistently gave higher germination percentages in darkness than in light. Although seeds with exposed embryos were still inhibited by light, exposing the embryos greatly increased germination. They concluded that the inhibitory effect of light was directly on the embryo itself.

Length of the light period as well as temperature is also an important factor that influences germination. Black and Wareing (1955) found that European white birch (Betula pubescens Ehrh.) seed will germinate at 15 C only under long day conditions, eight daily cycles being required to induce germination. However, at 20 to 25 C germination occurred following a single exposure to light for only 8 to 12 hours. More recently, Mayer and Poljakoff-Mayber (1963) and Hatano and Asakawa (1964) have presented comprehensive reviews of seed germination that covers light and temperature effects observed by a number of workers.

Several workers have observed that the light requirement of seeds decreases as seeds mature or afterripen. This was first noted by Thompson (1935). He concluded that increasing lengths of afterripening in positively photoblastic lettuce seed increased their sensitivity to light and decreased their photorequirement. Shuck (1936) also observed

that the photorequirement of immature, light-requiring lettuce seeds was greater than for mature ones.

McLemore (1966) found that with loblolly pine (Pinus taeda L.) seed, increasing lengths of afterripening resulted in higher percentages of germination in darkness, or a decrease in light requirements. He found that stratification periods in excess of 100 days were necessary to eliminate all light requirements. Fujii and Yokohama (1965) also reported that the photorequirement for the germination of Eragrostis (love-grasses) seeds decreased with the progress of afterripening.

In addition to temperature and afterripening effects on light sensitivity, several chemicals have been demonstrated to interact with light in controlling germination. E. H. Toole et al. (1955b) reported that one lot of lettuce seed imbibed and exposed to red irradiation gave only 30 percent germination. This value was greatly increased, however, by imbibing the seed in a 0.2 percent solution of potassium nitrate before the irradiation process. Miller (1956) presented data that demonstrated similarities of kinetin and red light effects. He believed that the two may react through the same biological mechanism. Leff (1964) found that kinetin interacted with light in the promotion of germination of lettuce seed. In the dark, kinetin had only a slight stimulating effect. She found the optimal condition for the study of this interaction was the use of small quantities of light after 7 hours of soaking in a 5×10^{-5}M solution of kinetin.

Khan and Tolbert (1965) noted that the inhibition of lettuce seed germination by materials similar to coumarin and xanthatin was reversed by a combination of red light plus kinetin, but not by either red light or kinetin alone. V. K. Toole and Cathey (1961) reported three separate effects of gibberellin on photosensitive seeds of lettuce and peppergrass. First, gibberellin caused light requiring seeds to germinate in total darkness. Second, gibberellin removed certain temperature blocks to germination and prevented the onset of dormancy imposed by high temperature. Finally, they noted that, when suboptimal levels of light and gibberellin were used, gibberellin caused a higher percentage of seeds to germinate at a given energy of red light.

Forest Tree Seed

Although most of the work subsequent to Borthwick and his co-workers' discovery of the photoreversible process in germination has been conducted with seeds from herbaceous species, this phenomenon has recently been demonstrated in seeds of some forest species. Nyman (1963) and Hatano and Asakawa (1964) have given rather comprehensive reviews of the photoreversibility of forest tree seed. Prior to their reviews, Jones (1961) compiled a list of seed from forest species which were known to be influenced by light. More recently, Barton (1967) has prepared a bibliography of over 20,000 references dealing with seeds. Some of these references deal with light effects on seed germination and a few pertain to forest tree seed.

The earliest reference to the need for light in germination of southern pine seed was made by Nelson (1940). She noted that seeds from loblolly, shortleaf (Pinus echinata Mill.), slash (Pinus elliottii Engelm.), and longleaf pine all germinated better in diffuse light than in darkness. Aside from this reference, there is a dearth in the literature concerning light effects of germination of longleaf pine seed. Other references in the literature were traced back to Nelson's original observation.

As noted earlier for seeds of herbaceous species, there is often an interaction between imbibiton temperature and light effects on germination of tree seed. Eliason and Heit (1940) first noted this in studying the dormancy of Scotch pine (Pinus sylvestris L.) seed. Although Barton (1928) first noted the beneficial effect of holding imbibed seeds at 3 to 5 C (hereafter referred to as stratification) in breaking dormancy of loblolly and shortleaf pine seed, V. K. Toole et al. (1958) noted that highest germination percentages with this species were obtained when the light treatments in the red region followed stratification. They reported approximately 50 percent germination after 1 to 4 days imbibition at room temperature. As the period of imbibition increased to 64 days, progressively higher percentages of seeds germinated in response to the same light treatment. They found that a long period of imbibition in darkness at 15 C or lower, i.e. stratification, is needed to enable the maximum number of seeds to germinate in response to a single light treatment. Treatment by repeated brief light exposures for 12 successive days had the same

effect as a single exposure after a long period of stratification. V. K. Toole et al. (1958) also reported that promoted loblolly pine seeds can be inhibited by a brief exposure to far-red irradiation. Thus, they were the first to demonstrate the reversible photoreaction of red and far-red light on germination of pine seed, although Hashimoto, Shihira, and Isikawa (1954) and Iwakawa and Kotani (1954) had reported that red light promoted germination of Japanese red (Pinus densiflora Sieb. & Zucc.) and Japanese black (Pinus thunbergiana Franco) pine seeds. Later, V. K. Toole et al. (1962) concluded that stratification at 5 C was more effective than 15 C for loblolly and white pine (Pinus strobus L.) seeds. Working with Virginia pine (Pinus virginiana Mill.) seed, V. K. Toole et al. (1961) reported that very little germination occurred in darkness at any temperature tested unless the seeds were stratified at 5 C for a period prior to placing them at a germination temperature of 25 C. Germination of the seeds was promoted by red light and inhibited by far-red light. About 1×10^6 ergs/cm^2 was needed for conversion of 50 percent of either PR or PFR.

Yelenosky (1961) reported that although seeds of yellow birch (Betula lutea Michx.) and paper birch (Betula papyrifera Marsh.) normally require stratification to break dormancy, the seeds of these species germinated in a water medium under artificial light without stratification. Earlier, Redmond and Robinson (1954) found a water soluble substance in yellow birch seed coats that inhibited growth of embryos. Since the seed would germinate readily in light, but not in darkness, they believed that the substance lost its inhibitory properties

when exposed to light. Wareing and Black (1958b) also noted that unstratified seeds of European white birch would break dormancy when imbibed seeds were exposed to light.

Borthwick, Toole, and Toole (1965) reported that the germination of seeds from the princess tree (Paulownia tomentosa (Thunb.) Baill.) required the action of PFR for many hours. This form disappeared in darkness before finishing its action and had to be regenerated by irradiation with red light. They accomplished this by continuous irradiation for 50 to 100 hours or by giving the seeds a succession of brief illuminations alternating with several hours of darkness. In this manner, the PFR continued acting in darkness until it disappeared.

Jones (1961) stated that, in general, intensity of light was relatively unimportant in the germination of tree seed, but length of the photoperiod had a pronounced effect. He also noted that the pine seed used in his studies had to be preconditioned by imbibing water before light had any effect on germination. He reported that germination of loblolly pine seed was better with photoperiods of 12 and 16 hours than with an 8-hour period. Lengthening the light exposure from 8 to 16 hours doubled germination of these seed, but increasing light intensity from 1600 to 3200 lux (approximately 150 to 300 foot candles) had little or no effect. Slash pine seed germinated approximately the same under 8-, 12-, and 16-hour photoperiods. When these seeds were germinated in darkness, a 15-minute exposure to direct sunlight doubled germination. He also found that exposure of slash pine seed to red light for 10 minutes doubled the germination obtained in total darkness.

Woods (1963) reported that single light exposures as short as 1/2000 of a second resulted in substantial increases of loblolly pine seed germination in darkness. Germination of unexposed seed was less than 10 percent in one test, and 41 percent following the flash of light. Although he did not determine the quantity or quality of light used, the intensity was undoubtedly quite high since he used an Ultrablitz Jet II strobe light for the short exposure. The loblolly pine seed used by Woods had been stratified for 60 days.

As noted earlier, the light requirement of loblolly pine seed can be completely overcome by stratification for slightly over 100 days. However, McLemore (1964) demonstrated that if white light, having a red component, was supplied during the stratification process, maximum germination would be obtained in darkness following stratification for approximately 50 days.

Ackerman and Farrar (1965) reported that germination of jack pine (Pinus banksiana Lamb.) and lodgepole pine (Pinus contorta Dougl.) seeds was reduced at all temperatures by the exclusion of light, but could be promoted by either a single exposure or by daily exposures greater than a minimum length. They also found a strong interaction between the light and temperature requirements of both species.

In studying light effects on germination of Douglas-fir (Pseudotsuga menziesii (Mirb.) Franco) seed, Richardson (1959) demonstrated that the germination of unstratified seed could be greatly accelerated by exposure to long photoperiods (16 to 24 hours) at temperatures of 14 to 20 C, but not at 26 C. Total germination, however, was unaffected. He also

found that gibberellic acid at 5 ppm accelerated germination in both light and darkness but, again, did not affect total germination. Richardson concluded that, in germination, gibberellic acid does not interact with light but acts as a general germination stimulant.

Johnson and Irgens-Moller (1964) reported that exposure of unstratified Douglas-fir seed to red light increased rate of germination while similar exposures to far-red light decreased the rate. The effects of red and far-red light were reversible. They also reported that exposure of dry seed to red light for 12 hours, followed by imbibition and germination in darkness, also increased the rate of germination.

Huss (1961) extracted seed from samples of Scotch pine cones in complete darkness. Samples from the same lots were extracted in light. Results of his tests showed that germination in darkness proceeded much slower than in light. However, no great differences in production of seedlings appeared between dark- and light-extracted seed when they were sown in the nursery. Apparently ignoring the aspect of imbibition prior to light treatment, which undoubtedly took place in the nursery, he concluded that light supplied during the practical treatment of cones and seed is sufficient to produce a maximum number of seedlings.

Nyman (1961 and 1963) reported that stratification of Scotch pine seed for 1 month eliminated their light requirement for germination. He noted that complete germination in darkness could be induced by a limited length of irradiation given after an imbibition period of 6 to 12 hours. Contrary to most other workers' findings, he stated that

unimbibed seeds were also sensitive to irradiation. Similar results have also been reported by Furukawa (1956) for seeds of Japanese red and Japanese black pine.

Although several studies have been instituted within the last 10 years relative to the effects of light on germination of various tree seed, only a few have dealt with pine seed. None of the studies have investigated the influence of light on germination of longleaf seed.

MATERIALS AND PROCEDURES

Seed Collection and Processing

Four separate lots of longleaf pine seed were used in each of the four different studies involved in this investigation. The four lots represented individual-tree collections, with each lot coming from a single tree. The trees were located on the J. K. Johnson Tract (Section 4, T 2 N., R 3 W.) of the Palustris Experimental Forest in Rapides Parish, Louisiana. They were open-grown, second-growth trees with large crowns and averaged approximately 45 years of age, 15 inches in diameter at breast height, and 75 feet in height.

Cones were collected from the trees on October 20, 1965, when they were completely mature but before any scales had started cracking. Specific gravity of the cones at this time was approximately .80--well below the value of 0.89 that is generally accepted as the index of maturity for longleaf pine cones (Wakeley 1954). All cones were collected by climbing the trees.

Seeds were extracted from all lots of cones within a week after collection. Those to be used in test no. 1 were extracted at a room temperature of approximately 22.5 C. in an air-conditioned darkroom. Cones were placed inside deep cardboard boxes with a black cloth covering the top of the boxes to prevent entry of light in the event of a light being accidentally turned on in the darkroom. These cones had opened sufficiently to release their seed after 5 days.

Seeds for the other three tests were extracted in a small, experimental cone kiln. The kiln was heated by propane gas and air was circulated over the cones by a squirrel-cage blower with a capacity of 1,500 cubic feet of air per minute. Controls for the kiln, the burner, and blower are depicted in Figure 2. Figure 3 shows a front view of the kiln cabinet with a drawer removed. Individual lots of cones and seed were kept separate by removable partitions in the drawers shown in Figure 3. Temperature within the kiln during the extraction process stayed within the range of 35 to 40 C. Static pressure was maintained at 1 inch of water. All of the cones were completely open after 2 days of drying, but the seeds were dried for an additional day in order to reduce their moisture content to approximately 10 percent. (All moisture levels were computed as a percentage of the oven-dry weight of seed after 24 hours of drying at 100 C in a forced-air, electric oven.)

Seeds for test no. 1, extracted by air drying in the darkroom, were dewinged individually by pinching the wings from the seed. This was accomplished by using a safe-green light. Seeds for the other three studies that were extracted in the cone kiln were dewinged by hand rubbing. They were cleaned of trash by running them through a small, laboratory model FS-24 aspirator manufactured by the Superior Separator Company. In order to assure that only sound seeds were utilized in all four tests, all lots of seed were cleaned to 100 percent soundness by floating off all empties and partially filled seed in n-pentane. The buoyancy of longleaf seed is so great that

Figure 2. Blower-heater unit and controls for cone kiln. A 1,500 c.f.m. squirrel cage blower, powered by a one-half horsepower electric motor, forces gas-heated air through the kiln.

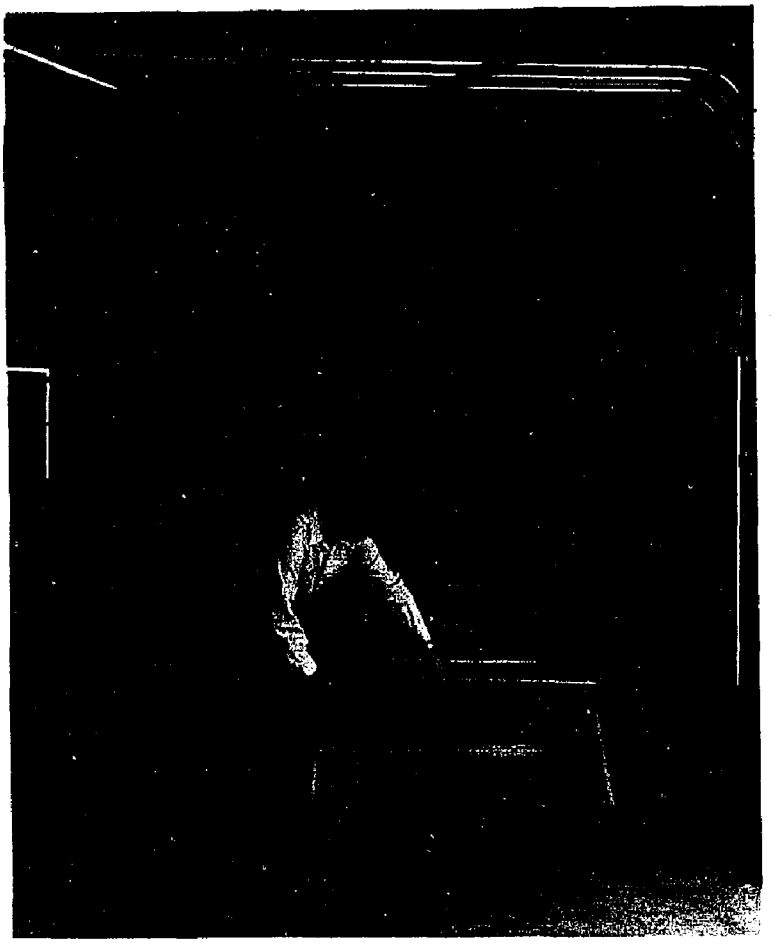


Figure 3. Cabinet portion of cone kiln with drawers removed. Each drawer has two partitions for keeping different lots of cones separate.

even sound seed will float in most liquids. However, the specific gravity of n-pentane is only 0.62 at 25 C, which is low enough to permit filled longleaf seed to sink (Fig. 4). McLemore (1965) demonstrated that this method of separating full and empty longleaf pine seed was not harmful to viability. Although seeds for the present studies remained in the pentane for less than 1 minute, his tests showed that soaking seed for as long as 4 hours was not detrimental.

Germination tests with seed extracted in the darkroom for study no. 1 were initiated immediately following extraction. Seeds for the three other studies were sealed in moisture-proof polyethylene bottles, following the cleaning process, and stored at -5 C until the tests were started.

Germination Tests

All germination tests in the four series of studies were conducted with 100-seed samples from each of the four single-tree lots, or replications. The 100-seed samples were placed in two germination dishes, each containing 50 seeds. The germination dishes were made of clear plastic and measured 12 x 12 x 3 centimeters. They were fitted with slip-top covers that prevented dessication but did not make an air-tight seal. During stratification and germination in darkness, the two dishes for each replication were kept inside a light-proof bag (Fig. 5). The cloth bags were made of a double thickness of black sateen and measured approximately 30 x 45 centimeters. Although the double thickness was sufficient to exclude light, the bags were folded over the tops of dishes, giving six thicknesses of the cloth rather than two.

