

RESEARCH NOTES



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**TSUGA CANADENSIS (L) CARR. GERMINATION
STIMULATED BY RED LIGHT WHILE INHIBITED BY
GIBBERELLIN AND KINETIN**

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Tsuga canadensis (L) Carr. germination stimulated by red light while
inhibited by gibberellin and kinetin

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ABSTRACT

Preliminary tests revealed daily treatment of stratified Tsuga canadensis (L) Carr (eastern hemlock) seed with one-half hour exposures of red light increased germination by 39 percent after 28 days over seeds kept in total darkness. Contrary to studies with other species, treatment of stratified seed with 1 mM GA or 0.05 mM kinetin inhibited germination, with or without red light treatment. At the concentration tested, the data suggest that GA is not directly involved in the red light mediated process of germination in eastern hemlock. Further testing is necessary to determine the specific role of these growth regulations in the stratification process of eastern hemlock seeds.

Additional Key Words. Eastern hemlock, seed, dormancy, light quality, growth regulators.

The presence of a phytochrome mediated system in the germination process of many species has been well documented as noted by Spruit and Mancinelli (1969). Further, it has been determined that many of the germination responses observed by irradiation with red light have also been observed when seeds are treated with gibberellin (GA) (Burdett and Vidaver, 1971; Kohler, 1966) or with a GA-kinetin combination (Bewley and Fountain, 1972; Khan, 1971). The little work done with seeds of tree species has produced results similar to those for light sensitive seeds such as lettuce. Seed germination of Pinus Strobus L. is significantly increased with short exposures of red light (Toole, et al., 1962) as in Pinus taeda L. (McLemore, 1971; Toole, et al. 1962). In addition, repeated or continuous exposures to red light increased germination within a given period of time and reduced the time necessary for seed stratification (McLemore, 1971; Toole, et al., 1962). Venator (1972) found that application of exogenous GA to seeds of low vigor Pinus caribaea var. hondurensis Barr. and Golf. significantly increased germination. Similar results were obtained by McBride and Dickson (1972) with Fraxinus americana L. Further, they found that the necessary stratification period for optimal germination could be reduced from 60 to 30 days by treatment with 1 to 100 ppm GA.

Most work on the effects of red light and growth regulators has been done with shade intolerant species. However, Olson et al. (1959) has shown that Tsuga canadensis (L.) Carr. (eastern hemlock) is also light sensitive but they did not attempt to determine the active wavelength. Results from preliminary tests in this study revealed that red light is active in stimulating germination of eastern hemlock, while a commonly used concentration of GA₃ and kinetin inhibits rather than enhances germination.

MATERIALS AND METHODS

Seeds of eastern hemlock were collected from trees near Houghton, Michigan during October of 1972 and were stored in a sealed dry container at 5° C. One group of seeds were removed from storage and stratified in moist sand at 5° C for 70 days, as recommended by Olson et al. (1959). In January, 1973 both stratified and unstratified seed were soaked for one hour in solutions containing either 1.0 mM GA₃, 0.05 mM kinetin, 1.0 mM GA₃ plus 0.05 mM kinetin, or distilled water. The seeds were divided into lots of 100, placed on two Whatman No. 1 filter papers in covered 9 cm Petri dishes, and moistened with 2 ml. of the appropriate solution. These concentrations are commonly used by other workers for similar experiments (Burdett, 1972; Burdett and Vidaver, 1971; Khan, 1971; and Venator, 1972). Two replications of each solution treatment were placed in growth chambers having either brief exposures to red light or complete darkness. The red light treatment consisted of daily one-half hour exposures to red light (peak at 615 nanometers and a half peak height bandwidth of 60 nanometers) at a rate of 0.056 g-cal/cm²/min. Temperature was maintained at 18° C as suggested by Olson, et al. (1959). Germinated seeds were counted in dim green light daily for 50 days and the filter paper moistened with distilled water when necessary (approximately every 5 to 6 days).

