ROLE OF STRATIFICATION, TEMPERATURE, AND LIGHT IN FRASER FIR SEED GERMINATION

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Abstract. Fraser fir *(Abies fraseri)* seed germination was examined as affected by cold-moist stratification, temperature, and light. There were strong interactions among these factors in the germination response. Germination was accelerated by cold-moist stratification and/or warm germination temperatures. Stratification periods up to 12 weeks improved germination at low temperatures (8-hr/16-hr thermoperiod of 20°/10°C), whereas 8 weeks was sufficient for seed germinated at high temperatures (30°/20°C). Germination was influenced by temperature regime, but temperature and light sensitivity decreased with increased durations of stratification. Except at low temperatures, a close relationship existed between daily heat input and germination. Maximum germination occurred at 500 to 600 degree-hours per 24-hr cycle. A 1-hr daily light treatment during the 8-hr temperature cycle broadened the temperature range for optimum germination. For a 42-day germination period, the effect of a daily 1-hr light treatment was essentially equivalent to 4 weeks stratification at 4°C.

Fraser fir *(Abies fraseri)* is indigenous to restricted areas of the Southern Appalachians (13), and is used commercially for Christmas trees, ornamentals, and Yuletide greenery. Although propagation can be achieved by both sexual and asexual means, commercial regeneration is limited to seed propagation. Despite widespread commercial use of sexual propagation there is little information available concerning the influence of various pre-germination treatments, such as cold-moist stratification. One report dealing specifically with seed stratification of this species concluded the practice was of doubtful commercial value (16). Similarly, little is known about such environmental factors as light and temperature on the germination process. Therefore, the objective of the following studies was to examine the effects of cold-moist stratification, temperature, and light on Fraser fir seed germination.

MATERIALS AND METHODS

Two experiments were conducted. Experiment 1 examined the role of cold-moist stratification on Fraser fir seed germination; Experiment 2, the influence of temperature on

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seed germination. Earlier work with Fraser fir (5) indicated that a 1-hr daily light seed treatment would significantly improve 42-day germination, so this treatment was included in both experiments to determine how it would influence the relationships between stratification and temperature. No attempt was made to quantify the relationship between light (duration, quality, or illuminance) and germination.

Seed was collected in the fall, 1981, by the North Carolina Forest Service from Roan Mountain (36° 01′ N. lat.; 82° 05′ W. long.; elev. = 1900 m). Damaged, undersized, or resinous seeds, as well as those appearing abnormal, were removed by hand prior to initiation of the experiments. Cutting tests and tetrazolium tests indicated germinative capacity of the graded seed to be 74 and 77%, respectively (1).

Seeds were germinated in 9-cm, covered, glass Petri dishes containing moist blotters. Dishes scheduled to receive a daily light treatment were placed inside black sateen cloth bags immediately after sowing. Dishes were randomized on metal trays, and placed in Pfeiffer germinators maintained within ±1°C of the set point. Relative humidity was approximately 100% in each germinator. Germination counts were recorded every 3 days for 42 days and seeds were considered germinated when radicals were ≥2 mm in length. Seeds with reversed embryos and multiple or abnormal radicles were not included in germination counts. Seeds were sprayed every 3 days with an aqueous suspension of benomyl containing 300 mg per liter (1). Approximately 0.8 ml of liquid was applied on each occasion, and appeared not to result in excessive moisture in the dishes. For dark-treated seeds, germination counts and benomyl applications were carried out under a green safelight known not to affect germination.

An 8-hr/16-hr thermoperiod was utilized, and seeds receiving a light treatment were subjected to a 1-hr daily illumination (measured with a cosine corrected LICOR LI-185 quantum/radiometer/photometer) from cool-white fluorescent lamps during the 8-hr cycle. The photosynthetic photon flux density (400 to 700 nm) was 21 to 27 μmol $m^{-2}s^{-1}$ (1.6 to 2.0 klx). Because germinators were not equipped with lights, the light treatment was imposed by setting trays on benches adjacent to the germinators.

Experiment 1: Stratification, temperature, and light. Dry sand was seived through a 16-mesh screen, and the fine separate retained. One hundred graded seeds were mixed with 40 ml moist sand (10 dry sand:1 water, by vol.) and placed in a 500 ml nonvented, polyethylene freezer bag. A total of 80 bags were prepared and placed in a dark 4°C cold-room for 0, 4, 8,

12, or 16 weeks. At the designated intervals, 16 randomly selected bags were removed. Seeds were separated from sand by flushing with tap water in a colander and were then transferred to dishes. Eight dishes were placed on an 8-hr/16-hr temperature regime at 30°/20°C; 8 at 20°/10°C. Within each temperature regime, 4 dishes received a 1-hr daily light treatment; 4 did not.

