

Laser activation of phytochrome-controlled germination in *Pinus banksiana*

ROBERT A. CAMPBELL¹ AND DONALD J. DURZAN²

Forest Ecology Research Institute, Canadian Forestry Service, Environment Canada, Ottawa, Ont., Canada

Received May 18, 1979³

Accepted August 21, 1979

CAMPBELL, R. A., and D. J. DURZAN. 1979. Laser activation of phytochrome-controlled germination in *Pinus banksiana*. Can. J. For. Res. 9: 522-524.

A 100 ms exposure from a 5 mW helium-neon laser was sufficient to potentiate fully phytochrome-controlled germination in *Pinus banksiana* Lamb. The energy requirement for the brief, high-intensity light exposure was comparable to that for a much longer, low-intensity light exposure. As a result, the laser may be valuable in studies of the germination process and the primary mode of action of phytochrome.

CAMPBELL, R. A., et D. J. DURZAN. 1979. Laser activation of phytochrome-controlled germination in *Pinus banksiana*. Can. J. For. Res. 9: 522-524.

Une exposition de 100 ms à un laser de 5 mW à l'hélium-néon s'avère suffisante pour conditionner complètement la germination dépendante du phytochrome chez le *Pinus Banksiana* Lamb. L'énergie requise pour un bref éclaircissement à forte intensité est comparable à celle d'un éclaircissement plus long à faible intensité. Conséquemment le laser peut s'avérer utile pour les études portant sur la germination et sur le mode d'action primaire du phytochrome.

[Traduit par le journal]

Introduction

Light is required for the germination of *Pinus banksiana* seed (1). That this light requirement is mediated by the pigment phytochrome is demonstrated by the following: (i) germination in darkness is less than 20%; (ii) if seeds are imbibed in darkness, then given a brief exposure of far-red light (730 nm) and returned to darkness, germination is 1-15% (i.e., less than in continuous darkness); (iii) if seeds are imbibed in darkness, then given a brief exposure of red light (660 nm) and returned to darkness, germination is 80-95%; and (iv) the effects of the two different light qualities are reversible (e.g., if the red light exposure is immediately followed by a far-red exposure then subsequent germination in the dark is less than 20% (12; Campbell unpublished).

In this laboratory we have been studying various aspects of the germination process in *P. banksiana* (4, 9). We became interested in the phytochrome control of germination as a means of separating events associated with germination per se from those associated with imbibition. The following experiment was carried out to determine whether germination could be induced by much shorter light exposures

than had been previously reported if a very intense light source such as a laser were used. If germination could be induced by a very brief exposure, it would be much easier to detect the very first metabolic events associated with germination and also possibly to determine the primary mode of action of phytochrome.

The ability to potentiate rapidly would also be useful in the study of rapidly expressed phytochrome-controlled processes. For example, electrical potential changes in oat coleoptiles have been observed 15 s after light treatment (10), and *Mougeotia* chloroplast movement has been observed in less than 1 min (7).

Materials and Methods

Pinus banksiana Lamb. seed was obtained from the seed bank at Petawawa Forest Experiment Station, Chalk River, Ontario (seed lot 70180). The seed was not extracted from the cones in the dark (cf. 11) but after it was received at the Forest Ecology Research Institute it was stored in a light-tight container at -18°C. All subsequent operations were carried out under a safelight. All treatments and subsequent germination were carried out at 25°C.

The safelight consisted of light from two Westinghouse F15T8/G green fluorescent lamps filtered through a Kodak safelight filter No. 7. The ends of the tubes were covered with black tape to minimize infrared emission. To test the effectiveness of the safelight, imbibed seeds previously exposed to red light, far-red light, or no pretreatment were exposed to the green light for 60 min. In no case was there any detectable effect of the green light on subsequent germination in the dark.

The helium-neon laser (Spectra-Physics model 134) had

¹Present address: Pest Control Section, Ministry of Natural Resources, Maple, Ont., Canada L0J 1E0.

