J. Aquat. Plant Manage. 25: 8-10

Seed Germination in *Myriophyllum Spicatum* L.

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ABSTRACT

Newly formed seeds of *Myriophyllum spicatum* L. collected from Pat Mayse Lake, Paris, Texas, were germinated on moistened filter paper under white, red (700 and 725 nm), green (520 nm), and blue (445 nm) light (at an intensity of 4.5 $\mu E \cdot m^{-2} \cdot sec^{-1}$) as well as in darkness. As many as 97% of the seeds germinated under red light (725 nm). Blue light significantly inhibited germination and almost no seeds germinated in darkness. A significant decrease in germination occurred under 50 cm of lake water at a light intensity of 4.5 $\mu E \cdot m^{-2} \cdot sec^{-1}$ but increased under 9.0 $\mu E \cdot m^{-2} \cdot sec^{-1}$. Seed germination can be inhibited by an extreme increase or decrease in light intensity. The significance of these findings for the *in situ* importance of sexual reproduction in milfoil is discussed.

Key words: Light intensity, light quality, milfoil, phytochrome, sexual reproduction.

INTRODUCTION

Several studies (Ministry of Environment, 1981; Grace and Wetzel, 1978; Patten, 1955) have indicated that seeds of Eurasian watermilfoil (*Myriophyllum spicatum* L.), hereafter called milfoil, are fertile. However, some authors (Patten, 1956; Smith *et. al.*, 1967; Amundsen, 1978) contend that the sexual reproduction is not important in the spread of the weed into new areas since there is an apparent absence of seedling survival *in situ*.

The ability of seeds of some aquatic macrophytes to germinate can be greatly affected by the restriction of light (Gopal and Sharma, 1983; Else and Tiemer, 1984). Very little data have been reported with regard to milfoil seed germination under different environmental stress conditions.

This study investigated light quality and quantity effects on milfoil seed germination.

MATERIALS AND METHODS

Seeds were gathered from a milfoil stand in Pat Mayse Lake located 24 km north of Paris, Texas in Lamar County. The milfoil was observed flowering in mid-September 1985 and seeds were collected on October 30. The seeds were left attached to the plant by breaking of 8-10 cm lengths of the stems with seed heads and taken to the lab to be placed in glass jars filled with tap water. The jars were placed near a window which allowed them to receive sunlight and the room was continuously lighted with fluorescent bulbs.

An initial germination test was made which suggested both a high viability and an influence of light quality on germination of newly formed seeds. Experiments were then done to determine specific effects of light on the germination of the seeds.

Seeds were placed in petri dishes (12 seeds per dish on filter papers moistened with distilled water and placed in a growth chamber. A photoperiod of 14:10 hrs (light:dark) at a constant temperature of 24 C was maintained. The seeds were exposed to 6 different qualities of light for a period of 72 hrs. The light was supplied by 40 watt Sylvania fluorescent bulbs and the different qualities were achieved by placing cellophane filters over the petri dishes. Emission spectra of the filters were determined by placing strips of each in a Coleman-Hitachi No. 124 spectrophotometer and observing the wavelengths of transmittance. Maximum transmittance of the filters were 445 nm, 520 nm, 700 nm and 725 nm. One set of seeds was kept enclosed in a cardboard box and another allowed to receive white light. The same energy level (4.35 $\mu E \cdot m^{-2} \cdot sec^{-1}$ 0.18 μE) was achieved for each dish under the light by covering them with varying thicknesses of cheese cloth. The seeds were left in natural clusters of four. There were 3 replicates for each exposure.

Another experiment was designed to test for seed germination under a column of lake water. Transparent plexiglass cylinders (13 cm in diameter) were affixed to flat bases and stood upright. Seeds (12 per exposure) were placed in petri dishes, wrapped in one layer of cheese cloth, and placed in the bottom of each cylinder. Water taken from the site of the milfoil stand in Pat Mayse was added to each cylinder to a depth of 50 cm. All cylinders were placed in a room with the same photoperiod and temperature as in the earlier experiment. Three replications of exposure to white light at 4.5 $\mu E \cdot m^{-2} \cdot sec^{-1}$ at the bottom of the water column were conducted and one set of seeds was exposed to 9.0 $\mu E \cdot m^{-2} \cdot sec^{-1}$.