Experiment 2: Temperature and light. Half the seeds were stratified for 33 days at 4°C, while the other half were kept in a sealed polyethylene bag (4 to 6% moisture content) at −17°C. Stratified seed was removed from the cold room, air dried overnight at room temperature (22°C) to facilitate handling, and graded and counted into dishes, each with 70 seeds. Twenty temperature regimes were established, each consisting of one of 4 different temperatures (15°, 20°, 25°, and 30°C) during the 8-hr cycle in factorial combination with one of 5 different temperatures (10°, 15°, 20°, 25°, and 30°C) during the 16-hr cycle. Temperatures during the 8- and 16-hr cycles should not be interpreted as day/night temperatures because half the seeds were germinated in darkness and the remainder received only 1 hr of light during the 8-hr cycle. Eight dishes of stratified seed and 8 dishes of nonstratified seed were randomly assigned to each of the 20 temperature regimes. For each stratification treatment, 4 dishes, received a 1-hr daily light treatment; 4 did not.

Response surfaces (14) were developed using linear and quadratic effects of 8- and 16-hr temperatures and their respective interactions. Two-dimensional diagrams of percent germination were plotted for various combinations of 8- and 16-hr temperatures using as data points the maximum germination values for each of the 20 temperature regimes. This technique was similar to one taken earlier to describe growth of Fraser fir seedlings at different day/night temperatures (12). Percent germination was examined as a function of daily degree-hours. Using an 8-hr/16-hr thermoperiod, daily degree-hours were computed for the 20 temperature regimes as follows: (8 × short cycle temperature) + (16 × long cycle temperature). This approach was similar to one used earlier (11) to describe seedling growth of several conifer species as affected by different day/night temperatures. Periodic germination rate was calculated as total germination during each 3-day interval, divided by 3. In concept, this rate was calculated like a periodic growth increment in forest stands (6).

RESULTS

Stratification. Stratification of the seeds enhanced total germination, particularly at low temperatures (20°/10°C) and in darkness (Figures 1 and 2). Under such conditions, total germination after 42 days was increased significantly (t-test, 5% level) by stratification periods up to 12 weeks. At higher temperatures (30°/20°C), 8 weeks stratification was sufficient, especially for seed which received a daily light treatment (Figure 1). In general, germination accelerated with increased duration of stratification. For seed stratified 16 weeks and germinated at 30°/20°C, half the potential germination was realized by the 9th day, and germination was virtually complete by the 20th day (Figs. 1, 2, and 3). Even after 42 days, germination of nonstratified seed at low temperatures (20°/10°C) was less than half completed. At that time, germination was proceeding steadily at approximately 1% per day (Fig. 3).