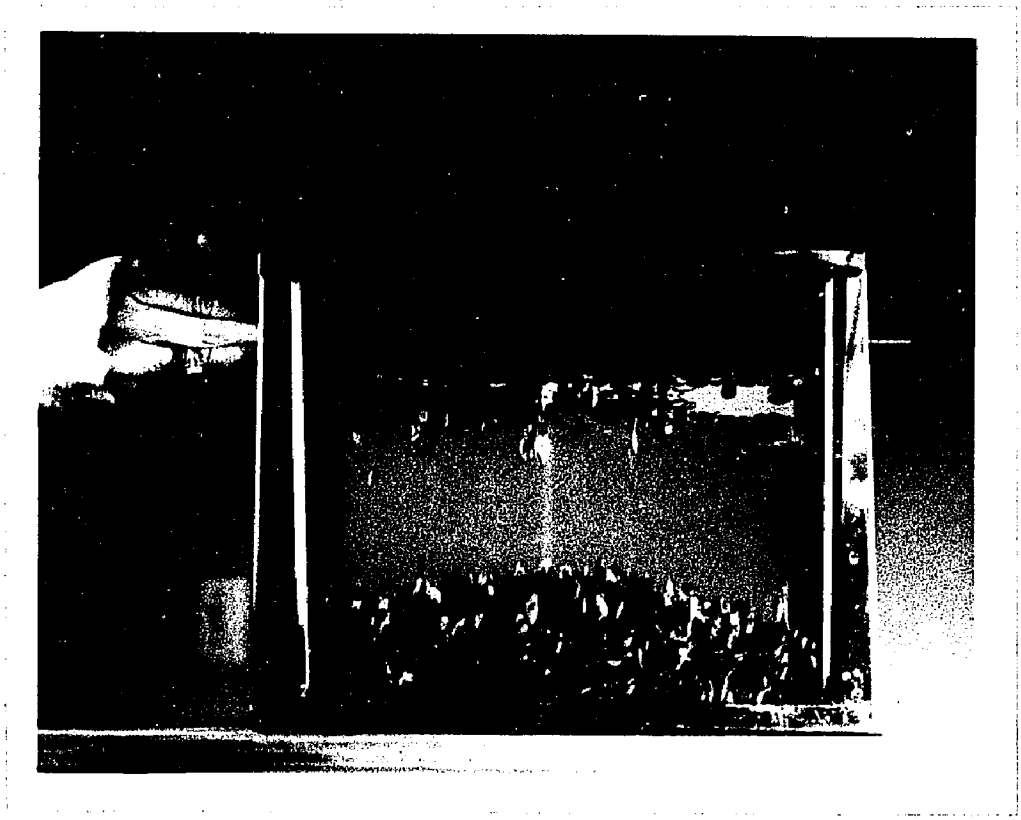


Figure 4. Separation of full and empty longleaf pine seed by flotation in n-pentane. Seeds that have sunk are 100 percent sound; those floating are empty.

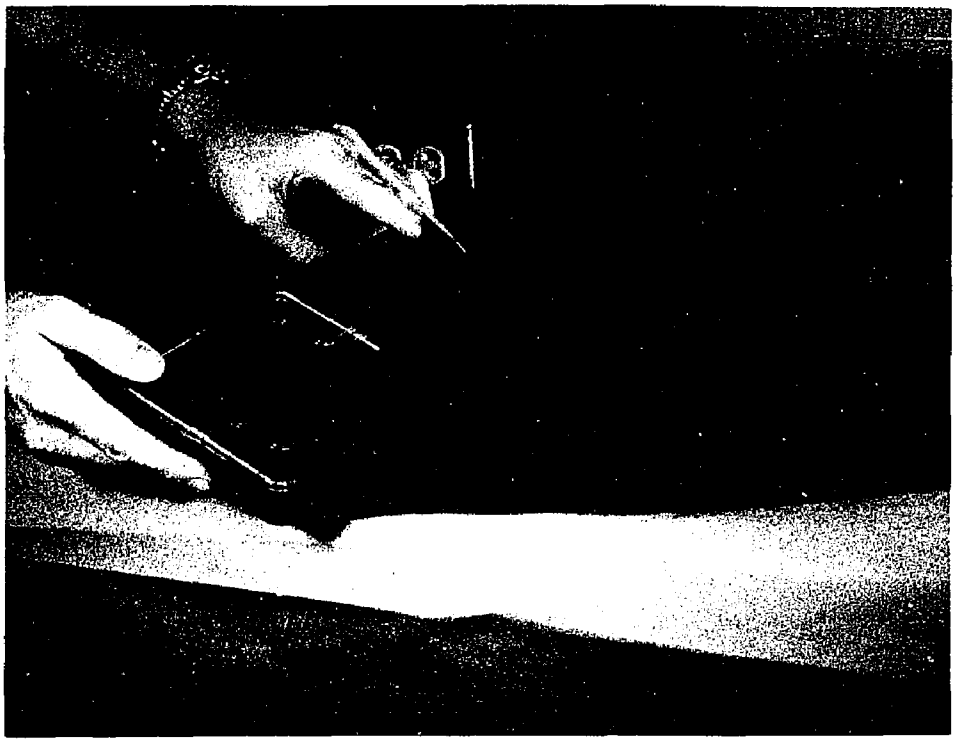


Figure 5. Placing a dish of seed into a black, light-proof, cloth bag.

The germination medium consisted of two parts granulated peat moss and one part sand. These two components were moistened, thoroughly mixed, and placed in the germination dishes to a depth of 1½ centimeters. After moistening with distilled water, pH of the medium was 5.8. Fifty seeds were placed in each dish by hand on the surface of the medium and the seed firmed down on the medium with a packing board cut to fit inside the dishes. The seeds and medium were then watered until saturated with a hand sprinkler. No excess water was left in the dishes. Immediately after wetting, the two dishes containing a 100-seed sample were placed inside one of the black, cloth bags.

Seeds receiving a stratification treatment were held in the light-proof bags inside a walk-in cooler at 1 C. In studies where different lengths of stratification were used, the dates that seeds were placed in stratification were arranged so that all seeds were moved to the germination temperature on the same day. Unstratified seeds were imbibed for 24 hours on the wet germination medium at 22.5 C prior to irradiation treatments.

Germination tests were conducted in the Alexandria Timber Management's seed testing laboratory (Fig. 6). Temperature of this room was maintained at 22.5 C $\pm$ 1 during the time of testing. The black, cloth bags containing dishes of seed were placed on shelves in the laboratory, one layer deep. Although each shelf in the laboratory is equipped with lights, the lights were turned off during the dark-germination phase of each test. The germination tests

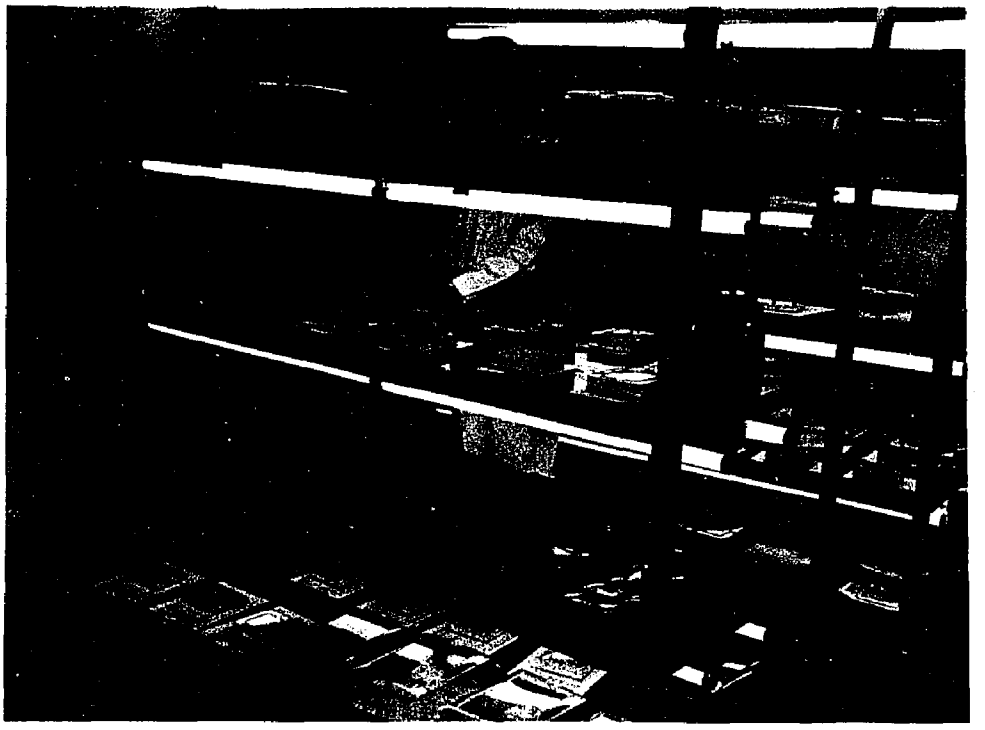


Figure 6. Laboratory where germination tests were conducted. Dishes on second shelf from bottom have been removed from black bags to determine potential germination in light.

in darkness generally lasted for 14 days. At the end of this period, the dishes were removed from the cloth bags and lights turned on over the shelves. Two, 30-watt, cool white, fluorescent light bulbs over each shelf provided approximately 120 foot-candles of light at the level of the seed. The photoperiod during this phase of the germination tests was 16 hours.

Germination counts were made periodically during both the dark- and light-germination phases. Seedlings were removed from the dishes at the time of these counts. A seed was considered as having germinated when the radicle had protruded from the seed coat for approximately ½ centimeter. Most of the time, however, cotyledons had emerged by the time seedlings were counted. Longleaf pine seedlings may reach the cotyledon stage within 2 or 3 days after protrusion of the radicle. Counts during the dark-germination phase of tests were made in a darkroom under a safe-green light. This was the same light mentioned earlier in connection with extraction and cleaning of seed for study no. 1. It will be described in the following section.

Lights and Filters

Three different lights were used during the course of the studies: a safe-green, red, and far-red light.

The safe-green light, used in the cleaning process of dark-extracted seed and for germination counts in the darkroom, consisted of a 20-watt, fluorescent light bulb wrapped with a piece of green gelatin filter. Preliminary tests with the safe-green light showed

that seeds do not respond to irradiation from this source. In one series of tests involving 4,800 loblolly pine seeds, germination in complete darkness during the entire period averaged 12.5 percent, while germination of seed exposed to the safe-green light for 5 minutes per day during the 14 days of testing averaged 12.4 percent. In another series of tests with 3,200 longleaf pine seeds, comparable values were 9.1 and 9.5 percent for tests conducted in complete darkness and exposed to the safe-green light, respectively.

Withrow and Price (1953) have described the process for preparation of gelatin filters to isolate narrow regions of the spectrum. The specific piece of gelatin used was obtained from the Smithsonian Institution's Division of Radiation Biology. Transmission of light as measured with a Beckman DK-2 spectrophotometer was in the range of 530 to 580 nm with a sharp peak at 550. Transmission at the peak was only 15 percent; hence the safe-green light used in these studies was very dim, measuring approximately 1 foot-candle on a Weston light meter. Irradiation intensity from the light was less than 100 ergs/cm^2/sec. This intensity was measured with a model 65 radiometer manufactured by Yellow Springs Instrument Company, Yellow Springs, Ohio.

The YSI model 65 radiometer consists of a thermistor bolometer, a compensating thermistor, a range attenuator, a chopper stabilized D.C. amplifier, and a meter readout. The thermistors operate in a Wheatstone Bridge with voltage output directly proportional to radiant energy. This signal is then scaled, amplified, and presented on the

meter. The meter reads in either ergs/cm^2/sec or milliwatts per cm^2. The sensor consists of two legs of a bridge circuit, each of which is a thermistor. One thermistor is attached to a metallic target which is coated with a special absorptive material (Krylon 1602) and is used as the receptor for radiant energy. The second thermistor is shielded from the radiation but is exposed to the ambient temperature as a reference. Intensity of irradiation from the red and far-red light sources was also measured with this instrument.

The red light source used in the studies described here consisted of a bank of eighteen, 96-inch, T8, cool white, fluorescent tubes manufactured by Westinghouse. This light was filtered through two layers of 300 MSC red cellophane manufactured by Brooks Paper Company, St. Louis. Two layers of the red cellophane stops essentially all irradiation in the visible portion of the spectrum below 580 nm. Above this point, there is a sharp increase in transmission to 660 nm. A large proportion of the radiation from fluorescent lights is in the red region of 580 to 680 nm, and very little in the region beyond 700 nm. Therefore, the double thickness of red cellophane over a fluorescent light source results in a fairly narrow band of light with a sharp peak at 660 nm. Seeds were irradiated at a distance of 1.1 meters from the lights. Relative intensity of light at the level of the seeds was about 25 foot-candles, but the irradiation intensity was approximately 2,600 ergs/cm^2/sec. V. K. Toole _et al_. (1961 and 1962) reported an intensity of 6,000 ergs/cm^2/sec for a light of almost identical construction.

The far-red light source used in these studies was obtained by filtering the light from three, 300-watt, internal-reflector, incandescent floodlights through two layers of 300 MSC red cellophane, two layers of 300 PC dark blue cellophane, and 6 cm of water. The 300 PC blue cellophane is no longer manufactured, but the material used in these studies was obtained from Dr. H. A. Borthwick who had purchased it from Brooks Paper Company. As mentioned earlier, two layers of the red cellophane stops essentially all irradiation with wave lengths shorter than 580 nm. The two layers of blue cellophane effectively blocks out irradiation in the red portion of the spectrum from 580 to 680 nm. Hence, the only light admitted through this system of filters had wave lengths greater than 680 nm, which is in the far-red portion of the spectrum. Moreover, incandescent lights emit a high proportion of radiation in this region. Water in the system served to filter out infra-red or heat waves which are emitted by incandescent lights. The 6 cm of water used removed essentially all waves longer than 780 nm. Hence, the far-red light system consisted of waves in the range of 680 to 780 nm with a high peak at 730 nm. Seeds were exposed at a distance of 1.1 meters from the light source. Visible light at this level was quite low, measuring only 1 foot candle, but the intensity of irradiation was over 9,000 ergs/cm^2/sec.

In constructing the far-red light source, the three floodlights were mounted inside a bottomless plywood box. The sides of the box were lined with glass, and a piece of glass was fitted over the bottom to hold water. The only appreciable irradiation filtered out by glass is in the ultra-violet region of the spectrum.

Since the box containing the floodlights was light-proof, except for the glass bottom, provision had to be made for preventing over-heating due to the 900 watts of lights inside the box. This was accomplished by mounting an exhaust fan in one end of the box and cutting a vent in the other end. A series of black baffles at both ends maintained the lightproof condition, yet permitted air to be drawn through the box by the exhaust fan. Since fluorescent lights give off little irradiation in the infra-red range, heating of the red light source posed no problem.

Both the red and far-red lights were located in a darkroom equipped with a light-lock so that anyone could enter or leave the room while an irradiation treatment was in progress without admitting light from the outside. Each of the lights were equipped with a Universal timer with luminous dials that could be set for periods ranging from 1 second to 60 minutes. For some of the irradiation treatments which lasted 64 minutes, the timer was set for an additional 4 minutes immediately following a 60-minute period of irradiation.

Seeds that received irradiation treatments under the red and far-red lights were brought into the darkroom in the black, cloth bags. Dishes of seed were then removed from the bags and placed on a table beneath the lights and the timer set for the prescribed length of time. Upon completion of irradiation treatments, dishes were replaced in the black bags and moved to shelves in the germination room.

Methodology for the Four Studies

Study No. 1

The purpose of this study was to evaluate the effect of light on unimbibed longleaf pine seed. As noted earlier, seeds from four different trees were extracted and dewinged in a darkroom. The moisture content of fully imbibed longleaf seed generally exceeds 70 percent, computed on a dry weight basis, and the moisture level of freshly extracted seeds is often over 30 percent, even though these seeds appear dry on the surface. Because of the high moisture content of freshly extracted seed, each of the single-tree lots were divided in half for a drying treatment. One sample was dried in darkness to a low moisture level and the other sample remained undried.

Drying in complete darkness was accomplished by enclosing the dewinged seed in a loosely rolled, black, cloth bag and placing the bag in a forced-air, ventilated oven at 38 C for 24 hours. Moisture determinations of dry and nondry seed from each of the four lots showed the following results:

	Percent moisture	
	Nondry seed	Dried seed
Lot 1	36.4	6.4
Lot 2	33.8	5.2
Lot 3	34.9	8.2
Lot 4	35.8	5.9

Each of the eight samples listed above were again divided in half for an irradiation treatment and a dark control. There were approximately 150 seeds in each of the resulting 16 subsamples. One hundred seeds from each of the eight subsamples to receive a light treatment were placed in a single layer in a clean, dry, plastic dish. The dishes of seeds were placed on a shelf in the testing room at 22.5 C and exposed to 120 foot-candles of light for 23 hours. This was followed by a 1-hour exposure to the red light to insure that all phytochrome in the seeds, if present, had an opportunity to be converted to the PFR form. The other eight samples of seed were kept in darkness during this time.

Following the irradiation treatment, all seeds were placed on the germination medium and moistened as described earlier. Seeds that were not exposed to light were placed in germination dishes under the safe-green light in the darkroom. All seeds in this study were tested in darkness in the black, cloth bags. This series of tests was started on October 26, 1965. After 28 days of testing in darkness, all dishes were removed from the bags and placed under lights in the seed laboratory for an additional 35 days when germination was judged to be essentially complete.

The effect of drying and the irradiation treatment on dark germination was evaluated by an analysis of variance with a split-plot design. The four single-tree lots of seed were considered as blocks (replications); dried and undried seed constituted the major plots; minor plots consisted of irradiated and unirradiated seed. The

following is a breakdown for degrees of freedom in the analysis:

Source of Variation	Degrees of Freedom
Blocks (seed lots)	3
Drying treatment (D)	1
Error I	3
Irradiation treatment (I)	1
D x I	1
Error II	6
Total	15

Arcsin $\sqrt{\text{percentage}}$ transformation of dark-germination was used in the analysis. Duncan's (1955) Multiple Range Test (referred to as Duncan's MR test in succeeding tables) was used to determine treatment differences between arrayed means.