²Present address: The Institute of Paper Chemistry, P.O. Box 1039, Appleton, WI, U.S.A. 54911.

³Revised manuscript received August 4, 1979.

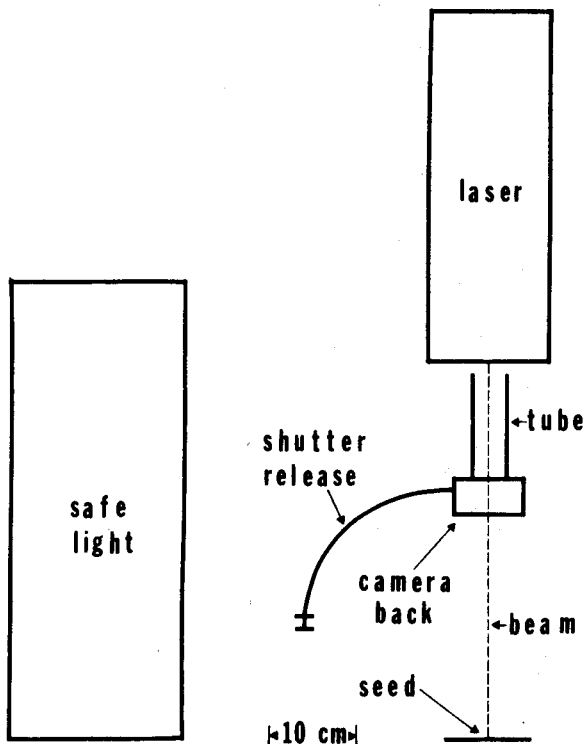


FIG. 1. Set up for treating seeds with laser light. The laser was left on continuously. The camera lens was replaced with a black tube to trap stray light. To prevent beam movement due to shutter vibration, there was no direct contact between the camera and the laser. The beam, when it struck the seeds on the target, was calculated to have a diameter of 1.54 mm and an area of 1.86 mm².

a rated output of 5 mW of CW red (632.8 nm) light. The beam had an exit diameter of 0.8 mm at $1/e^2$ points, random polarization, a TEM₀₀ spatial mode, and a divergence of 1.1 mrad. The far-red light source consisted of a mixture of incandescent and fluorescent light passed through a 3 mm thick Corning CS-7-69 filter (35% T at 730 nm, 0.1% T at 700 nm, 0.03% T at 660 nm). The power measured with the far-red sensor of an International light IL-150 meter was 350 $\mu\text{W}\cdot\text{cm}^{-2}$. The light used to germinate seeds under continuous light produced a flux of 240, 110, 210 $\mu\text{W}\cdot\text{cm}^{-2}$ in the red, far-red, and blue portions of the spectrum, respectively.

Methods for Laser Experiment

Sixteen 0.180 gm lots of seed were weighed out (about 50 seeds per lot) and placed in 50 mm \times 12 mm plastic Falcon dishes each containing 1 layer of Whatman No. 1 filter paper. One millilitre of distilled water was added simultaneously to each dish. Two dishes were placed under continuous light to act as light controls. Two dishes were placed in light-tight containers to act as dark controls. After 1 h of imbibition the remaining dishes were given a 20 min exposure of far-red light (The purpose of this was to reduce the initial germination potential so that the effect of low doses of laser light could be detected more easily). The dishes were returned to the dark for 17 h. The set-up for the laser treatments is shown in Fig. 1. Individual seeds

were placed on the target and an exposure made with a focal plane camera shutter. Approximately 25 seeds were treated at each shutter speed, followed by another 25 at each speed, etc. Four lots of 25 seeds were treated with 0, 2, 4, 10, and 20 ms of laser light. (For the 0 s treatment individual seeds were placed on the target for approximately 1 s with the laser on and the shutter closed in either the cocked or released position). Two lots of 25 seeds were treated with 100 and 1000 ms. Each seed lot was put in a fresh petri dish containing wetted filter paper and incubated in the dark. After 7 days the germination was scored.