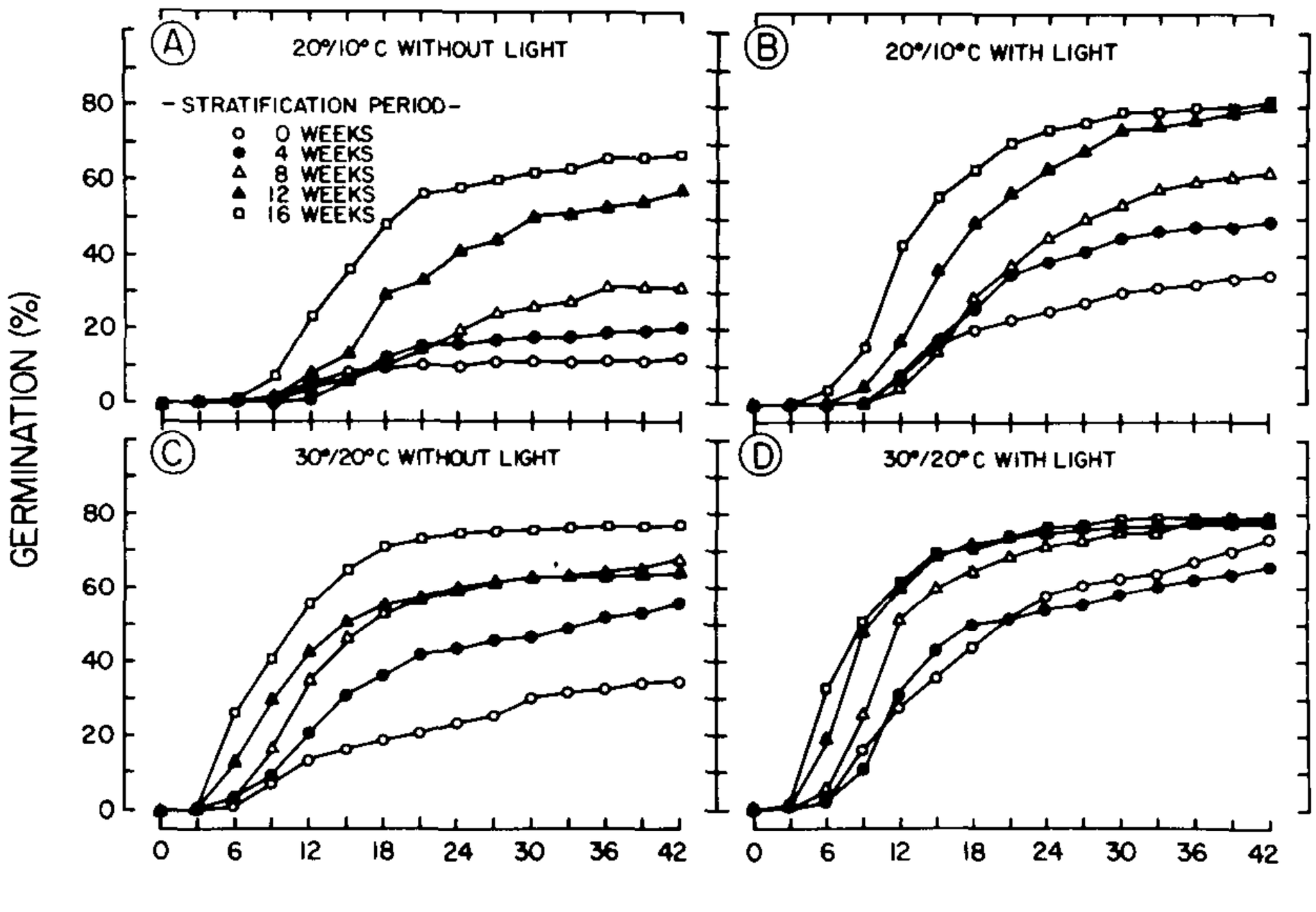

Figure 1. (Expt. 1) Influence of cold-moist stratification, temperature, and light on Fraser fir seed germination. (A) germinated in darkness at 20°/10°C; (B) germinated at 20°/10°C with a 1-hr light treatment during the 8-hr, 20°C cycle; (C) germinated in darkness at 30°/20°C; (D) germinated at 30°/20°C with a 1-hr light treatment during the 8-hr, 30°C cycle. Legend in (A) applies to all figures.

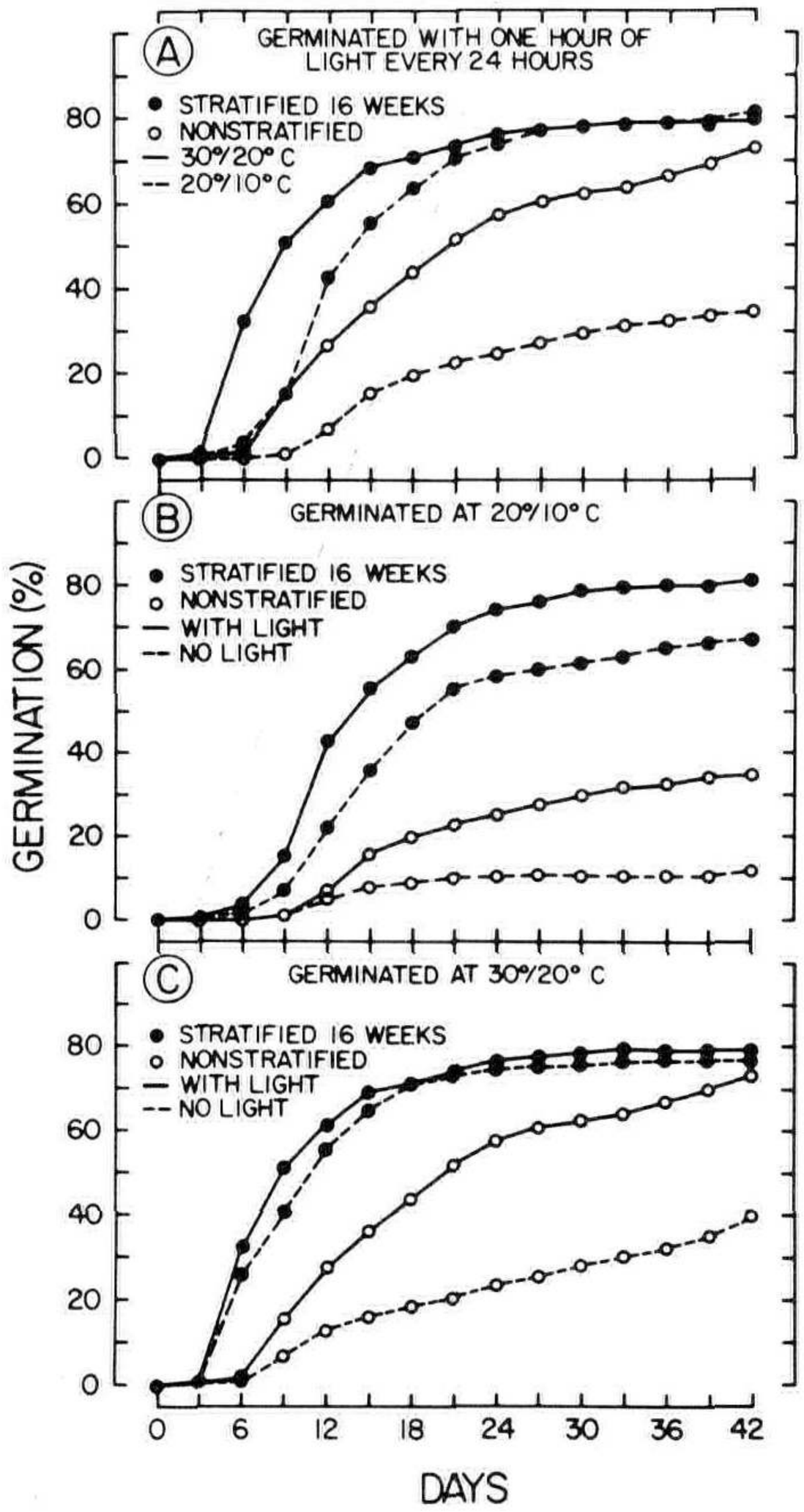

Figure 2. (Expt. 1) Total germination of Fraser fir seed after 42 days as affected by cold-moist stratification, temperature and light. (A) germination as affected by stratification and temperature for seed which received a 1-hr light treatment during the 8-hr temperature cycle; (B) germination at 20°/10°C, with and without a daily 1-hr light treatment during the 8-hr, 20°C cycle, as affected by stratification; (C) germination at 30°/20°C, with and without a daily 1-hr light treatment during the 8-hr, 30° cycle, as affected by stratification.