Study No. 2

As noted earlier, seed for the second, third, and fourth studies were dried in the cone kiln during the extraction process. Moisture content of the individual-tree lots was uniformly low, averaging 10.1 percent. Specific moisture levels of the four lots were:

Lot No.	Percent Moisture
1	11.6
2	7.6
3	11.7
4	9.6

The purpose of the second test was to evaluate light- and dark-germination of longleaf pine seed stratified for different lengths of time. Duplicate 100-seed samples from each of the four lots were stratified for 7, 14, and 28 days. Stratification was accomplished in complete darkness at 1 C as described earlier. In addition, an unstratified 100-seed sample that had been imbibed at 22.5 C for 24 hours was also tested for each lot. The stratification treatments were timed so that all were completed and the seed moved to the germination temperature on the same day -- December 28, 1965.

Upon completion of the pregermination treatments, one of the 100-seed samples from each lot and stratification treatment was removed from the cloth bag for a test of germination in light under the standard laboratory conditions described earlier. The other sample was left in the bag to germinate in darkness. After 14 days of testing, seeds in the dark tests were removed from the black bags to determine their germination potential. Analysis of the results was made on the basis of germination obtained during the first 14 days, however. Results from study no. 1 had indicated that germination in darkness was essentially complete after this length of time.

This study also had a split-plot design. As in all studies, the four seed lots were considered as blocks. The various lengths of stratification constituted the major plots and germination condition (light vs. darkness) the minor plots. The following is a breakdown for degrees of freedom in the analysis:

Source of Variation	Degrees of Freedom
Blocks (seed lots)	3
Stratification treatments (S)	3
Error I	9
Germination environment (G)	1
S x G	3
Error II	12
Total	31

Germination percentages after 14 days of testing were transformed to arcsin $\sqrt{\text{percentage}}$ in running the analysis. Duncan's (1955) Multiple Range Test was used to determine treatment differences between arrayed means.

Study No. 3

The objective of this study was to determine if germination of longleaf pine seed can be promoted and inhibited through the photo-reversible phytochrome system, and the amount of light necessary for this process.

All lots of seed were subjected to the same stratification treatments as in study no. 2. Twelve, 100-seed samples from each lot received each stratification treatment in complete darkness, with unstratified seed being imbibed overnight at 22.5 C. A 100-seed sample from each lot and stratification treatment received each of the following ten irradiation treatments prior to being transferred to the germination temperature for testing in darkness.

Treatment No.	Red Light (660 nm)	Far-red Light (730 nm)
	Minutes	
1	¼	0
2	1	0
3	4	0
4	16	0
5	64	0
6	64	¼
7	64	1
8	64	4
9	64	16
10	64	64

In addition, one sample served as a dark control, receiving no irradiation and being tested in darkness. The twelfth sample constituted the light control. These seed received no irradiation prior to testing, but were germinated in the light under standard laboratory conditions.

Due to the number of samples involved in this study and the length of time required for the irradiation treatment, tests with all lots of seed could not be instituted on the same day. Hence, all seeds from a particular lot, or replication, were irradiated and the tests started on the same day, but there was an interval of 1 week between initiation of tests for each of the four different lots. Irradiation treatments were applied to seed from lot no. 1 on December 28, 1965. Tests of lots 2, 3, and 4 were started on January 4, 11, and 18, 1966, respectively.

After 14 days of testing, all dishes of seed were removed from the black bags and potential germination was determined by continuing the tests in light. As in test no. 2, the 14-day results were used in analyzing results. Arcsin $\sqrt{\text{percentage}}$ transformation of the

data was used in conducting the analysis of variance. The analysis had a 4 x 12 factorial design with the following breakdown for degrees of freedom:

Source of Variation		Degrees of Freedom
Blocks (seed lots)		3
Treatments		47
Stratification treatment (S)	3	
Irradiation treatment (I)	11	
S x I	33	
Error		141
Total		191

Least significant differences (LSD) between means of transformed percentages were computed for distinguishing between treatments. Since the number of comparisons made with LSD cannot exceed the degrees of freedom for treatments, 47 comparisons were selected to be made. For each length of stratification, germination of the dark control was compared with irradiation treatments 1 through 5, consisting of increasing lengths of exposure to red light and no exposure to far-red light. This accounted for 20 of the comparisons. An additional 20 comparisons were made between treatment no. 5 (64R - 0FR) in each stratification period with treatments 6 through 10, wherein the exposure to red light was held constant at 64 minutes but lengths of the far-red exposure were increased. The dark control for each stratification period was also compared with

irradiation treatment no. 10 (64 R - 64 FR), accounting for four more of the degrees of freedom. Finally, comparisons were made between ranked means of the four stratification treatments, accounting for the final three degrees of freedom.

Study No. 4

The objective of this study was to investigate the potential for germination of longleaf pine seed to be repeatedly promoted and inhibited by red and far-red light. Periods of stratification and irradiation used in this series of tests were dependent on results obtained from study no. 3.

Two lengths of stratification were selected that appeared to condition longleaf seeds to a highly light-sensitive state. The two periods chosen were 7 and 14 days. Eight, 100-seed samples from each of the four lots were subjected to each length of stratification in complete darkness. Immediately following these treatments, all seeds received a high enough level of irradiation with red light to convert most of the phytochrome to the active, PFR form. The length of exposure to the red light source was determined to be 4 minutes from study no. 3. Immediately following the 4 minutes of irradiation in the 660 nm range, one of the 100-seed samples for each lot from each of the stratification treatments was returned to the black, cloth bag for germination in darkness. The seven remaining samples were then irradiated with far-red light in the 730 nm range for 4 minutes to convert the phytochrome to the PR, or inactive form. Again, a 100-seed sample from each stratification treatment was removed and

placed in a cloth bag for germination in darkness. This process of alternate irradiations with red (R) and far-red (FR) light for 4-minute periods was continued in the following manner until all samples were exhausted:

Sample Number	Irradiation Treatment
1	R
2	R FR
3	R FR R
4	R FR R FR
5	R FR R FR R
6	R FR R FR R FR
7	R FR R FR R FR R
8	R FR R FR R FR R FR

Since only 14 dishes (seven 100-seed samples) could be irradiated conveniently under the far-red light at one time, each lot and stratification treatment were irradiated and germination tests started on different dates in 1966 according to the following schedule.

Lot No.	Days of Stratification	Date Test Started
1	7	February 4
1	14	" 11
2	7	February 10
2	14	" 17
3	7	February 14
3	14	" 21
4	7	February 16
4	14	" 23

As in previous studies, the dark-germination tests lasted for 14 days at which time all seeds were placed in light to determine potential germination. An analysis of variance with a 2 x 8 factorial design was conducted with arcsin $\sqrt{\text{percentage}}$ transformations after the 14 days of testing in darkness. As in all studies, seed lots were considered as blocks. The following is a breakdown for degrees of freedom used in the analysis.

Source of Variation		Degrees of Freedom
Blocks (seed lots)		3
Treatments		15
Stratification treatment (S)	1	
Irradiation treatment (I)	7	
S x I	7	
Error		45
Total		63

Orthogonal comparisons were made to determine the effectiveness of the repeated photoreversibility process. In these comparisons, germination of all seeds receiving terminal irradiation treatments with the red light source (1, 3, 5, and 7) were compared with those receiving a final irradiation with far-red light (2, 4, 6, and 8). Treatment 1, which was a single, 4-minute period of irradiation with red light, was compared with treatment no. 7, which included seven alternations of 4-minute periods of red and far-red light with a terminal exposure to red light. Finally, treatment 2, which

consisted of a single exposure to red light followed by a single exposure to far-red light, was compared with treatment no. 8, which included eight alternations between the two light sources with a terminal exposure to far-red light. The following outline summarizes the comparisons made:

Comparisons	Irradiation treatment nos. 1	2	3	4	5	6	7	8
1, 3, 5, & 7 vs. 2, 4, 6, & 8	+	-	+	-	+	-	+	-
1 vs. 7	+						-	
2 vs. 8		+						-

The same three comparisons were made for both lengths of stratification. Hence, only 6 of the 15 degrees of freedom for treatments were utilized in making the orthogonal comparisons. While 9 other comparisons could have been made, those listed above were considered to be the only ones which were of interest.

RESULTS AND DISCUSSION

Study No. 1

Results of this study showed that light is extremely important in the germination of longleaf pine seed. Moreover, in order to elicit a response from light the seed must be in a moist condition.

After 28 days of testing in darkness, germination of both dry and nondry, unirradiated seed averaged less than 2 percent (Table 1). When dry seeds were exposed to light, germination averaged only 5 percent and did not differ significantly from that of unirradiated seed (Table 2). However, when nondry seeds were exposed to light and subsequently tested in darkness, germination of the four lots averaged 90 percent. Although these seeds were in a nondry condition when the irradiation treatment was applied, they were not imbibed. As previously noted, moisture content of these seed was about 35 percent, whereas completely imbibed seeds reach levels of 70 percent or above.

These results may constitute a partial explanation of Nyman's (1963) conclusions that irradiation of dry Scotch pine seed influences germination. Although his seeds were unimbibed when the irradiation treatments were applied, it is possible that enough moisture was retained by the seed to allow a response to the light treatment. It is recognized at this point that the moisture level of Scotch pine seeds used by Nyman was approximately 6 percent. However, Scotch pine

Table 1. The effect of light treatment and moisture on germination of longleaf pine seed extracted in darkness

Lot no.	Moisture level	Germination after 28 days in darkness		Germination after 35 additional days in light	
		Unirradiated	Irradiated	Unirradiated	Irradiated
		Percent			
1	Dry	6	13	97	99
2	Dry	0	2	99	98
3	Dry	0	6	81	96
4	Dry	2	0	84	82
Avg.		2	5	90	94
1	Nondry	4	95	97	97
2	Nondry	0	94	98	100
3	Nondry	0	93	92	97
4	Nondry	1	77	88	100
Avg.		1	90	94	98

Table 2. Analysis of variance for differences in dark germination of longleaf pine seed in study no. 1

Source of variation	Degrees of freedom	Sum of squares	Mean square	F
Blocks (seed lots)	3	343.09	114.36	13.04* [1/]
Drying treatment (D)	1	3,612.91	3,612.91	411.96**
Error I	3	26.30	8.77	
Irradiation treatment (I)	1	5,356.41	5,356.41	120.10**
D x I	1	3,921.58	3,921.58	87.93**
Error II	6	267.61	44.60	
Total	15	13,527.90		

Duncan's MR Test of Treatment Means [2/]

Treatment	Nondry Unirradiated	Dry Unirradiated	Dry Irradiated	Nondry Irradiated
Transformed mean	4.32	5.56	10.86	72.22

[1/] Throughout this paper, * denotes significance at the .05 percent level, and ** denotes significance at the .01 level. Values which are not significant at the .05 level are designated by N.S.

[2/] Means underscored by the same line are not significantly different at the 0.05 level. See table 1 for actual means of germination percentages.

seeds are quite small, averaging about 75,000 seeds per pound, while longleaf seeds are several times as large, averaging 4,500 seeds per pound. The greater number of seeds per pound in Scotch pine would result in a proportionately greater amount of surface area and, hence, a greater proportion of seed coat material per unit weight of seed. For example, the seed coats of longleaf pine constitute approximately 22 percent of the weight of the seed while coats of slash pine seed that average 14,500 per pound account for over 40 percent of the total seed weight. Since proportionately less moisture is contained in the coats of pine seed than in the megagametophyte and embryo, 6 percent moisture in Scotch pine seeds may well correspond to a much higher level in longleaf.

Although the difference between germination of irradiated and unirradiated, dry seed in this study was not significant, there was a tendency for seed exposed to light in the dry condition to germinate best. When moisture contents of dried seeds from the individual lots are examined in conjunction with the germination percents obtained, evaluation of the results is simplified. The following tabulation lists moisture levels of dried and undried seed from the four single-tree lots, together with germination percentages obtained after the irradiation treatment.

Lot no.	Percent moisture	Percent germination	Percent moisture	Percent germination
1	6.4	13	36.4	95
2	5.2	2	33.8	94
3	8.2	6	34.9	93
4	5.9	0	35.8	77

It is noted that germination of lots 2 and 4 was lowest and that these two lots also had the lowest moisture level. However, lot 3 had the highest moisture content, yet germination of these seed was lower than for lot 1.

Undoubtedly, some of the variation is explained by differences between lots. The analysis of variance showed that differences between lots were significant at the 0.05 level (Table 2). Undried seed from lot 4 exposed to light accounted for a substantial part of this variation. In this case, moisture content of the seed was 35.8 percent, and was intermediate to the other lots. Germination in darkness was only 77 percent while that of the other three lots was 93 percent or greater, indicating a difference between seed from individual trees. Evidence to be presented later supplements this observation of greater dormancy in seed from lot 4.

Seeds from all lots and treatments showed a high potential for germination upon being transferred from darkness to light after 28 days of testing (Table 1). Radicles of seed that had failed to germinate in darkness started to protrude after about 7 days in light and germination exceeded 90 percent for most treatments when the tests were terminated after 35 days in light. Seeds that had failed to germinate after this period of time -- noticeably in lots 3 and 4 -- were sound and appeared viable upon cutting. Apparently, the holding of these seeds in an imbibed condition in darkness at 22.5 C for 28 days during the dark-germination phase threw them into a deep state of dormancy. This corresponds with the findings of the other research workers mentioned earlier, that the active form of phytochrome (PFR) shifts to the inactive form in darkness.

The highly significant interaction between drying level and irradiation treatment shows that these two factors are dependent on each other. Detailed data pertaining to the time course of germination for seed used in study no. 1 are presented in Appendix Table 16.

Study No. 2

Results of this study again showed the superiority of longleaf pine seed germination in light over that obtained in darkness. Perhaps of greater importance, it also showed the strong interrelationship between stratification (imbibition at a low temperature) and light. Finally, conclusive evidence was obtained to demonstrate the different reactions of seed from single-tree lots.

Germination of seed tested in light was consistently higher than for seed tested in complete darkness (Table 3). Moreover, when seeds were tested in light, the percentage of germination remained fairly constant, regardless of length of stratification. The overall average was over 96 percent, which indicates the high germination potential of lots used in these studies. When seeds were tested in darkness, however, there was an increase in germination with each lengthening of the stratification period.

Table 3. Germination of longleaf pine seed in light and darkness following various periods of stratification

Lot no.	Days of stratification			
	0	7	14	28
		Percent		
	Tested in darkness [1/]			
1	57	60	71	89
2	6	13	51	97
3	17	39	45	92
4	8	3	17	42
Avg.	22	29	46	80
	Tested in light			
1	98	98	99	97
2	93	97	97	94
3	95	98	97	98
4	93	99	94	96
Avg.	95	98	97	96

[1/] Length of test was 14 days.

The uniform, consistently high germination obtained in light, regardless of length of stratification, and the increase in dark-germination with each successive increase in stratification resulted in a significant interaction between length of stratification and germination environment (Table 4). This interaction is depicted graphically in Figure 7. 1/

This study showed the ability of seeds to germinate in darkness varies markedly between lots from individual trees. Although study no. 1 demonstrated that dark germination may be influenced by exposing seeds to light immediately after extraction from cones, seed for this and the two subsequent studies were extracted in a cone kiln where there was essentially no light. Admittedly, the seeds may have been exposed to light for brief periods during the drying process when drawers of the kiln were opened. Since all seeds were extracted and processed in the same manner, however, differences between lots in this study are believed to be due to individual-tree variation rather than any preferential exposure of one lot to light over that of another.

Germination of unstratified seeds in darkness ranged from 6 percent for lot 2 to 57 percent for lot 1. Seeds in lot 1 in study no. 1 also showed a tendency to germinate better in darkness than any other lot. Although germination of unstratified seed in lot 2 was the poorest of any, when these seeds were stratified for 28 days and

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1/ Freese (1956) has pointed out that interaction does not require that the trends actually reverse. For example, if A is much better than B in the presence of one treatment but only a little better in the presence of some other treatment this is a form of interaction.

Table 4. Analysis of variance for differences in germination of longleaf pine seed in study no. 2 after 14 days

Source of variation	Degrees of freedom	Sum of squares	Mean square	F
Blocks (seed lots)	3	1,487.54	495.85	9.95**
Stratification treat. (S)	3	1,974.81	658.27	13.21**
Error I	9	448.47	49.83	
Germination environment (G)	1	11,780.35	11,780.35	100.64**
S x G	3	1,987.07	662.36	5.66*
Error II	12	1,404.64	117.05	
Total	31	19,082.88		

Duncan's MR Test of Treatment Means [1/]

Stratification treat.	7	14	28	0	28	14	7	0
Germ. environment	Lt.	Lt.	Lt.	Lt.	Dk.	Dk.	Dk.	Dk.
Transformed mean	82.00	80.03	79.04	77.07	66.16	42.37	30.13	26.00

[1/] Means underscored by the same line are not significantly different at the 0.05 level. See table 3 for actual means of germination percentages.