RESULTS AND DISCUSSION

The effect of red light was to reduce the number of days required before the germination of stratified seed commenced and to increase total germination over that of the non-irradiated seed (Figure 1). Stratified seed treated with distilled water plus red light (irradiated control) commenced germination on the eleventh day compared to the fourteenth day for

those treated with distilled water and kept in the dark (non-irradiated control). By then eight percent of the irradiated control seeds had germinated. Once the seeds of either treatment commenced germination, the daily germination rate was essentially the same regardless of treatment until the eighteenth day, at which point the germination rate of the non-irradiated seeds began to decrease dramatically (Figure 1). After 28 days, germination of irradiated seeds was 9 percent higher (32 percent vs. 23 percent) than the non-irradiated seeds. This was found to be significantly different at the 0.05 probability level.

The results with stratified seed agree favorably with the work done by Toole et al. (1962) and McLemore (1971) with pine. The fact that the greatest germination was only 32 percent can be partially explained by the lack of viability in many of the seed in each lot. Forty-five percent of the seeds were non-viable (aborted embryo) in the stratified irradiated seedlots compared to thirty-eight percent for the stratified non-irradiated lots. If the non-viable seeds are excluded, then 58 percent of the viable stratified irradiated seeds germinated compared to 37 percent for the viable non-irradiated seeds. While still somewhat low, these figures agree reasonably with other work done on eastern hemlock germination (Olson, 1959).

Essentially no difference existed between the date of first germination or the final germination percentage of unstratified seed. All treatments began germination between the 42nd and 44th day following the start of the study, and all treatments had between 7 and 9 percent germination after 50 days. The nearly complete lack of germination with unstratified hemlock seed under red light indicates that physiological process system activated by red light is operative only after the seeds have become hydrated for a period of time. This is consistent with the findings of Hsiao and Vidaver (1971) who found that lettuce

seeds have to be somewhat hydrated before the phytochrome system can stimulate germination. Further, Olson et al. (1959) reported that rapid germination of eastern hemlock seed will not occur until the seed has been stratified for at least 40 days, with 70 days being ideal. Unstratified seed normally takes between 30 to 50 days before germination is initiated or about the same length of time as the minimum recommended period of stratification Olson-et al. (1959). Exactly how much stratification is necessary before red light will enhance germination is not known. Toole et al. (1962) has indicated that stratification time for optimal germination of pine can be reduced when red light is used. In addition, Olson et al. (1959) reported that white light reduces the length of time required for unstratified eastern hemlock seed to germinate. Therefore, it is not unreasonable to assume that optimum germination can occur with shorter stratification periods for hemlock as well as pine if red light is used.

Germination of stratified seed treated with GA, kinetin or a combination of both was significantly reduced (at the 0.05 level) from their respective controls regardless of light treatment (Figures 2 and 3). However, the level of reduction was not as severe in the irradiated seed (Figure 2) as in the non-irradiated seed (Figure 3). Further, there was no real difference among growth regulator treatments with a given light treatment. The final germination percentages for the irradiated seed averaged 22-, 21-, and 18- percent for the GA-kinetin combination, kinetin, and GA treatments respectively compared to 14-, 12-, and 11- percent for the same three respective treatments in the dark. In addition, the final germination percentage for the non-irradiated control seeds was 23 percent (Figure 3) only slightly higher than that of the irradiated GA-kinetin treated seeds (Figure 2).

Initiation of germination for seed treated with growth regulators was not substantially different from their respective controls (Figures 2 and 3). The greatest delay occurred with irradiated GA and kinetin treated seeds which had delayed germination of only two days when compared to their controls. Apparently the growth regulator treatments did not inhibit the commencement of germination within a given light treatment, only the daily germination rate thereafter.

The results indicate that treatment of seed with these concentrations of growth regulators causes a general inhibition of the physiological process leading to germination, regardless of the light treatment applied. The inhibition caused by GA is inconsistent with the findings of other workers who report that GA acts similar to red light - both enhancing germination of light sensitive seed. Ross and Bradbeer (1968) present evidence that the process of stratification itself enhances the production of GA in hazel nuts which leads to germination. Further, Burdett (1972) postulates that red light stimulates the production of natural GA which in turn activates the phytochrome system and therefore germination. If both theories apply for eastern hemlock, stratification and red light irradiation would result in the natural production of optimal GA concentrations in the seed, and any further addition of GA could increase the concentration to inhibitory levels. However, the stimulation of GA production by red light irradiation does not seem likely in this case. If GA synthesis was involved in the red light mediated process, it would seem logical that addition of exogenous GA would inhibit germination of irradiated seed to a greater degree than non-irradiated seed. This should occur since, in theory, irradiated seed would have produced more natural GA than non-irradiated seed thereby resulting in a greater inhibition when exogenous GA was applied.