Temperature. Temperature had a marked effect on seed germination; its influence was affected by the duration of stratification and the presence or absence of light (Figure 4). In 3 of the 4 stratification and light treatments, maximum germination occurred at an 8-hr/16-hr temperature regime of 15°/25°C; 20°/25°C in the fourth. Total germination was closely related to the temperature of the 16-hr cycle, as evidenced by

the vertical orientation of isolines. Response surfaces (not shown) accounted for 83 to 88% of the variation in 42-day germination. For seeds which received a daily 1-hr light treatment, total germination decreased sharply and steadily as the temperature of the 16-hr cycle fell below 15°C, and reached a minimum of 10 to 20% at 10°C (Figure 4). A similar but more gradual decline occurred in darkness when the temperature of the 16-hr cycle fell below 22°C. Constant temperatures approaching 30°C noticeably decreased germination, especially with a light treatment, and killed seedlings soon after germination. With stratified seed, the range of temperatures (16-hr cycle) for optimum germination was broadened by a 1-hr daily light treatment. Total germination of nonstratified, nonirradiated seed was not only much lower than that for other treatments, but there was also a less clearly defined relationship between germination and the level of the 8- or 16-hr temperature cycles.

In addition to being closely related to the temperature of the 16-hr cycle, total germination was also a function of daily heat input (Figure 5). Over the range of 280 to 720 degree-hours, the relationship was quadratic with maximum germination at 500 to 600 degree-hours per 24-hr cycle. The range for optimum germination was widest for stratified seed which received a daily light treatment. Germination decreased linearly as daily heat input exceeded 560 degree-hours. With the exception of stratified seed which did not receive light, the relationship between heat input and germination was less clear at heat inputs $\leq$400 daily degree-hours.

Temperature influenced time course of germination as well as periodic germination rate (Figure 3). Periodic germination of stratified seed peaked between the 3rd and 6th day at 30°/20°C; the 9th and 12th day at 20°/10°C — an average difference of 6 days (Figure 3). The same pattern was evident for nonstratified seed, except that each peak occurred about 3 days later.

Light. Although interpretations are limited concerning the effects of light, several conclusions are warranted. Fraser fir exhibited no obligate light requirement for seed germination, but germination was enhanced by a 1-hr daily light treatment (Figure 1). The stimulatory effect of light was most evident for nonstratified seed and at low germination temperatures (Figures 1, 2, and 4), and decreased with increased durations of stratification (Figure 1). At low germination temperatures (20°/10°C), a daily 1-hr light treatment significantly improved 42-day germination (t-test, 5% level) for seed stratified up to 12 weeks, whereas at 30°/20°C it significantly increased 42-day

germination only for nonstratified seed (Figure 1). A daily 1-hr light treatment did not affect the temperature for maximum germination but broadened the temperature range for optimum germination (Figure 4). This was most pronounced for seed stratified less than 8 weeks, particularly nonstratified seed (Figure 1). A daily 1-hr light treatment increased the maximum periodic germination, but did not alter time course of germination (Figure 3).

