Annual Germination Window in Oospores of *Nitella furcata* (Charophyceae).[1]

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ABSTRACT

Oospores of Nitella furcata subsp. megacarpa (Allen emend. Wood) were collected from an oospore bank in the sediments of Lake George, New York. Incubated at constant temperatures, all or nearly all of the oospores germinated when exposed to a brief pulse of red light when the annual window of germinability was open. The window seems related to the annual cycle of sediment temperatures. It is open in spring and closes with the onset of a secondary dormancy in the summer. Oospores in storage follow a parallel path if held at 18° C, a summer equivalent temperature; the window remains open indefinitely if the oospores are held at 4° C. Attention is drawn to the similarity of the cyclic window of germinability in seeds of summer annuals and oospores of N. furcata.

Key index words: germination window; Nitella furcata; oospores; photoactivation; secondary dormancy

INTRODUCTION

Oospores of charophytes are reported to germinate in a variety of environments with widely ranging success. In extensive tests starting with newly ripened oospores of Chara spp., Proctor(1960, 1962, 1967) reported germination percentages ranging from 0 to 95%. A primary dormancy of from 1 to 3 months was widely reported (Proctor 1960, 1967, Forsberg 1965, Wetzel and McGreggor 1968). The response to stratification was always favorable, whereas a heat treatment gave conflicting results among the investigations.

Light was first reported to be either unnecessary, or capable of activating only a minor fraction of the oospores (Proctor 1960, 1962, 1967, Forsberg 1965). Later Wetzel and McGreggor (1968) showed that of the 13% germinating oospores most required light. A sensitivity to red light was first reported by Maeda and Imahori(1968). Phytochrome was implicated as the photoreceptor by Takatori and Imahori (1971) who showed red/far-red photoreversibility.

Oospores of Nitella spp. accumulate in the sediments beneath and beyond the nitella meadow in Lake George, NY. Stross et al.(in litt.) reported densities up to $4 \times 10^{6} \cdot m^{-2}$ in the upper 2.0 cm of sediment, or $2 \times 10^{4} \cdot L^{-1}$, and denser than weed seeds in arable soils (Livingston and Allessio 1968, Wesson and Wareing 1969a,b). The purpose of this study was to determine germination requirements and the seasonal status of dormancy in oospores from lake sediments.

METHODS AND MATERIALS

Sediments containing oospores of Nitella furcata subsp.

megacarpa(Allen emend. Wood) were removed at night with an Ekman dredge from water depths of 7.5 to 9.0 m, within the nitella meadow in Smith Bay, Lake George, New York, (Stross 1979). A slurry of the sediments was passed through a series of sieves (US Standard #8, #25), with oospores being retained on the smallest (#50). The retentate was refined further by a "panning" process (Proctor pers. comm.) to remove less dense organic debris and empty oospores. The concentrate was held in 0.9 L, wide mouth jars and transported from the lake on ice. Stringent dark conditions were maintained throughout collection and testing. In the lab the material was used in experiments and stored at constant temperatures of 4 or 18° C. Field collections were made throughout the year.

Germination trials were made over a three-year interval on oospores that were recently collected or had been stored in constant temperature and darkness within the refined retentate. Each treatment contained a minimum of four replicates, each replicate contained 20 or 50 oospores. Oospores were counted out under a green safelight, except where indicated, and cultured on a 1.2% agar substratum, overlaid with membrane-filtered (0.6 µM pore size) water from Lake George. Culture vessels were acid-cleaned, shell vials (25 x 95 mm), or wide-mouth jars (112 mL). The "safelight" was constructed with 14 W cool white fluorescent lamp (General Electric), and two filters: a solution of $CuCl_2$ (0.293M) with a 10.0 cm pathlength and a piece of green acrylate (#2128, Caloric Plastics, New Jersey).

The oospores were incubated in walk-in growth chambers (Percival, Boone, Iowa) at a constant 13° C for experiments conducted up until May 1984 and thereafter at 18° C. They were irradiated for 15 hours each day with approximately 9.5 $\mu E \cdot m^{-2} \cdot s^{-1}$ daylight fluorescent light

after it had been filtered through red (Rohm & Haas #2424) plexiglas. No safelight was used in dark controls and in treatments designed to measure energy requirements. A preliminary measure of spectral sensitivity was carried out with narrow band interference filters (Schott, Duryea, Pennsylvania) at red (669nm), green (538nm) and blue (449nm) wavelengths. Light was supplied by two 75 W incandescent flood lamps (Ken-Rad), filtered through 5.0 cm of water. Photon fluences were measured with a quantum sensor (Li-Cor, LI-185; Lincoln,Nebraska) positioned at the level of the oospores. Spectral transmission of light by the interference filters and by the waters of Lake George was measured with a Li-Cor LI-1800UW spectroradiometer.

Germination was readily recognizable after 10 days at 18° C when the valves of the oospore were open and the primary cells for protonema and rhizoid were visible under 15 x magnification (Ross 1959). At 14 days the protonema contained chlorophyll. The minimum incubation time was 10 days. Percentage germination is based on the total number of viable oospores; ungerminated oospores were judged to be viable if starch grains were extruded from a turgid cell when squeezed with a forceps.