Seed Area Calculations

The average horizontal area of the upper surface of a *P. banksiana* seed was determined by projecting a photographic transparency of imbibed seeds onto a sheet of paper, tracing the images, cutting out the tracings, and weighing the cutouts. The area so calculated was 4.37 mm² with a standard deviation of 0.065.

Statistical analyses according to Goldstein (5) were performed.

Results and Discussion

The results are presented in Fig. 2. Comparison of bar graphs A and B indicates the light requirements for germination. Comparison of B with C indicates the inhibiting effect of the far-red pretreatment. The germination in treatment C is considerably higher than that found normally in our laboratory. It is possible that there was a slight light leak. Another possibility is that the seed coats of the softened, imbibed seeds were damaged by the extensive handling required in applying the treatment. Nyman (11) found a marked increase in dark

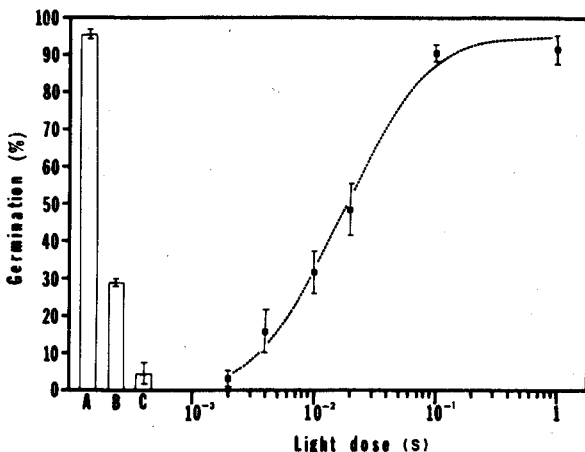


FIG. 2. The stimulation of *Pinus banksiana* seed germination by laser light: (A) germination in continuous white light; (B) dark germination; (C) germination after far-red light followed by 0 s of laser light; (D) germination after far-red light followed by laser light. Standard errors are given. Curve was fitted by probit analysis. Light dose values can be converted to millijoules per seed by multiplying the seconds by five.

germination of *Pinus silvestris* if the seed coats were punctured.

The data in Fig. 2 indicate that a laser flash of 100 ms is enough to fully potentiate germination. There is a significant stimulation at an exposure of 4 ms. Over the range of 3 to 90% germination (2 to 100 ms exposure) there is a linear variation of the probit with the log of the energy. A linear relationship has also been found for *Lactuca sativa* (2) and *Pinus silvestris* (11).

As the laser beam covered only about one-third of the upper surface of the *P. banksiana* seed, it is meaningless to express the light doses in millijoules per square centimetre as is usually done. For comparison, the ED₅₀ (dose required to induce 50% of the maximum germination) was calculated on a per seed basis for the laser data and for the data of Orlandini and Bulard (12). The former gave a value of 0.075 mJ/seed whereas the latter gave a value of 0.052 mJ/seed. The slightly higher value for the laser may be a result of the laser beam covering only one-third of the upper surface of the seed. Another possible explanation derives from the fact that the slow step in the conversion of P_r to P_{fr} has a half-conversion time of 2.3 s (8). As all of the laser exposures were considerably shorter than that, energy might have been lost because it could not be utilized fast enough. If that were the case, the slope of the probit versus log energy graph for the laser exposures should be greater than that for other light exposures given over periods of 10–300 s (2, 12). Furthermore, the laser probit versus log energy graph should not be linear but the slope should increase as the exposure becomes shorter. Neither of these is the case. The slope of the laser probit versus log energy graph is 1.9 probits per log unit. From the data of Borthwick et al. (2, 3) for lettuce, a slope of 1.9 was calculated. From the data of Orlandini and Bulard (12) for *P. banksiana*, a slope of 1.6 was calculated. The goodness of fit of the computed curve in Fig. 2 indicates that the slope does not increase as the exposure decreases.