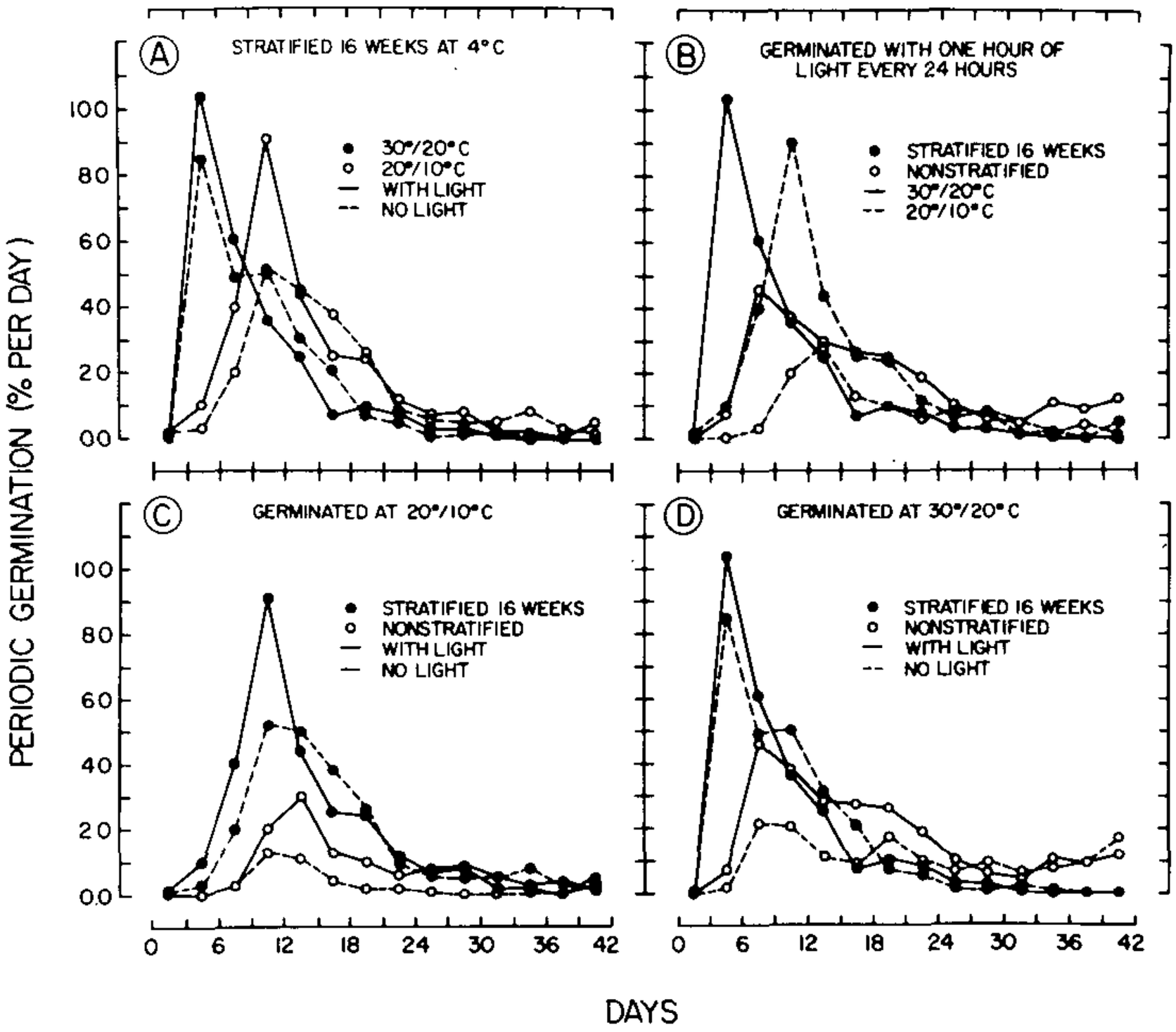

Figure 3. (Expt. 1) Periodic germination of Fraser fir seed as affected by cold-moist stratification, temperature and light. (A) germination under different temperature regimes, with and without a 1-hr light treatment during the 8-hr cycle, following 16 weeks stratification at 4°C; (B) germination as affected by stratification period and germination temperature for seed which received a daily 1-hr light treatment during the 8-hr cycle; (C) germination at 30°/20°C, with and without a daily 1-hr light treatment during the 8-hr, 30°C cycle, as affected by stratification period; and (D) germination at 20°/10°C, with and without a daily 1-hr light treatment during the 8-hr, 20°C cycle, as affected by stratification period. Each data point is plotted at the midpoint of the time interval for which it was calculated.

DISCUSSION

There were strong interactions among the 3 factors, as has been noted similarly for other conifer species (2, 3, 8, 17, 19). A case in point is the loss of sensitivity to temperature and

light with increased durations of stratification (Figures 1, 2, and 4). Even though stratification makes temperature a less critical factor and allows more rapid germination at low temperatures, the relationship is influenced by light. At short durations of stratification (0 to 4 weeks), light is apparently more important than stratification in broadening the range of temperatures for optimum germination of Fraser fir, and increasing total germination at low temperatures (Figure 4). In its effect on total germination, a 1-hr daily light treatment was approximately equivalent to 4 weeks of stratification at 4°C. For stratified seed, sensitivity to temperature was most pronounced in darkness (Figure 4). After only 4 weeks stratification, a 1-hr daily light treatment reduced temperature sensitivity to the extent that germination was relatively high for any constant temperature between 18° and 27°C. The same light treatment was of less consequence, however, following long periods of stratification (Figures 1 and 2).