The reverse was found, however, in that the total germination was less (therefore greater inhibition) for non-irradiated GA treated seed (11 percent) than it was for the irradiated GA treated seed (18 percent) (Figures 2 and 3). Further, even when germination of GA treated seed of both light treatments are compared to their respective controls, the non-irradiated seed still sustained a somewhat greater germination reduction than did the irradiated seed (Table 1).

Although the results indicate that GA synthesis may not be directly related to the red light mediated process in eastern hemlock, they are inconclusive about growth regulator involvement with the stratification process. All growth regulator treatments inhibited germination of all stratified seed to a substantial degree (Table 1). While irradiated seed were consistently inhibited to a lesser degree than non-irradiated seed (Table 1), the differences were generally not excessively large. It is therefore possible that the inhibition occurred simply because the stratification process had already produced sufficient GA and/or kinetin for germination, and the exogenous growth regulators resulted in inhibitory high concentrations. On the other hand, it is equally possible that these growth regulators operate in a different capacity entirely, or perhaps have no natural physiological function in the stratification process at all.

The lack of any detectable effect of growth regulators on germination of unstratified seed is equally inconclusive. These results suggest that the applied growth regulator concentrations were either too low to have any effect on germination or else they have no active role in the stratification process at all. Only a complete test using a series of GA and kinetin concentrations will determine the role or non-role of GA and kinetin in the germination process of eastern hemlock.

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TABLE 1. Percent reduction from controls in total germination of stratified seed after 28 days when treated with growth regulators.

	Control	GA	GA-kinetin	kinetin	Ave.
Irradiated	0	44	32	34	38
Non-irradiated	0	52	39	48	46