In the phytochrome system controlling chloroplast movement in *Mougeotia*, a single high-intensity red light flash in the "millisecond range" (sic) did not produce a significant response. However, when two successive flashes, each 50% intensity of the single flash, were separated by more than 3 ms there was a significant response which reached a maximum with an interval of 30 ms (6). If the phytochrome system controlling pine seed germination were the same, one would expect the slope of the probit versus log energy graph to increase as the exposure decreased below 30 ms. The fact that the slope does not increase suggests that the two systems may differ.

Helium-neon lasers have been used to activate phytochrome-controlled processes including seed germination (13). The emphasis in the previous work was the aspect of action at a distance which is made possible by the collimate nature of laser beams. The flux used was relatively low so that potentiation of responses did not occur any faster than with conventional low-intensity light sources. The present work shows that a high-intensity light source, such as a laser, can potentiate a phytochrome response very rapidly without a significant loss of efficiency. Thus, the laser should be a useful tool in seeking early metabolic events associated with germination. It may also have value in studies of the primary mode of action of phytochrome.

Acknowledgements

We thank Mr. B. S. P. Wang of Petawawa Forest Experiment Station for supplying the seed and Mr. J. French for technical assistance.

1. ACKERMAN, R. F., and J. L. FARRAR. 1965. The effect of light and temperature on the germination of jack pine and lodgepole pine seeds. Univ. Toronto Fac. For. Tech. Rep. 5.
2. BORTHWICK, H. A., S. B. HENDRICKS, M. W. PARKER, E. H. TOOLE, and V. K. TOOLE. 1952. A reversible photoreaction controlling seed germination. Proc. Natl. Acad. Sci. U.S.A. **38**: 662–666.
3. BORTHWICK, H. A., S. B. HENDRICKS, E. H. TOOLE, and V. K. TOOLE. 1954. Action of light on lettuce-seed germination. Bot. Gaz. (Chicago), **115**: 205–225.
4. DURZAN, D. J., A. J. MIA, and B. S. P. WANG. 1971. Effects of tritiated water on the metabolism and germination of jack pine seeds. Can. J. Bot. **49**: 2139–2149.
5. GOLDSTEIN, A. 1964. Biostatistics: an introductory text. MacMillan Co., New York, NY.
6. HAUPT, W., and N. BRETZ. 1976. Short-term reactions of phytochrome in *Mougeotia*. Planta, **128**: 1–3.
7. HAUPT, W., and H. ÜBEL. 1975. Zum mechanismus der phytochromwirkung bei der chloroplastenbewegung von *Mougeotia*. Z. Pflanzenphysiol. **75**: 165–171.
8. HENDRICKS, S. B., and H. A. BORTHWICK. 1967. The function of phytochrome in regulation of plant growth. Proc. Natl. Acad. Sci. U.S.A. **58**: 2125–2130.
9. MIA, A. J., and D. J. DURZAN. 1974. Cytochemical and subcellular organization of the shoot apical meristem of dry and germinating jack pine embryos. Can. J. For. Res. **4**: 39–54.
10. NEWMAN, I. A., and W. R. BRIGGS. 1972. Phytochrome-mediated electric potential changes in oat seedlings. Plant Physiol. **50**: 687–693.
11. NYMAN, B. 1963. Studies on the germination in seeds of Scots Pine (*Pinus silvestris* L.) with special reference to the light factor. Studia Forestalia Suecica No. 2 Skogshögskolan, Stockholm.
12. ORLANDINI, M., and C. BULARD. 1972. Photosensibilité des graines de *Pinus banksiana* Lamb. Biol. Plant. **14**: 260–268.
13. PALEG, L. G., and D. ASPINALL. 1970. Field control of plant growth and development through the laser activation of phytochrome. Nature (London), **228**: 970–973.