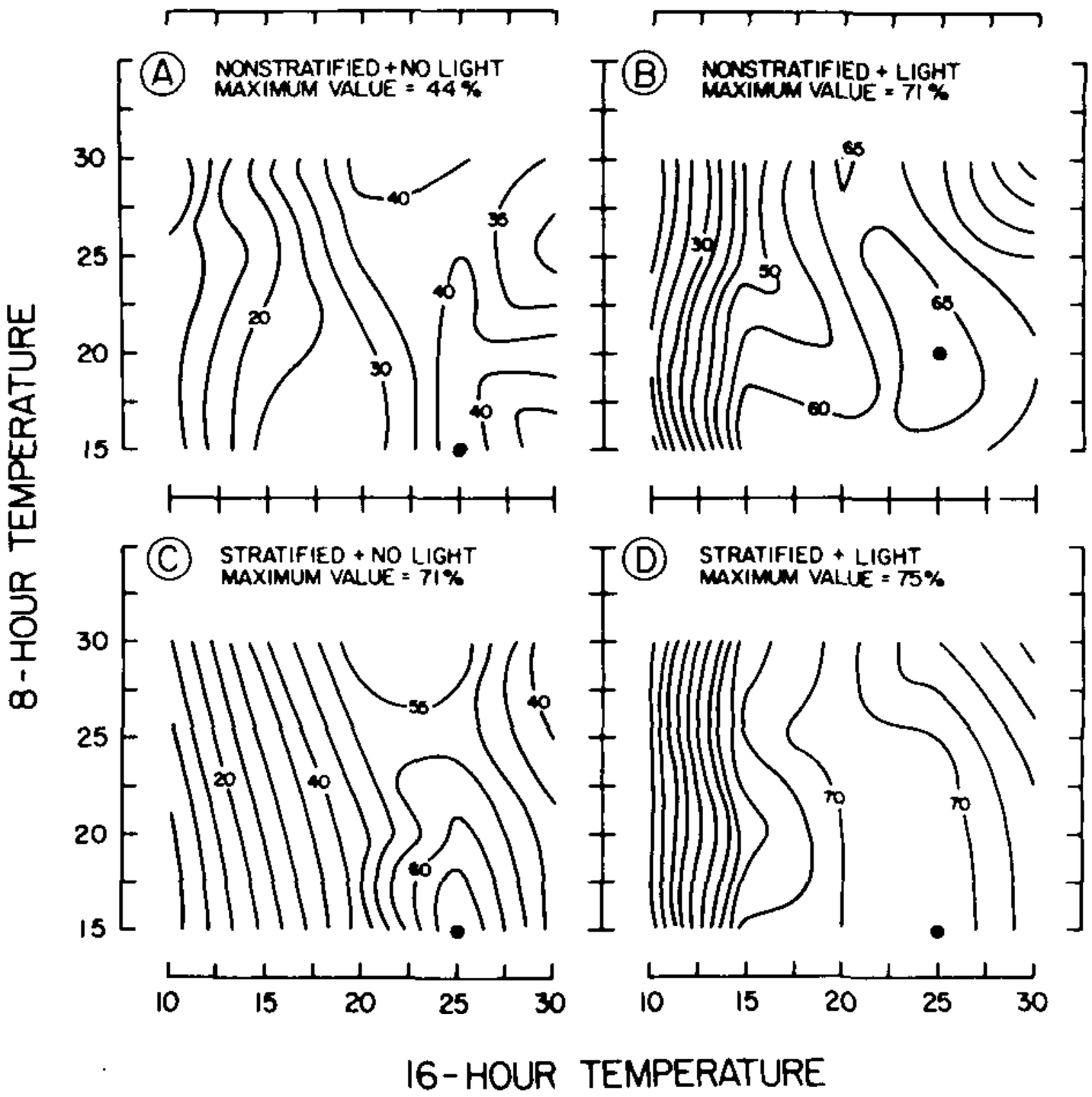

Figure 4. (Expt. 2) Germination of Fraser fir seed at different temperature regimes. The thermoperiod was 8 hr/16 hr. (A) nonstratified seed germinated in darkness, (B) nonstratified seed subjected to a daily 1-hr light treatment during the 8-hr cycle, (C) stratified seed germinated in darkness, (D) stratified seed subjected to a daily 1-hr light treatment during the 8-hr cycle. Stratification was for 33 days at 4°C. Maximum germination value signified by ●.