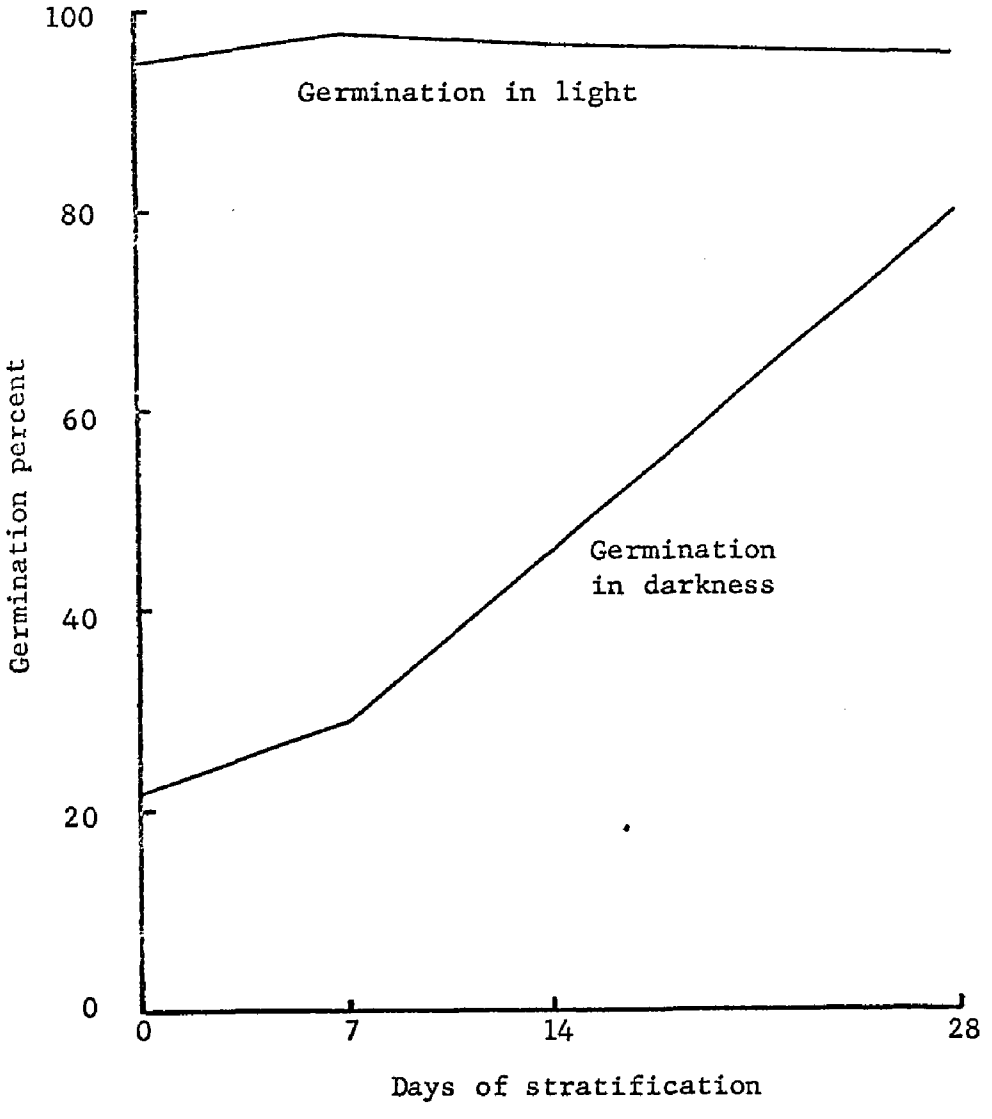

Figure 7. Germination of longleaf pine seed in light and in darkness after different lengths of stratification.

tested in darkness, germination was higher than for any other lot -- averaging 97 percent (Table 3). The poorest germination obtained in darkness after 28 days of stratification was with lot 4. Germination of these seed was only 42 percent. Dark-germination of seed from lot 4 was also low in study 1, even when undried seeds were irradiated and tested in darkness. This strengthens the evidence for variation in light requirements for seed from individual trees.

Results of this study indicate that 28 days of stratification was long enough to essentially eliminate the light requirement for germination of three of the four lots tested. When Duncan's multiple range test was applied to the arrayed treatment means, germination of seed stratified for 28 days and tested in darkness was not significantly different from that of all seed tested in light (Table 4).

After 14 days of testing, seeds in the dark-germination phase were placed in light to determine potential germinability. Seeds from all treatments in lots 1, 2, and 3 germinated quite promptly upon removal to light. After 14 days in light, germinability of these three lots ranged from 89 to 99 percent and averaged 94 percent (Appendix Table 17). These tests were terminated at this point. Seeds from lot 4, on the contrary, germinated quite slowly upon removal to light and averaged only 56 percent after 14 days of testing, having increased to this amount from an average of 18 percent at the end of the dark-germination test. Germination of these seed essentially stopped at this point and had reached an average of only 71 percent after a total of 80 days. At this time the seeds, which still

appeared to be viable, were stratified for 14 days in an attempt to determine whether they were nonviable or dormant. This resulted in an increase to 86 percent at the end of 101 days. These seeds continued to germinate, one or two at a time, until a total of 230 days (nearly 8 months) had elapsed when they averaged 99 percent germination. The tests for lot 4 were terminated at this time. The course of germination for this lot of seed is summarized in Table 5. Although the data in Table 5 shows an increase from 71 to 86 percent between 80 and 101 days of testing, it is noted that all of this germination occurred within a 7-day period -- between 94 and 101 days. The seeds were in stratification at 1 C between 80 and 94 days; consequently, no germination occurred during this period.

The foregoing example is dramatic evidence of the deep state of dormancy that can be induced in some lots of longleaf pine by holding the imbibed seeds in darkness at room temperature for 14 days. V. K. Toole and Borthwick (1966) have also reported that when seeds of Eragrostis curvula are held in an imbibed condition in darkness for as long as 4 days, subsequent germination in light is greatly reduced. Shuck (1936) and Nutile (1943) have reported similar findings with lettuce seed.

Detailed data pertaining to the time course for germination of all lots of seed used in this study are presented in Appendix Table 17.

Table 5. Cumulative germination of longleaf pine seed from lot 4 used in study no. 2

Days of strat.	Germ. at end of dark test	Germination after testing in light			
		14 days	80 days	101 days	230 days
	Percent				
0	8	43	66	92	99
7	3	58	65	76	100
14	17	64	79	90	98
28	42	59	75	88	98
Avg.	18	56	71	86	99

Study No. 3

In this study, as in study no. 2, germination of seed in darkness was much lower than in light. Also, each increase in length of stratification resulted in higher germination in darkness. Overall germinability for light-control seeds was 97 percent, whereas seeds kept in complete darkness averaged 48 percent (Table 6).

Perhaps of greater importance was the demonstration of promotion of germination with red light and inhibition with far-red light. Table 7 shows the transformed means of germination percentages by length of stratification and irradiation treatment.[2/] Each mean in the main body of the table consists of an average of the four lots of seed used. Table 8 shows that differences due to the stratification and irradiation treatments were significant at the 0.01 level. Differences between lots of seed were also significant at the 0.01 level. The analysis indicates there was no interaction between the stratification and irradiation treatments. Although increases in length of stratification generally resulted in higher germination, the promotion and reversal trends due to irradiation treatments were fairly consistent, regardless of length of stratification. Perhaps if longer periods of stratification had been used, the stratification x irradiation interaction would have been significant because germination of the dark control and all seeds receiving red light would have been uniformly high. Irradiation of seeds with red light for 15 seconds

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2/ Transformed values are presented in table 7 rather than percents because the LSD's were computed from the transformations.

Table 6. Germination of longleaf pine seed stratified for different periods and exposed to various lengths of irradiation with red and far-red light

Light treatment		Days of stratification				
Red	Far-red	0	7	14	28	Avg.
----Minutes---		----------Percent--[1/]----------				
Light control		96	95	98	98	97
Dark control		27	35	52	77	48
½	0	30	50	62	82	56
1	0	38	58	68	90	64
4	0	62	71	86	90	77
16	0	46	77	83	96	76
64	0	58	86	90	95	82
64	½	49	70	84	93	74
64	1	45	62	69	88	66
64	4	34	42	57	81	54
64	16	17	16	29	73	34
64	64	15	24	38	66	36
Avg.		43	57	68	86	

[1/] After 14 days of testing.

Table 7. Means of transformed germination percentages of longleaf pine seed used in study no. 3 [1/]

Light treatment		Days of stratification				
Red	Far-red	0	7	14	28	Avg.
---Minutes-----		----------Transformed mean----------				
Light control		80.47	79.06	84.24	83.30	81.77
Dark control		28.68	35.10	46.29	62.87	43.23
¼	0	31.94	44.60	53.45	67.56	49.39
1	0	37.13	50.75	58.53	73.48	54.97
4	0	53.44	59.70	68.82	72.62	63.64
16	0	42.23	63.17	69.34	78.87	63.40
64	0	50.59	69.75	72.51	77.50	67.59
64	¼	44.30	57.68	67.59	75.06	61.16
64	1	42.99	55.98	58.70	71.95	57.40
64	4	34.16	39.77	49.67	66.53	47.53
64	16	18.65	16.60	30.68	60.04	31.49
64	64	18.99	24.80	33.92	55.86	33.39
Avg.		40.30	49.75	57.81	70.47	

[1/] $LSD_{.05}$ for determining significant differences between means of irradiation treatments within each stratification period is 17.59.

$LSD_{.05}$ for determining significant differences between overall average of means of irradiation treatment is 8.79.

$LSD_{.05}$ for determining significant differences between overall average of means of stratification treatments is 5.07.

Table 8. Analysis of variance for differences in germination of longleaf pine seed used in study no. 3

Source of variation	Degrees of freedom	Sum of squares	Mean square	F
Blocks (seed lots)	3	21,804.11	7,268.04	45.97**
Treatments	47	64,790.02	1,378.51	8.72**
Stratification treat. (S)	3	23,531.91	7,843.97	21.23**
Irradiation (I)	11	36,916.44	3,356.04	49.61**
S x I	33	4,341.67	131.57	1.20 [1/] N.S.
Error	141	22,293.05	158.11	
Total	191	108,887.18		

[1/] Inverted ratio.

and 1 minute consistently resulted in a noticeable increase in germination over that of the dark control. Comparisons of these means by using the computed LSD showed that the differences were not statistically significant for individual periods of stratification, however. Undoubtedly this was due to the considerable amount of variation encountered combined with an insufficient number of replications. Nevertheless, it is felt that these increases, although not significant, are noteworthy. Four minutes of irradiation with red light resulted in significant increases in germination over the dark control for all periods of stratification with the exception of 28 days.

Periods of 16 and 64 minutes exposure generally resulted in little or no increase in germination over that obtained with 4 minutes of irradiation (Figs. 8 thru 11). This is strongly indicative that 4 minutes of exposure to a radiation intensity of 2,600 ergs/cm^2/sec in the region of 660 nm may be a saturation level. In fact, 16 minutes of exposure decreased germination in several instances. This decrease was particularly noticeable for unstratified seed (Fig. 8), and was largely due to the reaction of seed in lot 2. This phenomenon will be discussed later in more detail.

Since the interaction between length of stratification and irradiation treatments was not found to be significant, overall averages for each period of irradiation were computed and are listed in Table 7. In this case, where a larger number of observations resulted in a smaller LSD, exposure to red light for 1 minute resulted

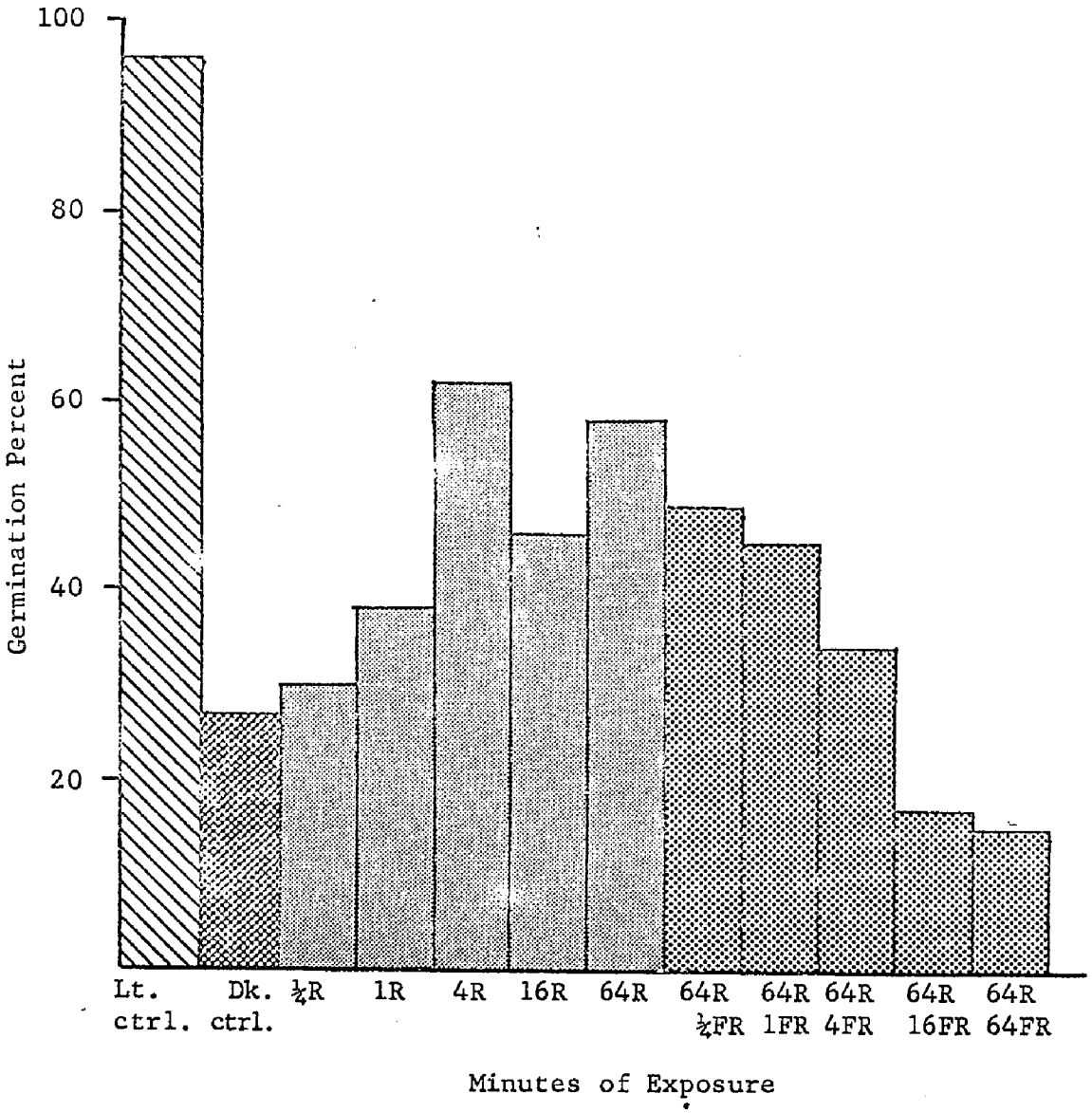

Figure 8. Germination of unstratified longleaf pine seed after 14 days in darkness following various exposures to red (R) and far-red (FR) light.

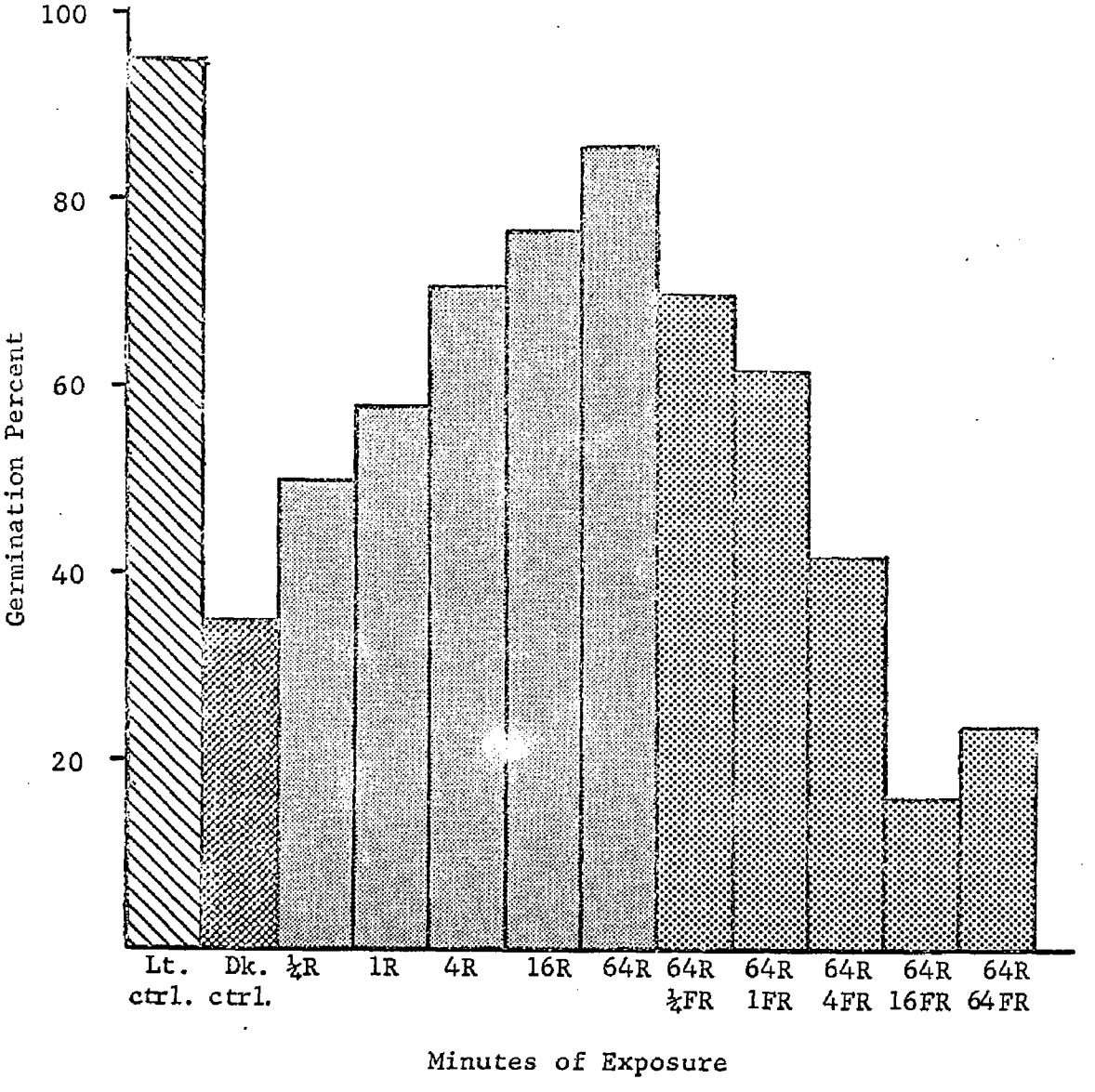

Figure 9. Germination of longleaf pine seed, stratified for 7 days, after 14 days in darkness following various exposures to red (R) and far-red (FR) light.