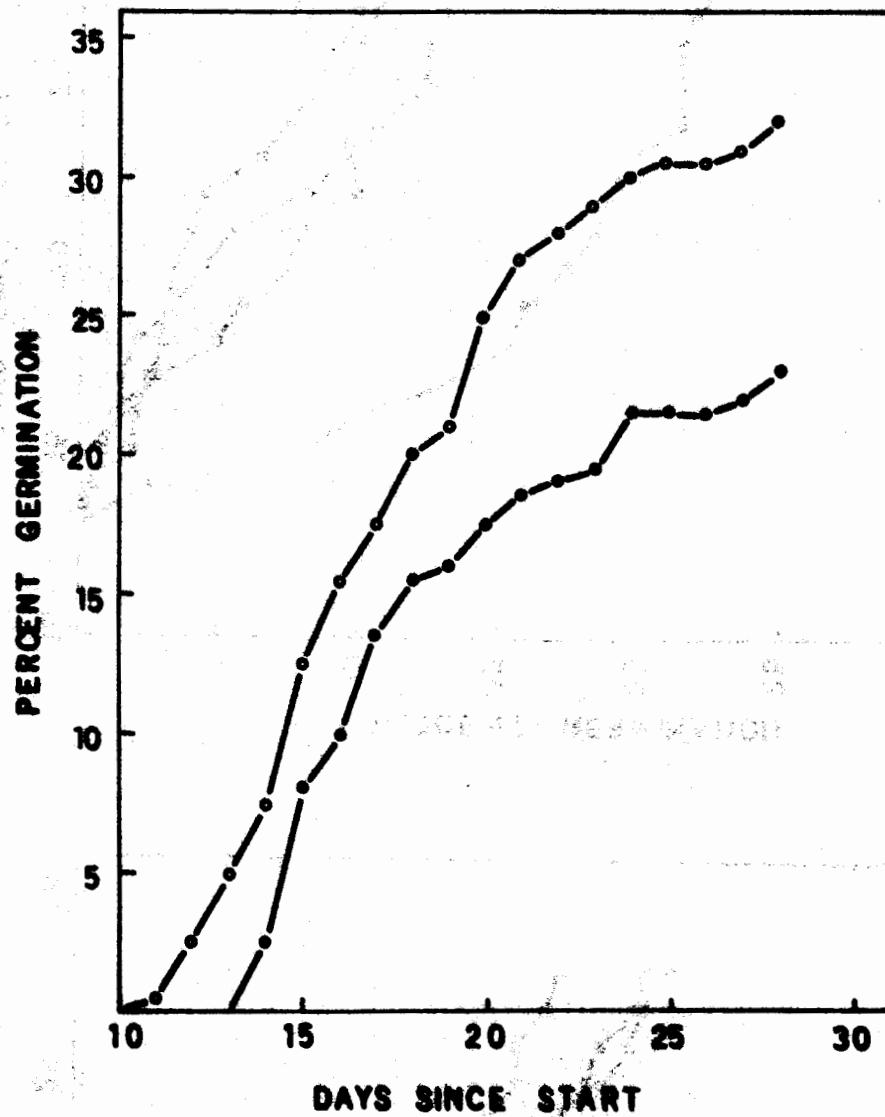


FIG. 1. Percent germination of stratified hemlock seed irradiated with red light for one-half hour each day (o-o) and seed maintained in darkness (●-●)

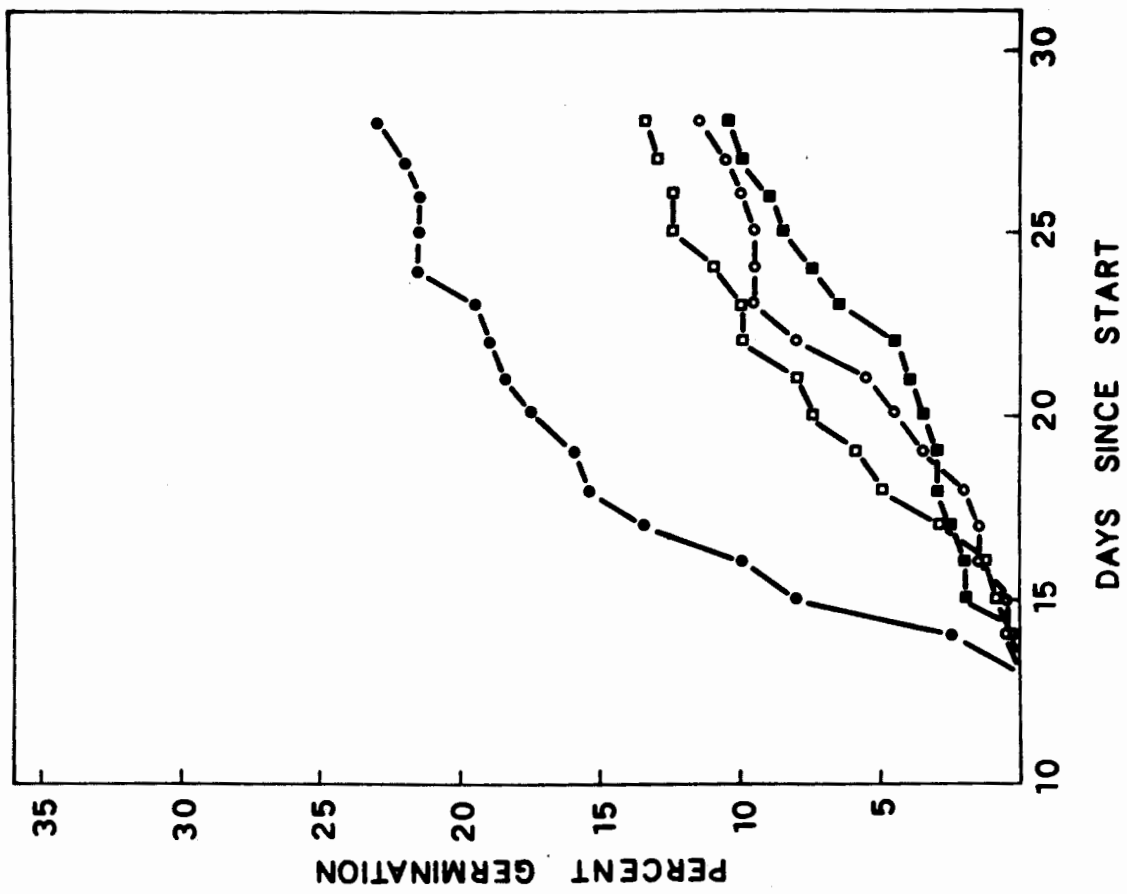


FIG. 2. Percent germination of stratified seed irradiated with red light and treated with either distilled water (●-●), a GA₃-kinetin combination (○-○), 0.05 mM kinetin (□-□), 0.05 mM GA₃ (■-■) or 1.0 mM GA₃ (▲-▲).