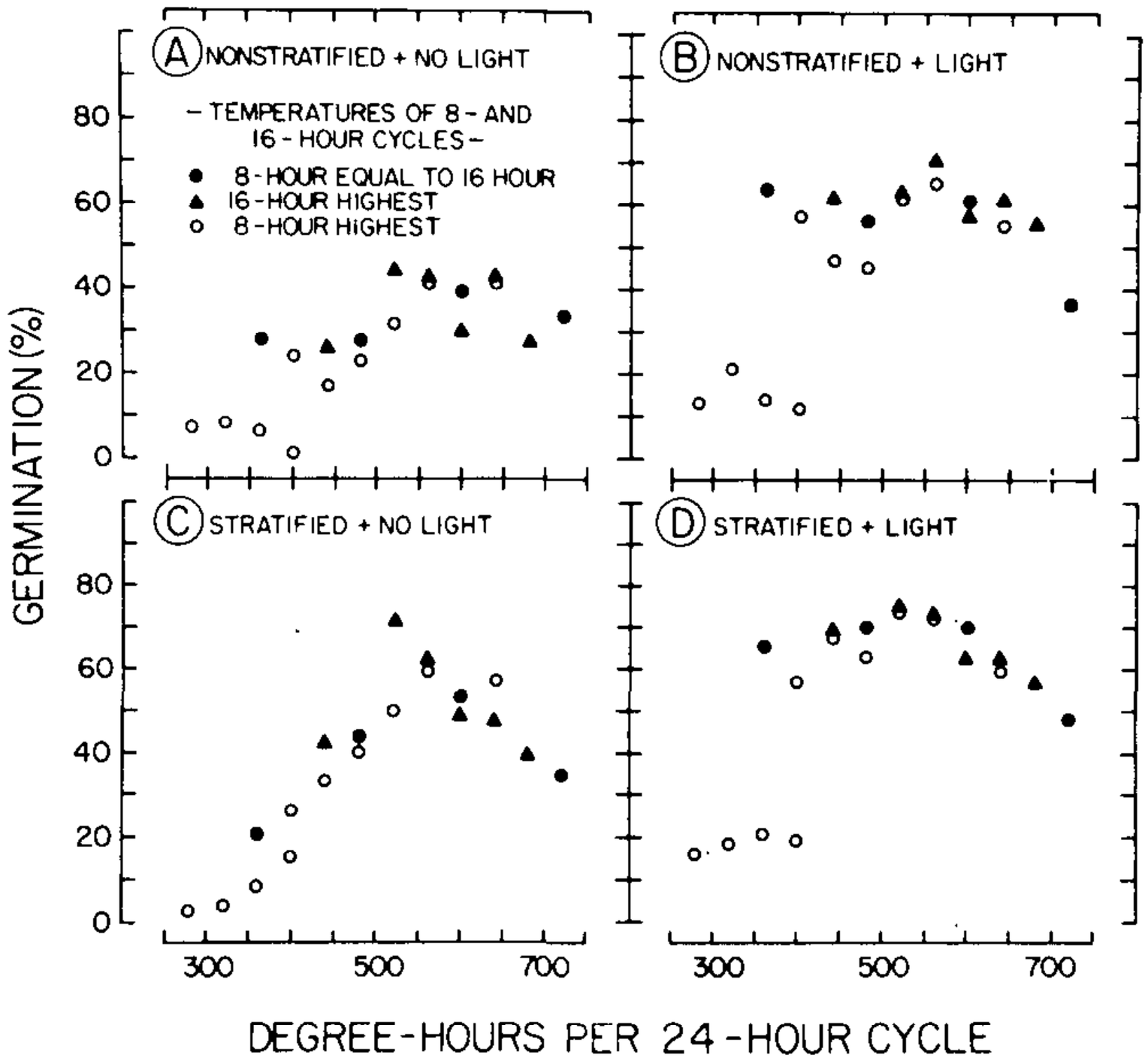

Figure 5. (Expt. 2) Germination of Fraser fir seed as affected by heat input during an 8-hr/16-hr thermoperiod. (A) nonstratified seed germinated in darkness, (B) nonstratified seed which received a 1-hr light treatment during the 8-hr cycle, (C) stratified seed germinated in darkness, (D) stratified seed which received a 1-hr light treatment during the 8-hr cycle. Stratification was for 33 days at 4°C. Legend in (A) applies to all figures.

The time required for germination to follow its course was quite variable, depending on the stratification period, germination temperature, and light treatment. This has implications in selecting the duration of laboratory germination tests. If tests are conducted at warm temperatures (30°/20°C) and include a daily 1-hr light treatment, they need not exceed 28 to 30 days for seed stratified 8 or more weeks; 42 days for seed stratified 4 weeks (Figure 1). Based on these experiments, it is not known how these limits would be affected by light treatments of longer duration. On the other hand, at lower temperatures (20°/10°C) and/or in darkness, more time is required, perhaps 60 to 90 days, to realize most of the potential germination for seed stratified 8 weeks or less. In outdoor nurseries — even those well below the elevation of natural stands, nonstratified Fraser fir seeds commonly germinate throughout the first growing season following planting; a few not until the second season. While this is a useful adaptation to survive weather fluctuations in the native habitat, it is troublesome to nursery-

men who prefer faster germination. Stratification causes rapid and complete germination in a relatively short time over a wide range of environmental conditions. Quick establishment and more uniform stands would facilitate scheduling of cultural treatments.

Minimum and maximum temperatures for germination were not determined. Forty-two-day germination was less than 20% at 15°/10°C (Figure 4). Earlier work indicated that germination of nonstratified seed was practically nil after 42 days at a constant 10°C and zero at 35°C, even with a daily 1-hr light treatment (5). Although these results agree well with those for other *Abies* and *Picea* species (4, 10, 15), seeds of many conifer species can germinate at temperatures between 0° and 5°C given sufficient time (2, 8, 9, 17, 18). At the other extreme, germination occurred at constant 30°C, but was below optimum (Figures 3 and 5), and germinants did not survive the 42-day test.

It is not possible to identify the optimum 8-hr temperature for germination, based on Experiment 2, because maximum germination occurred at the periphery of the data in 3 of the 4 stratification-light treatments (Figure 4). Perhaps germination would have been greater for 8-hr temperatures less than 10°C. The likelihood for this seems small for stratified seed because germination was virtually complete (based on tetrazolium test) after 42 days at 15°/25°C and obviously could not have been much greater than the observed maximum. The uncertainty is greater however, for nonstratified, nonirradiated seed, considering the low 42-day germination and the weak relationship between germination and temperatures of the 8-hr or 16-hr cycles.

Seed tended to mold during cold stratification periods >8 weeks. The type of fungus(i) was not identified, but observations suggested a form of *Rhizoctonia*. One would thus have to weigh the advantages of long stratification periods against the risk of losing seed as a consequence of mold. Fungicide treatments prior to stratification deserve study, and different stratification procedures might also diminish the problem. Other potential problems of long-term cold stratification might be the tendency for seed to germinate during treatment (2, 17) or to gradually lose viability at the high seed moisture content associated with treatment (7).