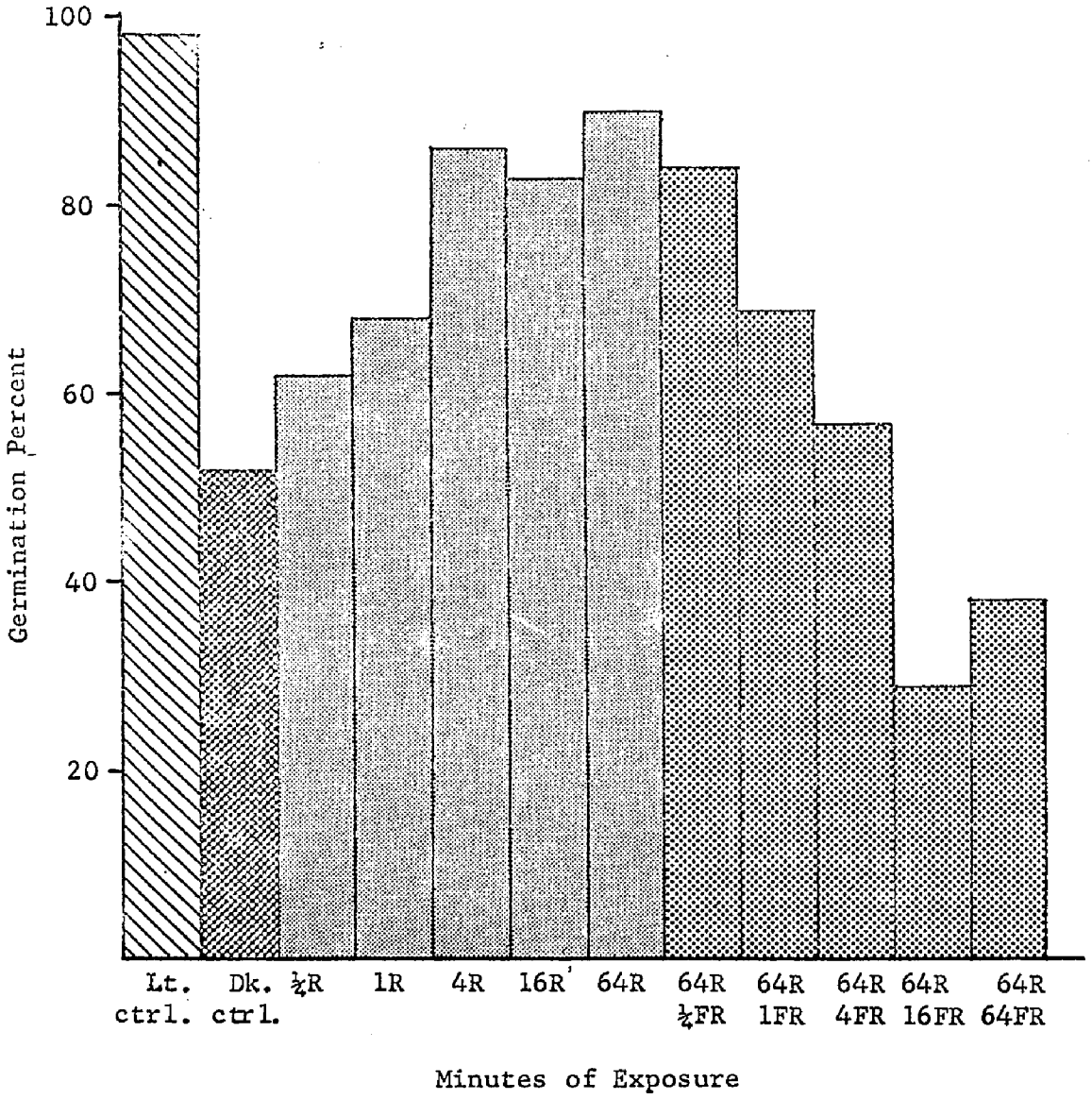

Figure 10. Germination of longleaf pine seed, stratified for 14 days, after 14 days in darkness following various exposures to red (R) and far-red (FR) light.

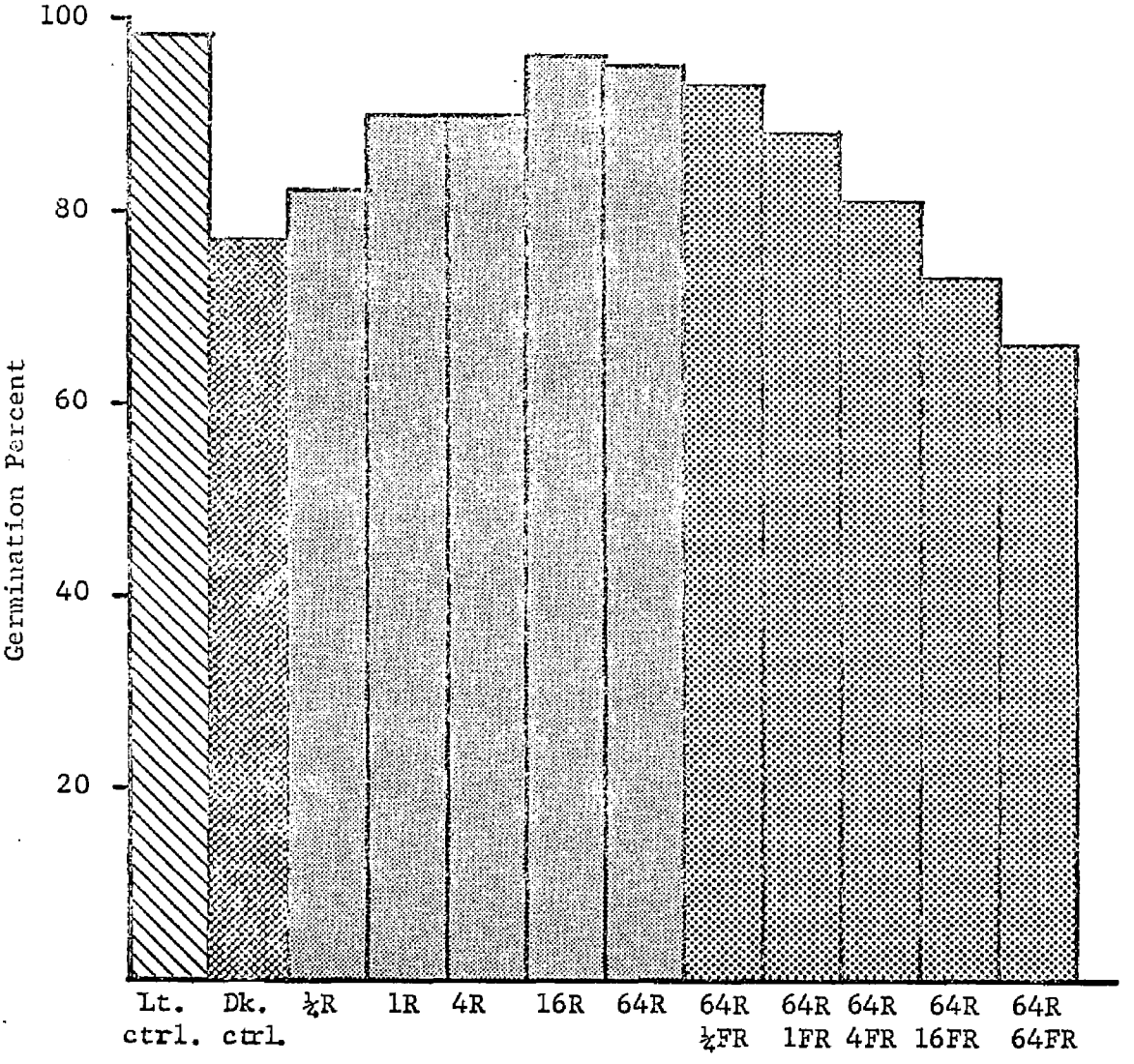

Figure 11. Germination of longleaf pine seed, stratified for 28 days, after 14 days in darkness following various exposures to red (R) and far-red (FR) light.

in a significant increase in germination over the dark control. The average germination for all dark-control seeds was 48 percent. Exposure to red light for 1 minute resulted in an overall average of 64 percent. Periods of 4 and 64 minutes increased this percentage to 77 and 82, respectively (Table 6).

After the seeds had been fully promoted by irradiation with red light for 64 minutes, increasing amounts of far-red light consistently decreased germination (Figs. 8 thru 11). Again, exposures for 15 seconds and 1 minute caused noticeable, although not significant, decreases. For the first three periods of stratification -- 0, 7, and 14 days -- 4 minutes of irradiation with far-red light caused significant reversals in germination. After 28 days of stratification, however, 64 minutes of far-red light were required to obtain a significant reduction in germination. This is further evidence that longer periods of stratification tend to lessen the control of germination by light treatments. Figure 11 depicts this evidence in a graphic manner. In this instance, where seeds were stratified for 28 days, the light requirement for most seeds was apparently overcome by the long period of stratification.

Since the intensity of irradiation from the far-red light source used in this study was slightly over 9,000 ergs/cm^2/sec, and exposure for 4 minutes was required to obtain significant reductions in germination, it would appear that a higher level of irradiation is needed for reversal than is required for promotion. Close examination of the data indicates that this may not be the case.

Figures 8 thru 11 show that 15 seconds to 1 minute of exposure to far-red light consistently decreased germination below that obtained after the 64-minute exposure to the red. Moreover, the 4-minute exposure to far-red light was sufficient to cause a drastic reduction. Little additional reversal was obtained by extending the far-red irradiation periods from 16 to 64 minutes. Indeed, in several instances exposure for 64 minutes resulted in higher germination than was obtained after 16 minutes. This response is again primarily atrributed to seed from lot 2, the aberrant lot mentioned earlier that showed decreases with prolonged exposure to red light.

Again, when the LSD computed for overall averages of the irradiation treatments was used for determining which treatments differed significantly, it was found that a 1-minute exposure to far-red light was sufficient to cause a significant reduction in germination from that obtained when seeds were exposed to 64 minutes of red light. The average germination of seeds receiving 64 minutes of red light was 82 percent; 64 minutes of red light followed by 1 minute of far-red light resulted in 66 percent germination. Extending the length of far-red irradiation to 4, 16, and 64 minutes resulted in germination percentages of 54, 34, and 36 percent, respectively (Table 6). It is noted that irradiation with far-red light for 16 minutes inhibited germination below the 48 percent level obtained in the dark controls.

The analysis of variance (Table 8) showed there were significant differences between individual lots of seed. Not only were there differences in the degree of response obtained from the various stratification and irradiation treatments, but the response itself varied between lots (Tables 9 thru 12). Lot 2 is an example of this diversity.

Extending the length of irradiation with red light generally resulted in increases in germination percentages, but there was a consistent decrease in germination of seeds in lot 2 between 4 and 16 minutes of exposure. This particular lot accounted for most of the decrease in germination between these two periods as shown in Figures 8 and 10. The drop in germination between 4 and 16 minutes was most pronounced with unstratified seed (Table 10). Unstratified seed from lot 2 exposed to 4 minutes of red light germinated 90 percent. When this exposure was lengthened to 16 minutes, germination was only 20 percent. Unstratified seed from lots 1 and 3 showed similar trends although the decrease between 4 and 16 minutes was not as pronounced as in lot 2.

The reason for the decrease in germination of seed between 4 and 16 minutes of exposure to red light is unknown. Personal communication with Dr. H. A. Borthwick and Mrs. V. K. Toole also failed to yield an explanation for the phenomenon. A supplementary test with seed from lot 2 was conducted to further evaluate the effects of red light on germination of this particular lot. In this test, seeds were imbibed

Table 9. Germination of longleaf pine seed in lot 1 after various stratification and irradiation treatments

Light treatment		Days of stratification				
Red	Far-red	0	7	14	28	Avg.
---Minutes----		----------Percent----------				
Light control		100	100	99	100	100
Dark control		41	45	62	84	58
¼	0	25	71	53	87	59
1	0	46	79	89	94	77
4	0	86	83	86	90	86
16	0	74	96	100	95	91
64	0	90	99	99	95	96
64	¼	81	92	96	96	91
64	1	97	100	98	98	98
64	4	95	94	97	96	96
64	16	64	63	78	93	74
64	64	39	63	73	95	68
Avg.		70	82	86	94	

Table 10. Germination of longleaf pine seed in lot 2 after various stratification and irradiation treatments

Light treatment		Days of stratification				
Red	Far-red	0	7	14	28	Avg.
---Minutes-----		----------Percent----------				
Light control		98	97	96	98	97
Dark control		58	67	87	95	77
¼	0	61	71	94	96	80
1	0	73	94	99	97	91
4	0	90	98	97	98	96
16	0	20	87	91	98	74
64	0	33	86	86	95	75
64	¼	26	76	91	93	72
64	1	51	83	84	88	76
64	4	12	27	59	91	47
64	16	1	2	10	75	22
64	64	19	21	49	80	42
Avg.		45	67	79	92	

Table 11. Germination of longleaf pine seed in lot 3 after various stratification and irradiation treatments

Light treatment		Days of stratification				
Red	Far-red	0	7	14	28	Avg.
---Minutes-----		----------Percent----------				
Light control		90	90	99	99	94
Dark control		3	22	44	84	38
¼	0	22	35	73	96	56
1	0	17	25	54	97	48
4	0	43	30	75	89	59
16	0	36	54	72	98	65
64	0	53	73	88	97	78
64	¼	23	45	73	92	58
64	1	16	29	55	98	50
64	4	24	42	68	92	56
64	16	3	1	24	86	28
64	64	1	11	29	67	27
Avg.		30	38	63	91	

Table 12. Germination of longleaf pine seed in lot 4 after various stratification and irradiation treatments

Light treatment		Days of stratification				
Red	Far-red	0	7	14	28	Avg.
---Minutes-----		----------Percent----------				
Light control		96	93	100	95	96
Dark control		7	7	15	44	18
¼	0	10	21	29	49	27
1	0	15	32	28	72	37
4	0	30	72	85	84	68
16	0	52	72	70	93	72
64	0	57	86	86	94	81
64	¼	65	66	76	92	75
64	1	15	34	39	66	39
64	4	3	4	5	44	14
64	16	1	0	5	37	11
64	64	1	0	0	22	6
Avg.		29	41	45	66	

in darkness for 24 hours at 22.5 C and exposed to various periods of irradiation under red light with the following results:

Minutes exposure	Percent germination
0	1
1	21
2	25
4	51
8	5
12	27
16	12
20	17
24	46
32	58
64	47
128	65

The above results show a sharp decrease in germination between 4 and 16 minutes of exposure, just as in the main study. However, the decrease between 4 and 8 minutes was even greater, and there was a substantial increase in germination between the 8- and 12-minute exposures. After 16 minutes, there was a consistent increase up to 32 minutes where germination apparently leveled off or showed a slight decline on up to 64 minutes of exposure. Irradiation for 128 minutes resulted in 65 percent germination -- the highest obtained for any length of exposure.

Results of this supplementary study provide strong evidence that the reversals noted with exposures to red light in the main study were not chance occurrences. It is believed that this reaction of longleaf pine seed to red light should be explored further in future research with numerous lots of seed from individual trees.

In addition to the aberrant reaction of lot 2 to irradiation with red light, an abnormal response was also obtained with these seed following exposure to far-red light for 64 minutes. Increasing lengths of irradiation in the far-red portion of the spectrum generally resulted in decreases of germination throughout the study. Table 10 shows a consistent increase in germination of seed in lot 2 after 64 minutes of exposure to far-red light from that obtained after 16 minutes. Similar increases were evident for seed in lot 3 stratified for 7 and 14 days, although the differences were not as pronounced as in lot 2. It is difficult to understand why short periods (16 minutes) of far-red light inhibit germination in these two lots of seed, while longer periods (64 minutes) increase germination. However, V. K. Toole and Borthwick (1966) have also reported that under certain conditions red light inhibits and far-red repromotes germination of Eragrostis curvula (love-grass) seeds.