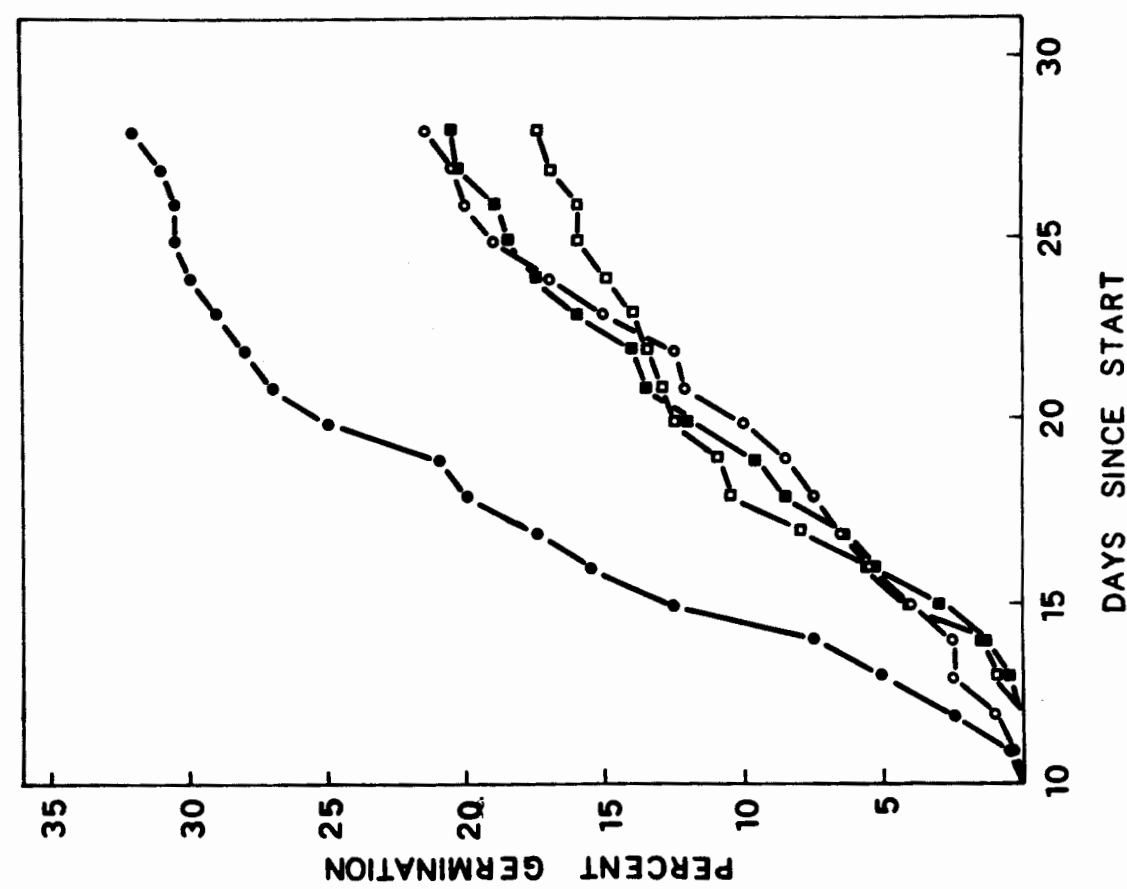


FIG. 3. Percent germination of stratified seed maintained in darkness and treated with either distilled water (●-●), a GA₃ - Kinetin combination (○-○), 0.05 mM kinetin (□-□), 0.05 mM GA₃ (■-■) or 1.0 mM GA₃ (▲-▲).