A means of capitalizing on the beneficial effects of long stratification periods would be fall sowing, thus allowing natural stratification during winter, but this practice is risky. Currently, most Fraser fir seed — much of it nonstratified — is sown in spring. To realize the benefits of cold-moist chilling,

while reducing the risks, the safest procedure for commercial growers appears to be 4 to 8 weeks of artificial stratification prior to spring planting.

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LITERATURE CITED

1. Adkins, C.R. 1983. Effects of selected fungicides, surface sterilants, and environmental factors on germination of Fraser fir seed. MS thesis, N. C. State Univ., Raleigh.
2. Allen, G.S. 1960. Factors affecting the viability and germination behavior of coniferous seed. IV. Stratification period and incubation temperature, *Pseudotsuga menziesii* (Mirb.) Franco. *For. Chron.* 36:18-29.
3. Asakawa, S. 1959. Germination behavior of several coniferous seed. *J. Jap. For. Soc.* 41:430-435.
4. Baldwin, H.I. 1934. Germination of the red spruce. *Plant Physiol.* 9:491-532.
5. Blazich, F.A. and L.E. Hinesley. 1980. Effects of temperature and light on Fraser fir seed germination. *Proc. Southern Nurserymen's Assoc. Ann. Res. Conf.*, 25th Annu. Rpt. p. 225-227.
6. Chapman, H.H. and W.D. Meyer. 1949. Forest Mensuration. McGraw-Hill, New York.
7. Danielson, H.R. and D.F. Grabe. 1973. Storage of noble fir seeds. *Proc. Assoc. Off. Seed Anal.* 63:161-165.
8. Edwards, D.G.W. 1969. Investigations on the delayed germination of noble fir. Ph.D. Thesis, Univ. of Washington, Seattle.
9. Franklin, J.F. and K.W. Kreuger. 1968. Germination of true fir and mountain hemlock seed on snow. *J. For.* 66:416-417.
10. Fraser, J.W. 1970. Cardinal temperatures for germination of balsam fir seed. *Can. For. Serv., Info. Rpt.* PS-X-22.
11. Hellmers, H. 1962. Temperature effect on optimum tree growth. pp. 275-287. *In* T.T. Kozlowski (ed.) Tree Growth. Ronald Press, New York.
12. Hinesley, L.E. 1981. Initial growth of Fraser fir seedlings at different day/night temperatures. *For. Sci.* 27:545-550.
13. Liu, T. S. 1971. A monograph of the genus *Abies*. National Taiwan Univ., Taipei, Taiwan.
14. Mead, R. and D.J. Pike. 1975. A review of response surface methodology *from a biometric viewpoint. Biometrics* 31:803-851.
15. Roe, E.I. 1948. Balsam fir seed — its characteristics and germination. *USDA For. Serv., Lake States For. Expt. Sta., Paper 11.*
16. Speers, C.F. 1962. Fraser fir seed collection, stratification, and germination. *Tree Planters' Notes* 53:7-8.
17. Stearns, F. and J.S. Olson. 1958. Interactions of photoperiod and temperature affecting seed germination in *Tsuga canadensis*. *Amer. J. Bot.* 45:53-58.
18. Stein, W.I. 1951. Germination of noble and silver fir seed on snow. *J. For.* 49:448-449.

19. Wang, B.S.P. 1960. The effects of stratification and incubation temperature on the germination of grand fir. MS thesis, Univ. of British Columbia, Vancouver, Canada.

LARRY KUHNS: Did you try Clorox to control the mold growth on your seeds?

CRAIG ADKINS: *Abies* seeds are very dirty and have many different fungal organisms on them. We tried Clorox and other surface sterilants but none of them worked. A very weak concentration of Benlate will control the fungal growth.

QUESTION BOX

The Question Box Session was convened at 9:15 a.m. with Ralph Shugert and Joerg Leiss serving as moderators.

MODERATOR LEISS: What herbicides are recommended for control of perennial weeds in containers.

MICHAEL DODGE: At White Flower Farm we believe very strongly that herbicide use in herbaceous perennials is not a good practice, because of the similarity between the weeds you are killing and the plants you are growing. Good husbandry is the answer. Prevent the weeds from growing by pasteurizing the compost and preventing weeds from growing in the beds on which the pots are standing. Weeds on the standing-out beds can be controlled by using a covering cloth or a combination of Roundup and Surflan to kill and prevent weeds. Keep the surrounding area weed-free to reduce blow-in of weed seeds.

JOERG LEISS: Fusilade does a good job on grasses.

MODERATOR LEISS: Would Surflan, applied at the recommended rate during the fall, have any adverse effect on rhododendrons or azaleas planted in the spring?

LARRY KUHNS: It should have no effect even if applied in the spring after planting at the recommended rate of 2 to 4 lb AIA.

CHARLIE PARKERSON: We use this material in an EC formulation on rhododendrons and are very satisfied with it.

MODERATOR LEISS: What herbicide would you use to control wild oats and downy brome in established evergreen and deciduous nursery stock, both pre- and post-emergence?

LARRY KUHNS: For post-emergence Poast or Fusilade can be used while for pre-emergence, Surflan in spring and Devrinol in the fall can be applied.