Downs (1964) reported that in seeds from some species of Bromeliaceae brief exposures to far-red light induced an appreciable number of seeds to germinate and continuous exposure to far-red light induced maximum germination. He also noted that in other species red light promoted germination and that the promotive effect was completely reversed by subsequent irradiation with far-red light. These findings are in partial agreement with results obtained with longleaf pine seed in this study, the exception being that some longleaf seeds are apparently promoted by long periods of exposure to far-red light rather than the brief, 2-minute exposures mentioned by Downs.

One particularly disconcerting occurrence in the germination of seed in lot 2 was the discrepancy between germination of dark-control seed in this study and in study no. 2. Germination of 0-, 7-, and 14-day stratified, dark-control seed from lot 2 in the present study was consistently higher than in study no. 2. (Stratification for 28 days virtually eliminated the light requirement of these seed in both studies and resulted in germination of 97 and 95 percent in studies 2 and 3, respectively.) For example, germination of unstratified, dark-control seed from lot 2 in study no. 2 was only 6 percent; in study no. 3 germination under comparable conditions was 58 percent. The seeds were handled in an identical manner in both instances and germination of the other three lots were quite similar in both studies for all periods of stratification. The reason for the discrepancy in lot 2 seed between studies is unknown, however, it is noted that in the supplementary study with these seed germination of the unirradiated sample was only 1 percent.

Another pertinent observation made in the present study was that in three of the four lots of seed used (lots 2, 3, and 4), exposure to far-red light consistently reduced germination below that obtained in the dark control. For example, seeds in lot 2 stratified for 14 days germinated 87 percent in complete darkness (Table 10). These seeds were subsequently promoted to 99 percent germination by a 1-minute exposure to red light, but were reduced to 10 percent germination after 16 minutes' exposure to far-red light. Numerous

other examples could be cited from the three lots of seed mentioned. Conversely, V. K. Toole (personal communication) has noted that in her work with seeds of Eragrostis, promoted seeds could not be inhibited below the dark-control level. It is recognized that the dark-control seed used in this study may have been promoted by light somewhat during the extraction and drying process.

Finally, it is noted that lots 1 and 2 reacted differently from lots 3 and 4 in the present study. A relatively high proportion of seeds in the two former lots germinated without exposure to light, while comparable treatments of the latter resulted in much lower percentages. Moreover, shorter periods of exposure appeared to be required for promotion and inhibition of germination in lots 1 and 2. Long periods of stratification and exposure to red light were required to obtain high germination in lots 3 and 4.

As noted earlier, all seeds were moved to light, after 14 days of testing in darkness, to determine potential germination. Seeds from lots 1, 2, and 3 immediately responded to the light and generally exceeded 95 percent germination within 14 days. As in study no. 2, seeds from lot 4 failed to give this response when moved to light. Although some germination was obtained after 14 days in light, many of the seeds appeared to remain in a dormant condition. Dead seeds will mold very quickly in germination dishes but seeds from lot 4 did not mold, indicating a condition of dormancy rather than nonviability. Germination in light was also noticeably slower for the dark-control, seed exposed to 16 minutes of red light, and for seed exposed to far-red light for 1 or more minutes.

Table 13 shows the time course for germination of unstratified seed, which was typical for all seeds in lot 4. Germination of the dark-control seeds in this instance was 7 percent after 14 days of testing in darkness. Removal to light resulted in a total of only 55 percent at the end of an additional 14 days of testing, i.e., approximately half of the seeds remaining at the end of the dark-germination phase failed to respond to light within 14 days. After 45 days in light, germination was still only 56 percent. At this time, all treatment-sublots that had not attained 95 percent germination were stratified for 14 days in an effort to induce further germination. The remaining seeds responded to this treatment and continued to germinate slowly for the next month until approximately 90 percent germination was attained. The test in light lasted for 195 days, at which time practically all samples had exceeded 95 percent germination. Detailed data concerning the time-course of germination for all lots of seed in study no. 3 are presented in Appendix Tables 18 to 21.

The slow germination of seed in lot 4 upon removal to light, coupled with similar evidence obtained from study no. 2, strongly indicates that holding these seeds in an imbibed condition in darkness at 22.5 C results in a lapse into dormancy. While PFR (the active form of phytochrome) is thought to shift to PR (the inactive form) in darkness, it would be expected that removal of the seeds to light would stimulate germination. Furthermore, Dr. Winslow Briggs of Stanford University (personal communication) has noted that in

Table 13. Cumulative germination of unstratified longleaf pine seed from lot 4 used in study no. 3

Irradiation treat.		Germ. at end	Germination in light (days)					
Red	Far-red	of dark test	14	45	66 [1/]	101	157	195
-----Minutes-----		----------Percent----------						
Light control		96	98	98	--	--	--	--
Dark control		7	55	56	69	80	96	97
¼	0	10	89	95	--	--	--	--
1	0	15	83	85	88	91	99	100
4	0	30	86	87	88	94	97	98
16	0	52	76	81	90	95	97	97
64	0	57	94	95	--	--	--	--
64	¼	65	97	97	--	--	--	--
64	1	15	72	78	83	91	94	94
64	4	3	66	73	82	88	91	92
64	16	1	69	77	80	88	93	95
64	64	1	59	77	83	93	95	97

[1/] Seeds were stratified for 14 days between 45 and 59 days of testing. Hence, the 66-day period was only 7 days after stratification was applied.

preliminary observations in vitro the PFR form of phytochrome in longleaf seed appears stable, unlike that in most other species of seed and seedlings observed to date. If this should be the case, it is quite possible that the type of dormancy induced in the seed in this study is not photodormancy. Failure of a large proportion of the seed to germinate in light substantiates this hypothesis. Moreover, response of these seed to stratification indicates thermodormancy rather than photodormancy.

Nevertheless, the present study did provide sufficient evidence to conclude that the light requirement of longleaf pine seed operates through the phytochrome system in which the promotive effect of irradiation with red light in the region of 660 nm is reversed by subsequent irradiation with far-red light having a wavelength of 730 nm.

Study No. 4

Results of this study demonstrated that germination of longleaf pine seed can be repeatedly promoted and inhibited by alternate exposures to red and far-red light. Figure 12 shows the typical effect of these irradiations on germination of seed. Germination after14 days in darkness is shown in Table 14 by treatment and lot number. An anslysis of variance of arcsin $\sqrt{\text{percentage}}$ transformations of the germination percentages showed that differences due to blocks, length of stratification, irradiation treatment, and the stratification x irradiation interaction were all significant at the 0.01 level of probability (Table 15).

Figure 12. Four dishes of longleaf seed showing the effect of various 4-minute exposures to red (R) and far-red (FR) light.

Table 14. Repeated promotion and inhibition of germination in longleaf pine seed by exposure to red (R) and far-red (FR) light

Sequence of exposures	Lot number 1	2	3	4	Avg.
4-minute periods	Percent				
Stratified 7 days					
R	96	96	91	81	91
R-FR	33	5	9	9	14
R-FR-R	97	94	91	76	90
R-FR-R-FR	38	13	11	10	18
R-FR-R-FR-R	95	98	91	84	92
R-FR-R-FR-R-FR	37	10	22	11	20
R-FR-R-FR-R-FR-R	99	91	94	77	90
R-FR-R-FR-R-FR-R-FR	31	8	18	6	16
Stratified 14 days					
R	100	96	91	59	86
R-FR	77	65	34	7	47
R-FR-R	99	94	87	65	86
R-FR-R-FR	69	53	40	2	41
R-FR-R-FR-R	97	95	89	49	82
R-FR-R-FR-R-FR	68	49	15	3	34
R-FR-R-FR-R-FR-R	100	95	92	66	88
R-FR-R-FR-R-FR-R-FR	59	32	29	5	31

Table 15. Analysis of variance for differences in germination of longleaf pine seed in study no. 4

Source of variation	Degrees of freedom	Sum of squares	Mean square	F
Blocks (seed lots)	3	6,828.45	2,276.15	45.40**
Treatments	15	30,779.16	2,051.94	40.93**
Strat. treatments (S)	1	467.54	467.54	9.33**
Irrad. treatments (I)	7	29,156.30	4,165.19	83.09**
S x I	7	1,155.32	165.04	3.29**
Error	45	2,255.87	50.13	
Total	63	39,863.48		

F Values for Orthogonal Comparisons

	Days of stratification	
	7	14
Treatments 1, 3, 5, and 7 vs. 2, 4, 6, and 8	25.05**	12.30**
Treatment 1 vs. 7	< 1 N.S.	< 1 N.S.
Treatment 2 vs. 8	< 1 N.S.	< 1 N.S.

Germination of seed from lot 1 was not inhibited to the same degree by far-red irradiations as that of the other three lots. This agrees with results obtained in study 3. Nevertheless, promotions and reversals were consistent. Seed in lot 1 stratified for 7 days germinated from 95 to 99 percent when the terminal 4-minute exposure was under the red light. When far-red light was administered last, germination of the same seed ranged from 31 to 38 percent. Differences between the irradiation treatments were most striking in lots 2 and 3 stratified for 7 days. Germination of these seed was consistently more than 90 percent following terminal exposures to the red source. When far-red light was applied last, germination averaged approximately 10 percent.

When the length of stratification was extended to 14 days, germination of seed receiving a final exposure to red light was essentially unchanged. On the other hand, germination of seed receiving terminal exposures to far-red was higher than when stratified for 7 days. This also agrees with results obtained in study 2, and partially accounts for the interaction between length of stratification and irradiation treatment. The significant interaction is also partially explained by the reaction of seed in lot 4. Oddly enough, germination of these seed was generally lower after 14 days of stratification than after 7 days. This was true regardless of whether the terminal irradiation treatment was with red or far-red light.

Orthogonal comparison of irradiation treatments 1, 3, 5, and 7 (terminal R) with 2, 4, 6, and 8 (terminal FR) showed the differences in germination to be significant at the 0.01 level. Figures 13 and 14 show the magnitude of these differences. Germination of seed following a single exposure to red light (treatment 1) did not differ significantly from those receiving four exposures of red alternately with three exposures to far-red (treatment 7). Moreover, differences in germination between treatments 2 and 8 were not significant, although Figure 14 shows a decreasing trend between the two.

The lack of significant differences in the comparisons noted above indicates that a 4-minute exposure to red light at the intensity used is sufficient for full promotion of phytochrome in the seeds to the PFR form. Conversely, 4 minutes of far-red light appears to result in complete reversal to the PR form. If conversion had not been essentially complete in either case, differences between treatments 1 and 7 or 2 and 8 would no doubt have been significant.

General Observations

All seeds that germinated in darkness in this series of studies had bright green cotyledons, while those germinated in light were darker green. Pine seeds are rather unusual in this respect in that chlorophyll develops in newly germinated seedlings in the absence of light. Another interesting aspect of the seedlings observed in the studies was the exceptionally long hypocotyls, due to etiolation, that resulted during dark-germination. The nature of longleaf pine seedlings in the juvenile stage is such that their

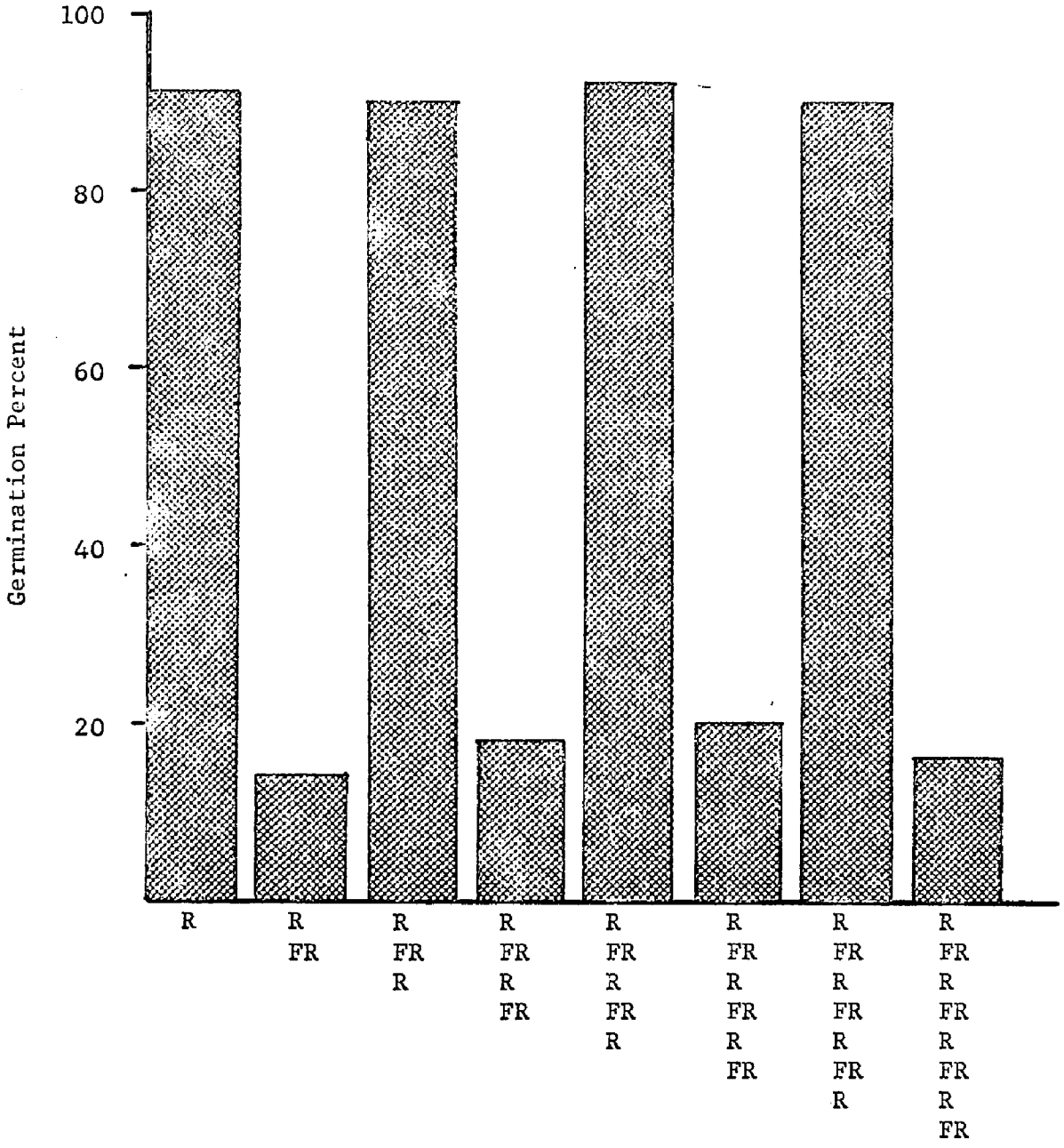

Figure 13. Germination of longleaf pine seed stratified for 7 days prior to indicated number of 4-minute exposures to red (R) and far-red (FR) light. All tests were conducted in darkness for 14 days.

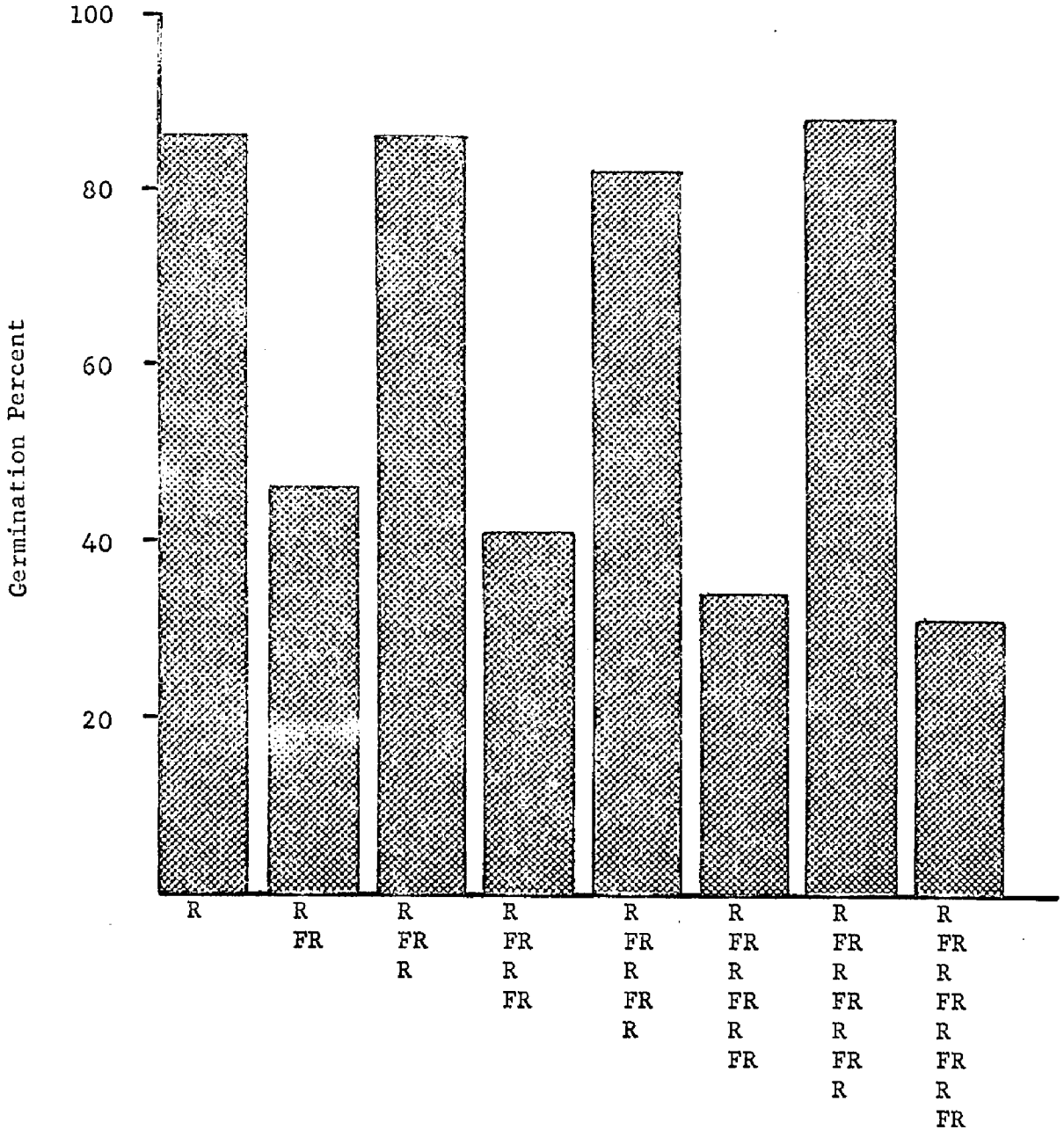

Figure 14. Germination of longleaf pine seed stratified for 14 days prior to the indicated number of 4-minute exposures to red (R) and far-red (FR) light. All tests were conducted in darkness for 14 days.

hypocotyls generally fail to elongate. This phenomenon, coupled with a failure of the terminal bud to elongate, results in the so-called "grass stage" which may persist for several years in longleaf seedlings. Hypocotyls of seedlings germinated in darkness in these studies showed a pronounced elongation, or etiolation, and often exceeded 1 inch in length within 3 to 5 days.