RESULTS AND DISCUSSION

Oospores of N. furcata germinated profusely in the laboratory when two conditions were met. Under standard conditions (see Methods) oospores required exposure to light at 13 and 18° C. Their response to light was restricted to an annual "window" of germinability. Peak germinability was found in the months of May and June 1984 when germination ranged from 79.0 to 95.0% (Fig. 1A).

The germination window is best defined with evidence of its closing in each of three years. Oospores incubated immediately after collection in July and September 1983 germinated at 25.1 and 0.0% , respectively. Collections on June 20 and July 28, 1984 yielded 81.0 and 0.0% germinating oospores, respectively. Finally, a collection from the meadow in September 1985 gave a germination of only 1.5% (Fig. 1A).

The window seemed to open in April 1984 (Fig. 1A). Oospores collected in early March 1984 showed no obvious germination until late April (not shown). An early April 1985 collection yielded a 97.2% germination in late April. Thus it seems safe to suggest the germination window is fully opened by late April.

Sediment temperatures similar to those of summertime were found to close the window of germinability. Oospores were collected in spring and stored in complete darkness at 4 and 18° C. Samples, removed at intervals from 4° C and incubated in the light, germinated throughout the summer and autumn at a minimum of 72.0% (Fig. 1B). In contrast oospores stored at 18° C in the dark became completely refractory to activation in July.

In the laboratory, encroaching secondary dormancy was reversed. One set of oospores was transferred from 18 to 13° C after becoming refractory. After approximately two months at 13° C, germination at 18° C in the light was 78.2%. Further storage, but at 4° C, resulted in minimum light germination of 89.3% over the next three months. No precise relationship was established, however, between length of warm storage and duration of the refractory phase.

Light exposure was necessary for germination. Dark germination was 5.0% or less while light exposed oospores germinated in excess of

85.0%. The sensitivity of oospores to light is illustrated by the safelight response. A 10-min exposure resulted in germinations of 40.0 to 50.0%.

Light pulses of a few minutes were as effective as the full photophase. A pulse of 0.3 Joule (J)$\cdot m^{-2}$ red light was saturating with a germination of 87.0%. A pulse of 0.065 $J \cdot m^{-2}$ red (669nm) light gave a 50.0% response, as estimated by regressing germination on the log of energy supplied (Schäfer and Fukshansky 1984). The oospores were less sensitive to the green (538nm), requiring 5.5 $J \cdot m^{-2}$ for 50.0% germination, and least sensitive to the blue (449nm), requiring 182.0 $J \cdot m^{-2}$ for a 50.0% germination.

To appreciate the photosensitivity, we extrapolated the results to the meadow in Lake George. With light transmission restricted to a 20 nm band, and a calculated attenuation coefficient, k_{660} = $0.48 \cdot m^{-1}$, a one second exposure would give a 50.0% germination of oospores at a depth of 12.2 m, or 2.2 m deeper than the deepest distribution of shoots of N. furcata in the meadow. A 50.0% germination might be expected at 29.2 m. if the oospores were allowed to accumulate the light signal for one hour, This depth is nearly twice the depth of the lower boundary of the meadow, which consists of N. flexilis(L.) Ag. (Stross 1979).

Oospores of other charophytes in the meadow also germinated profusely in the spring. They include N. acuminata A. Br. ex Wallm. , N. tenuissima (N. transilis T.F.A.), and Chara braunii Gm. Oospores of N. flexilis failed to germinate in limited testing. The germination window for N. acuminata remained open longest in the summer.

Conclusions. Oospores of N. furcata accumulate in the sediments beneath the nitella meadow in Lake George. Many obviously escape germination after passing through a primary dormancy and remain viable to depths of 6.0 cm and beyond in sediments of Lake George. Within the reservoir or bank, oospores undergo an annual cycle of secondary dormancy. Germination seems highly probable if the oospores are exposed to an appropriate light stimulus in spring when the annual window is open.

The light response is consistent with a low energy, phytochrome reaction. The oospores were 80 times more sensitive to red than to green light and 2800 times more sensitive to red than to blue. A 50.0% germination with 0.065 $J \cdot m^{-2}$ in the red indicates great sensitivity relative to typical phytochrome reactions measured in the laboratory (Smith 1975), including activation energies reported for seeds of Stellaria (Baskin and Baskin 1979). Light sensitivity approached the extremes reported for germination of seeds of Arabidopsis (Cone et al. 1985).

The germination characteristics of oospores in the meadow share some similarities to the seeds of summer annual weeds. Following a demonstration of light sensitivity (Sauer and Struik 1964), an annual cycle of true and conditional dormancy was described for the ragweed, Ambrosia (Bazzaz 1970). Ambrosia also remains sensitive to light if stored in the dark at low temperatures (Willemsen 1975, Baskin and Baskin 1980). Like the seeds, oospores of N. furcata are dormant when removed from the parent plant.

The light environment is pivotal in germination of N. furcata oospores. The question is whether a light reaction may determine depth zonation as noted earlier (Stross 1979). Implications based on

a light demand at one stage in the life cycle, would be worthy of further study.

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Fig. 1A, B. Mean germination (with 95% confidence limits) of Nitella furcata oospores exposed to light under standardized conditions (see Methods). A) Results from germination trials with field collected oospores. Incubations started on the day indicated (x-axis) which was within one week of collection. B) Influence of storage temperature on germination of oospores collected in May (4^{o} C) or June 1984 (18^{o} C). Points indicate time subsample was withdrawn and incubated.

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