In addition to the elongation noted above, hypocotyls of many dark-germinated seedlings showed a distinct curvature or hook that is commonly seen in other pine species. Seedlings displaying this curvature are shown in Figure 15. The hypocotyl hook is perhaps most often associated with germination of beans. Several workers (Klein, Withrow, and Elstad 1956 and Borthwick 1961 and 1965) noted that light influenced the straightening of hypocotyl hooks in seedlings as they emerged from the soil. Lack of light in the present studies apparently resulted in the formation of a hypocotyl hook, a phenomenon that is atypical in longleaf seedlings germinated in light because of their extremely short hypocotyl.

In addition to the development of hypocotyl hooks, germination of many of the longleaf seeds in this study was abnormal. Abnormal germination is defined as a seedling that has germinated but will obviously fail to become established. One of the most common types of abnormal germination, and the type most often observed in these studies, was a failure of the radicle to penetrate the germination medium. This was commonly observed in seedlings germinated in

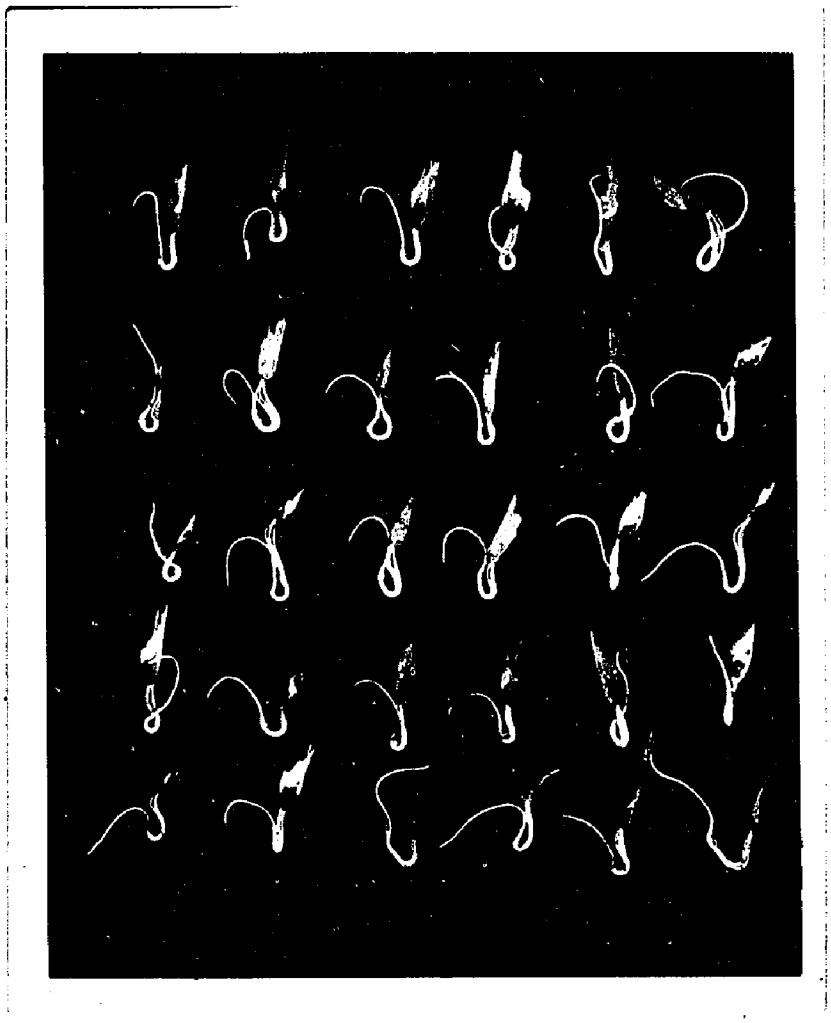

Figure 15. Longleaf pine seedlings, germinated in darkness, showing etiolation and crooking of hypocotyls and curving of radicles.

darkness. Figure 15 illustrates the aberrant growth of radicles of seedlings germinated in darkness. It is entirely possible that this effect was caused by the absence of light. Irvine and Freyre (1961) have demonstrated a diageotropic [3/] effect in roots of vanilla vines (Vanilla planifolia Andrews). Roots of this species grow horizontally in darkness and red or far-red light. They turn downward only in the presence of blue or white light -- white, of course, containing a blue component. In the absence of blue light, roots already growing downward changed directions in 12 hours and grew horizontally. Irvine and Freyre's findings may constitute an explanation for the abnormal germination of longleaf seed in darkness in the present studies.

3/ Diageotropism is defined as the orientation of plant parts at right angles to the pull of gravity.

SUMMARY AND CONCLUSIONS

The influence of light on the germination of longleaf pine seed was investigated in four separate studies. Seed used in the course of these studies were from four, single-tree collections.

Study no. 1 demonstrated that seeds dried to a moisture content of less than 10 percent fail to respond to irradiation treatments. Seeds used in this study were extracted from cones in a darkroom. Half of the seeds were dried and the other half were left undried. Samples of dry and undry seeds were exposed to irradiation from white light and red light in the 660 nm range, while other samples were left in darkness. Germination was subsequently tested in darkness. The only appreciable germination obtained was from the undry seeds exposed to light. The undry seeds were not imbibed, but had moisture contents of approximately 35 percent upon extraction. Results of this study indicated that this moisture level was sufficiently high to permit a response of seed to irradiation since dark-germination of seed receiving this treatment averaged 90 percent. All dry seeds and undry seeds that were not exposed to light essentially failed to germinate -- averaging less than 10 percent. This affords conclusive evidence that light is necessary for germination of longleaf pine seed and that the seeds must have a high moisture level in order to respond to the light.

Study no. 2 showed that seeds extracted in a cone kiln and handled in a normal manner (including drying to less than 10 percent moisture) also have a light requirement for germination. Although there was considerable variation between the individual-tree lots, seed tested in darkness averaged 22 percent germination while those tested in light averaged 95 percent. This study also indicated that stratification of longleaf pine seed reduced the light-requirement for germination. Each increase in length of stratification resulted in an increase in dark germination. Germination of seed stratified for 7, 14, and 28 days averaged 29, 46, and 80 percent, respectively. These averages would have been considerably higher without seed from lot 4. Germination of this lot of seed was only 42 percent in darkness after 28 days of stratification. Moreover, a deep state of dormancy was induced in these seed during the period of testing in darkness. Germination of seeds from the first three lots averaged 94 percent after 14 days upon removal to light. Seed from lot 4 stratified for 28 days had reached only 59 percent germination within a comparable period of time. Stratification was required to promote germination of these seed, and then germination was not judged to be complete until after testing in light for 230 days.

Study no. 3 demonstrated that germination of longleaf pine seed in darkness can be promoted by short exposures to red light, with a wavelength of approximately 660 nm, and inhibited by irradiation with far-red light in the region of 730 nm. Unstratified seed and seed

stratified for 7, 14, and 28 days were exposed to red light for periods of ¼, 1, 4, 16, and 64 minutes. In addition, other samples of seed irradiated with red light for 64 minutes were exposed to far-red light for the same periods as listed above. When all lots and periods of stratification were considered, exposure to red light for 1 minute was sufficient to cause a statistically significant increase in germination over that of the dark control. Germination of the dark control averaged 48 percent, while exposure to red light for 1 minute resulted in an average germinability of 64 percent. Likewise, a 1-minute exposure to far-red light resulted in a significant decrease in germination from that obtained after 64 minutes of irradiation with red light. Germination of the latter averaged 82 percent, while that of the former was 64 percent. Irradiation with far-red light for 16 minutes consistently lowered germination below that of the dark control.

Seed from lot 2 showed an unusual response to the irradiation treatments used in study no. 3. Four minutes of red light resulted in essentially complete germination of these seed, but a lengthening of the exposure to 16 minutes resulted in a marked inhibition. A supplementary test demonstrated that an even greater decrease was obtained after only 8 minutes of exposure. The same lot of seed could also be inhibited by 16 minutes of far-red light and repromoted to a certain degree with a 64-minute exposure. The reaction of this lot of seed was in sharp contrast to that of the other lots where increasing lengths of red light generally resulted in increased germination while lengthening of the far-red exposures gave progressively lower germination.

Study no. 3 also demonstrated that most longleaf pine seed stratified for 28 days lost their light requirement and germination could not be controlled by exposures to red and far-red light.

Results of the fourth, and final, study showed that germination of longleaf pine seed can be repeatedly promoted and inhibited by alternate exposures to red and far-red light. In this study, seed stratified for 7 and 14 days were given alternate, 4-minute exposures to red and far-red light, up to eight consecutive periods. When the terminal exposure was to red light, germinability generally exceeded 90 percent; when the terminal exposure was under far-red light, average germination of seed stratified for 7 days was only 17 percent and that of seed stratified for 14 days averaged 38 percent.

This series of studies present evidence that the germination of longleaf pine seed is controlled through the phytochrome system. Exposure to red light in the region of 660 nm shifts the phytochrome to the active, far-red aborbing form (PFR) while exposure to far-red light results in a reversion to the inactive red-absorbing form (PR).

While the studies in this investigation provided information to supply answers to the questions for which they were designed, several problems were encountered that are left unexplained. This is often the case in research. Specifically, further research is needed to answer questions in the following areas:

1. More needs to be known about the reaction of seed from individual trees to light since there were large variations between lots in the present studies. Several questions immediately come to the forefront here. Is the light requirement a heritable characteristic? If so, is this trait passed on from one generation to another through the male or female parent, or both? Is this characteristic dominant or recessive? It may be possible to eventually obtain a strain of this species that is not light-requiring through a process of mass selection and breeding. This has apparently occurred in the case of most agricultural crops since they are generally nondormant and do not require light for germination.

2. Inhibition of germination by intermediate (16 minutes) exposure to red light was a rather unique response obtained with seed in lot 2. Since there were slight trends of this reaction in lots 1 and 3 also, this may indicate that this is a common occurrence in longleaf pine seed. More research is needed to determine if this is the case.

3. Promotion of germination in seed from lot 2 by prolonged exposure to far-red irradiation is another peculiarity of longleaf seed that warrants further investigation. Although this phenomenon has been reported for seeds of some Bromeliaceae (pineapple family) by Downs (1964), his work showed that brief exposures to far-red light

induced an appreciable number of seeds to germinate and longer exposures induced maximum germination. In the present studies, short periods of far-red light inhibited germination of seed from lot 2 while longer exposures (64 minutes) partially repromoted germinability.

In spite of the discrepancies noted above, the series of studies in the present investigation did show that germination of longleaf pine seed can be promoted by irradiation with red light and inhibited by far-red light. Moreover, this alternation can be repeated many times with the same seed and is characteristic of the photoreversible reaction of phytochrome. The ability to control germination varies greatly between single-tree lots of seed and is dependent on length of stratification.

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APPENDIX

Table 16. Cumulative germination of longleaf pine seed in study no. 1

Lot no.	Moisture condition	Exposed to light	Days of test 6	8	10	14	21	28 1/	35	42	49	63
			Percent									
1	Dry	Yes	0	0	2	10	13	13	58	90	97	99
1	Dry	No	1	1	1	1	5	6	67	89	95	97
1	Nondry	Yes	48	68	76	88	94	95	96	96	96	97
1	Nondry	No	1	1	2	4	4	4	51	89	95	97
2	Dry	Yes	0	1	1	1	2	2	93	96	98	98
2	Dry	No	0	0	0	0	0	0	70	81	96	99
2	Nondry	Yes	38	56	78	90	90	94	99	99	100	100
2	Nondry	No	0	0	0	0	0	0	74	91	95	98
3	Dry	Yes	0	0	0	3	4	6	61	88	96	96
3	Dry	No	0	0	0	0	0	0	71	76	81	81
3	Nondry	Yes	25	48	71	83	93	93	93	95	96	97
3	Nondry	No	0	0	0	0	0	0	26	77	91	92
4	Dry	Yes	0	0	0	0	0	0	25	43	73	82
4	Dry	No	0	0	0	0	1	2	32	57	75	84
4	Nondry	Yes	10	20	47	64	74	77	86	93	100	100
4	Nondry	No	1	1	1	1	1	1	34	62	81	88

1/ Seeds were moved to light after 28 days of testing in darkness.

Table 17. Cumulative germination of longleaf pine seed in study no. 2

Lot no.	Days of strat.	Germ. environ.	Days of testing 4	6	8	10	14 [1]	21	28	230
			Percent							
1	0	Dark	19	31	42	52	57	88	92	--
		Light	58	75	93	95	98	100	100	--
	7	Dark	34	35	41	57	60	86	95	--
		Light	91	93	97	98	98	98	98	--
	14	Dark	51	54	60	66	71	84	97	--
		Light	90	98	99	99	99	99	99	--
	28	Dark	55	65	76	85	89	94	99	--
		Light	83	87	92	97	97	97	97	--
2	0	Dark	1	1	1	5	6	80	89	--
		Light	2	27	76	90	93	94	94	--
	7	Dark	0	0	0	8	13	88	94	--
		Light	61	82	93	97	97	97	98	--
	14	Dark	19	24	27	49	51	84	91	--
		Light	76	88	93	96	97	97	97	--
	28	Dark	89	90	92	96	97	99	99	--
		Light	91	93	93	93	94	95	95	--
3	0	Dark	0	4	7	15	17	79	89	--
		Light	16	40	77	94	95	95	95	--
	7	Dark	4	4	7	33	39	95	98	--
		Light	58	84	92	97	98	98	98	--
	14	Dark	22	29	31	39	45	89	93	--
		Light	70	87	96	97	97	97	97	--
	28	Dark	71	80	87	92	92	96	96	--
		Light	91	93	97	98	98	98	98	--

[1] Seeds were moved to light after 14 days of testing in darkness.

Table 17. (Cont.)

Lot no.	Days of strat.	Germ. environ.	Days of testing							
			4	6	8	10	14 [1/]	21	28	230
			Percent							
4	0	Dark	1	3	3	8	8	32	43	99
		Light	4	13	61	90	93	94	94	--
	7	Dark	0	1	2	2	3	44	58	100
		Light	25	61	87	97	99	99	99	--
	14	Dark	5	10	13	16	17	45	64	98
		Light	35	67	84	94	94	95	95	--
	28	Dark	24	29	37	41	42	56	59	98
		Light	60	83	93	95	96	96	97	--

Table 18. Cumulative germination of longleaf pine seed from lot 1 used in study no. 3

Length of strat.	Light treat. Red	Light treat. Far-red	Days of test 6	8	10	14 [1/]	21	28
Days	--Minutes---		----------Percent----------					
0	Light control		83	95	97	100	100	100
	Dark control		17	20	36	41	80	95
	¼	0	14	14	24	25	75	93
	1	0	30	34	42	46	80	99
	4	0	60	74	84	86	95	97
	16	0	59	64	72	74	92	97
	64	0	69	77	83	90	94	96
	64	¼	51	61	71	81	90	94
	64	1	69	86	91	97	99	99
	64	4	64	78	91	95	99	99
	64	16	26	52	61	64	88	97
	64	64	11	27	36	39	79	95
7	Light control		93	99	100	100	100	100
	Dark control		23	26	39	45	85	98
	¼	0	55	59	66	71	89	100
	1	0	58	66	78	79	94	98
	4	0	69	74	83	83	93	98
	16	0	87	90	93	96	98	98
	64	0	80	92	95	99	100	100
	64	¼	79	86	89	92	97	99
	64	1	81	87	93	100	100	100
	64	4	73	84	90	94	97	98
	64	16	33	48	58	63	87	95
	64	64	35	48	56	63	93	100
14	Light control		91	97	99	99	99	99
	Dark control		43	48	58	62	85	97
	¼	0	37	42	48	53	85	93
	1	0	68	75	85	89	97	98
	4	0	62	70	79	86	96	98
	16	0	84	92	97	100	100	100
	64	0	87	94	97	99	99	99
	64	¼	74	86	91	96	97	98
	64	1	80	86	93	98	98	98
	64	4	68	84	93	97	100	100
	64	16	47	65	75	78	93	99
	64	64	38	57	69	73	93	98

[1/] Seeds were moved to light after 14 days in darkness.

Table 18. (Cont.)

Length of strat.	Light treat. Red	Light treat. Far-red	Days of test 6	8	10	14 [1/]	21	28
Days	--Minutes---		----------Percent----------					
28	Light control		95	97	99	100	100	100
	Dark control		58	73	81	84	92	96
	¼	0	61	72	83	87	92	95
	1	0	65	78	84	94	98	99
	4	0	61	73	83	90	98	98
	16	0	76	87	94	95	97	97
	64	0	68	79	89	95	97	98
	64	¼	65	80	88	96	98	98
	64	1	74	87	93	98	100	100
	64	4	64	84	90	96	98	98
	64	16	65	79	87	93	96	98
	64	64	70	84	89	95	96	96

Table 19. Cumulative germination of longleaf pine seed from lot 2 used in study no. 3

Length of strat.	Light treat. Red	Light treat. Far-red	Days of test 6	8	10	14 [1/]	21	28
Days	--Minutes---		----------Percent----------					
0	Light control		74	91	96	98	98	98
	Dark control		19	39	57	58	93	94
	¼	0	12	30	57	61	96	96
	1	0	25	57	66	73	86	90
	4	0	31	69	87	90	97	97
	16	0	9	11	13	20	73	91
	64	0	19	24	26	33	89	93
	64	¼	19	22	24	26	83	93
	64	1	33	38	47	51	97	98
	64	4	10	11	12	12	80	90
	64	16	1	1	1	1	84	96
	64	64	1	3	16	19	80	86
7	Light control		80	92	93	97	97	98
	Dark control		20	46	64	67	96	96
	¼	0	45	65	71	71	88	90
	1	0	79	89	93	94	95	96
	4	0	82	96	98	98	98	98
	16	0	83	86	87	87	97	98
	64	0	74	83	86	86	96	100
	64	¼	63	72	74	76	93	97
	64	1	70	78	81	83	96	97
	64	4	20	25	26	27	97	99
	64	16	2	2	2	2	94	98
	64	64	2	12	20	21	95	98
14	Light control		95	96	96	96	97	97
	Dark control		73	82	86	87	94	95
	¼	0	86	91	94	94	96	96
	1	0	93	98	99	99	99	99
	4	0	94	96	97	97	97	97
	16	0	85	88	90	91	97	97
	64	0	80	83	85	86	95	97
	64	¼	84	90	90	91	97	97
	64	1	79	81	83	84	99	99
	64	4	54	54	57	59	97	99
	64	16	10	10	10	10	83	90
	64	64	24	37	49	49	88	94

[1/] Seeds were moved to light after 14 days in darkness.

Table 19. (Cont.)

Length of strat.	Light treat. Red	Light treat. Far-red	Days of test 6	8	10	14 1/	21	28
Days	--Minutes---		----------Percent----------					
28	Light control		98	98	98	98	98	98
	Dark control		89	92	95	95	98	98
	¼	0	92	94	95	96	97	97
	1	0	89	96	97	97	98	98
	4	0	96	98	98	98	98	98
	16	0	95	97	98	98	98	98
	64	0	94	95	95	95	100	100
	64	¼	90	93	93	93	99	99
	64	1	85	85	86	88	94	95
	64	4	88	90	91	91	97	97
	64	16	72	73	74	75	95	95
	64	64	75	77	80	80	92	95

Table 20. Cumulative germination of longleaf pine seed from lot 3 used in study no. 3

Length of strat.	Light treat.		Days of test					
	Red	Far-red	6	8	10	14 [1/]	21	28
Days	---Minutes--		----------Percent----------					
0	Light control		61	81	83	90	96	96
	Dark control		1	1	1	3	91	95
	¼	0	14	18	19	22	88	95
	1	0	10	12	12	17	94	98
	4	0	28	40	41	43	79	84
	16	0	22	30	32	36	84	94
	64	0	42	48	51	53	89	92
	64	¼	19	23	23	23	80	92
	64	1	9	12	13	16	86	94
	64	4	12	21	21	24	87	92
	64	16	1	1	1	3	85	94
	64	64	1	1	1	1	94	100
7	Light control		69	83	85	90	96	96
	Dark control		13	20	21	22	93	96
	¼	0	27	34	34	35	90	95
	1	0	15	20	20	25	90	99
	4	0	25	28	29	30	81	90
	16	0	42	49	49	54	85	93
	64	0	56	62	68	73	95	98
	64	¼	30	34	34	45	86	93
	64	1	23	24	24	29	84	93
	64	4	26	34	35	42	94	99
	64	16	1	1	1	1	87	93
	64	64	3	9	10	11	94	98
14	Light control		90	96	98	99	99	99
	Dark control		32	40	40	44	91	96
	¼	0	68	71	73	73	92	96
	1	0	47	49	51	54	95	97
	4	0	66	69	72	75	96	97
	16	0	61	63	68	72	97	100
	64	0	79	85	85	88	94	96
	64	¼	64	68	68	73	88	92
	64	1	49	50	51	55	86	94
	64	4	58	67	67	68	94	99
	64	16	24	24	24	24	85	97
	64	64	23	29	29	29	98	100

[1/] Seeds were moved to light after 14 days in darkness.

Table 20. (Cont.)

Length of strat.	Light treat. Red	Light treat. Far-red	Days of test 6	8	10	14 1/	21	28
Days	--Minutes---		----------Percent----------					
28	Light control		95	98	98	99	99	100
	Dark control		74	82	82	84	98	100
	¼	0	93	95	96	96	99	99
	1	0	96	97	97	97	100	100
	4	0	86	87	88	89	97	98
	16	0	91	96	97	98	98	98
	64	0	95	95	96	97	98	98
	64	¼	89	90	91	92	96	96
	64	1	93	95	98	98	99	100
	64	4	89	91	91	92	97	99
	64	16	74	77	80	86	99	99
	64	64	65	66	67	67	95	96

Table 21. Cumulative germination of longleaf pine seed from lot 4 used in study no. 3

Length of strat.	Light treat. Red	Light treat. Far-red	Days of test 6	8	10	14 [1/]	21	28	59 [2/]	80	98	115	209
Days	--Minutes---		----------Percent----------										
0	Light control		9	69	92	96	98	98	98	--	--	--	--
	Dark control		2	4	6	7	32	55	56	69	77	80	97
	¼	0	2	4	8	10	68	89	95	--	--	--	--
	1	0	6	8	11	15	69	83	85	88	91	91	100
	4	0	4	13	19	30	70	86	87	88	93	94	98
	16	0	11	32	46	52	71	76	81	90	94	95	97
	64	0	12	31	45	57	88	94	95	--	--	--	--
	64	¼	15	42	54	65	90	97	97	--	--	--	--
	64	1	6	12	15	15	52	72	78	83	89	91	94
	64	4	1	2	3	3	49	66	73	82	86	88	92
	64	16	0	0	0	1	47	69	77	80	86	88	95
	64	64	0	0	0	1	34	59	77	83	89	93	97

1/ Seeds were moved to light after 14 days in darkness.

2/ Samples that had not attained 95 percent germination at the end of 59 days were stratified for 14 days to encourage additional germination.

Table 21. (Cont.)

Length of strat.	Light treat. Red	Light treat. Far-red	Days of test 6	8	10	14	21	28	59	80	98	115	209
			Percent										
7	Light control		31	77	85	93	95	95	95	--	--	--	--
	Dark control		4	6	7	7	56	72	75	85	89	91	100
	¼	0	10	14	18	21	75	91	95	--	--	--	--
	1	0	11	24	28	32	73	84	85	88	91	93	100
	4	0	28	56	65	72	96	99	100	--	--	--	--
	16	0	19	63	68	72	90	93	93	93	95	96	100
	64	0	26	62	73	86	95	96	96	--	--	--	--
	64	¼	16	50	61	66	79	82	83	85	87	87	97
	64	1	16	30	31	34	58	67	66	70	75	77	95
	64	4	1	2	3	4	55	89	92	94	95	95	100
	64	16	0	0	0	0	35	61	64	77	88	89	100
	64	64	0	0	0	0	66	88	89	93	93	94	99

Table 21. (Cont.)

Length of strat.	Light treat. Red	Light treat. Far-red	Days of test 6	8	10	14	21	28	59	80	98	115	209
Days	--Minutes---		----------	----------	----------	----------	Percent	----------	----------	----------	----------	----------	----------
14	Light control		41	90	100	100	100	100	100	--	--	--	--
	Dark control		12	14	15	15	61	79	84	91	92	93	100
	¼	0	17	26	28	29	76	91	95	--	--	--	--
	1	0	11	17	22	28	82	95	96	--	--	--	--
	4	0	49	73	82	85	95	98	98	--	--	--	--
	16	0	31	57	67	70	93	95	95	--	--	--	--
	64	0	41	68	82	86	97	98	98	--	--	--	--
	64	¼	35	56	68	76	94	95	96	--	--	--	--
	64	1	30	35	37	39	73	81	83	89	92	94	100
	64	4	1	1	3	5	32	56	60	77	86	89	100
	64	16	5	5	5	5	52	80	91	94	97	98	98
	64	64	0	0	0	0	64	84	89	93	97	97	100

Table 21. (Cont.)

Length of strat.	Light treat.		Days of test										
	Red	Far-red	6	8	10	14	21	28	59	80	98	115	209
Days	--Minutes---		----------Percent----------										
28	Light control		75	93	95	95	96	97	97	--	--	--	--
	Dark control		39	42	42	44	66	81	89	93	95	96	98
	¼	0	38	41	43	49	83	94	97	--	--	--	--
	1	0	54	65	67	72	92	98	99	--	--	--	--
	4	0	70	77	80	84	93	96	98	--	--	--	--
	16	0	59	81	85	93	99	99	99	--	--	--	--
	64	0	74	87	92	94	97	98	98	--	--	--	--
	64	¼	69	83	84	92	95	97	97	--	--	--	--
	64	1	56	62	66	66	79	87	90	97	97	98	99
	64	4	34	40	43	44	79	89	94	96	96	96	98
	64	16	32	37	37	37	59	81	86	91	93	94	100
	64	64	19	22	22	22	65	81	84	93	94	95	100

Table 22. Cumulative germination of longleaf pine seed from lot 1 used in study no. 4

Sequence of exposures	Days of test				
	7	10	14 [1/]	21	28
4-minute periods	Percent				
	Stratified 7 days				
R	78	89	96	99	100
R-FR	14	16	33	85	97
R-FR-R	77	84	97	98	98
R-FR-R-FR	19	22	38	91	99
R-FR-R-FR-R	77	90	95	98	99
R-FR-R-FR-R-FR	25	27	37	92	99
R-FR-R-FR-R-FR-R	95	99	99	99	99
R-FR-R-FR-R-FR-R-FR	20	25	31	87	99
	Stratified 14 days				
R	100	100	100	100	100
R-FR	71	73	77	95	99
R-FR-R	98	99	99	99	100
R-FR-R-FR	60	63	69	92	99
R-FR-R-FR-R	95	97	97	97	97
R-FR-R-FR-R-FR	62	65	68	92	99
R-FR-R-FR-R-FR-R	95	98	100	100	100
R-FR-R-FR-R-FR-R-FR	49	50	59	86	99

1/ Seeds were moved to light after 14 days of testing in darkness.

Table 23. Cumulative germination of longleaf pine seed from lot 2 used in study no. 4

Sequence of exposures	Days of test				
	7	10	14 [1/]	21	28
4-minute periods	Percent				
	Stratified 7 days				
R	95	96	96	98	98
R-FR	3	4	5	83	91
R-FR-R	94	94	94	97	97
R-FR-R-FR	7	10	13	95	98
R-FR-R-FR-R	97	98	98	98	98
R-FR-R-FR-R-FR	4	6	10	96	97
R-FR-R-FR-R-FR-R	89	91	91	95	95
R-FR-R-FR-R-FR-R-FR	5	6	8	87	95
	Stratified 14 days				
R	94	95	96	97	98
R-FR	63	64	65	94	98
R-FR-R	93	94	94	98	98
R-FR-R-FR	50	53	53	95	95
R-FR-R-FR-R	93	94	95	98	98
R-FR-R-FR-R-FR	48	48	49	98	99
R-FR-R-FR-R-FR-R	94	95	95	99	99
R-FR-R-FR-R-FR-R-FR	30	30	32	97	97

[1/] Seeds were moved to light after 14 days of testing in darkness.

Table 24. Cumulative germination of longleaf pine seed from lot 3 used in study no. 4

Sequence of exposures	Days of test 7	10	14 1/	21	28
4-minute periods	Percent				
	Stratified 7 days				
R	77	88	91	100	100
R-FR	8	8	9	97	99
R-FR-R	80	87	91	99	99
R-FR-R-FR	8	8	11	94	97
R-FR-R-FR-R	80	90	91	99	99
R-FR-R-FR-R-FR	15	21	22	98	99
R-FR-R-FR-R-FR-R	86	93	94	98	99
R-FR-R-FR-R-FR-R-FR	13	17	18	99	100
	Stratified 14 days				
R	82	85	91	97	97
R-FR	30	31	34	98	98
R-FR-R	80	83	87	96	98
R-FR-R-FR	39	40	40	99	99
R-FR-R-FR-R	84	87	89	97	97
R-FR-R-FR-R-FR	15	15	15	96	97
R-FR-R-FR-R-FR-R	83	89	92	99	99
R-FR-R-FR-R-FR-R-FR	26	28	29	99	99

1/ Seeds were moved to light after 14 days of testing in darkness.

Table 25. Cumulative germination of longleaf pine seed from lot 4 used in study no. 4

Sequence of exposures	Days of test								
	7	10	14 [1/]	21	28	35 [2/]	56	70	144
4-minute periods	Percent								
Stratified 7 days									
R	68	77	81	86	89	89	93	97	--
R-FR	6	8	9	39	44	44	73	94	--
R-FR-R	70	73	76	91	92	93	98	100	--
R-FR-R-FR	10	10	10	54	64	65	82	93	--
R-FR-R-FR-R	73	77	84	91	91	91	97	99	--
R-FR-R-FR-R-FR	8	11	11	60	73	74	86	97	--
R-FR-R-FR-R-FR-R	68	73	77	86	90	90	95	97	--
R-FR-R-FR-R-FR-R	6	6	6	54	65	67	76	95	--
Stratified 14 days									
R	45	58	59	75	79	80	82	90	98
R-FR	4	6	7	55	62	63	69	83	98
R-FR-R	54	63	65	84	88	88	89	91	99
R-FR-R-FR	2	2	2	56	73	73	83	86	99
R-FR-R-FR-R	38	47	49	81	90	90	90	92	100
R-FR-R-FR-R-FR	3	3	3	69	79	79	81	86	100
R-FR-R-FR-R-FR-R	52	64	66	81	88	89	90	90	97
R-FR-R-FR-R-FR-R	3	5	5	59	68	69	71	77	91

[1/] Seeds were moved to light after 14 days of testing in darkness.

[2/] Seeds were stratified for 14 days after tests had run for 35 days.

VITA

Bobbie F. McLemore was born on May 22, 1932, on a farm near Jasper, Texas. He was the ninth of nine children born to Ivy Augustus and Kate Elizabeth (Sims) McLemore.

He graduated from Jasper High School in May, 1949, and entered the Agricultural and Mechanical College of Texas in September, 1949, receiving a Bachelor of Science degree with a major in botany in June of 1953.

Immediately following graduation he entered the United States Army with a commission of Second Lieutenant and served in Korea. He was released from active duty on April 15, 1955, and employed by the Texas Agricultural Experiment Station at Beaumont, Texas, until September, 1955, when he entered the Graduate School in Forestry at Michigan State University. After completing one academic year at Michigan State University, he was employed by International Paper Company until September, 1956, when he enrolled in the Graduate School at Louisiana State University.

McLemore was awarded a Master of Forestry Degree by Louisiana State University in August, 1957, and immediately employed by the Southern Forest Experiment Station at Alexandria, Louisiana. While working at Alexandria, he took several courses at Louisiana College in Pineville, Louisiana. From September, 1961, until September, 1962, he was detailed

to work in the Agricultural Research Service's seed laboratory at Beltsville, Maryland. During this time, he completed a year of academic study in the botany department at the University of Maryland.

He is married to the former Bobbiline Marshall and has two children, Kent Robert, 11 years old, and Brenda Lynn, 8 years old. McLemore is presently a candidate for the Doctor of Philosophy degree in the School of Forestry and Wildlife Management at Louisiana State University.

EXAMINATION AND THESIS REPORT

Candidate: Bobbie Frank McLemore

Major Field: Forestry

Title of Thesis: The Influence of Light on Germination of Longleaf Pine Seed

Approved:

Thomas Hansbrough
Major Professor and Chairman

Max Goodrick
Dean of the Graduate School

EXAMINING COMMITTEE:

Bryant A. Bateman

Paul y Burns

Norwin E. Linnartz

Leon C. Standifer

Date of Examination:

July 8, 1967