A third experiment was done to observe seed germination under a series of different light intensities. Seeds were prepared as in the first experiment and exposed to four discreet intensities of white light (14.0, 7.0, 3.5, and 1.25 $\mu E \cdot m^{-2} \cdot sec^{-1}$.

RESULTS AND DISCUSSION

The milfoil seeds proved to be highly viable. Under favorable conditions, a 97.2% germination rate was observed. However, seed germination can be greatly affected by light quality. The mean percent germination ranged from 77.8% to 97.2% for those seeds exposed to wavelengths above 500 nm. Seeds exposed to light of 445 nm and those left in the dark had noticeably lower germination rates of 30.5% and 5.6% respectively as indicated in Figure 1.

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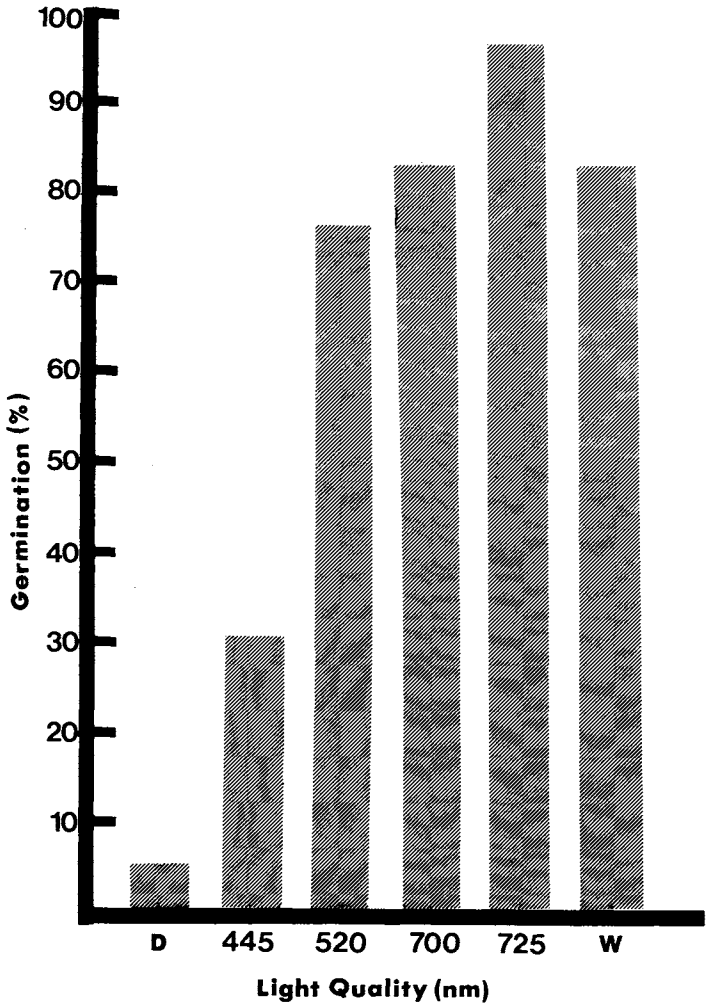

Figure 1. Mean percentages of seed germination of milfoil evaluated under six different light qualities at 24 C.

Applying the Kruskal-Wallis (Chi Square Approximation) Test to the data, it was concluded that a statistically significant difference existed between mean percentages of germination among the 6 treatment groups ($X^2=16.68$; $P=0.005$ at an alpha level of 0.05). The 2-tailed Dunnet's Multiple Range Test was used to locate the source of variation, designating the white light treatment as the control. The test distinguished 3 statistically significant groups as was expected and is presented in Table 1. These nonparametric tests were used because the data did not prove to be normally distributed.

These results indicate a strong preference for the longer wavelengths, especially within the red range. The data also indicate an inhibition of germination by blue light and the near failure of any seed to germinate in darkness.

Experiments in a simulated *in situ* light environment confirm the results of the filter experiments. Table 2 reveals a near total failure of seeds to germinate under 50 cm of lake water with light intensity comparable to that maintained in the filter experiments. Only after the light intensity was doubled from 4.5 to 9.0 $\mu E \cdot m^{-2} \cdot sec^{-1}$ was any appreciable germination obtained. These results suggested a substantial loss of red light occurring within the first half meter of water.

TABLE 1. RESULTS OF THE NONPARAMETRIC STATISTICAL ANALYSES CONDUCTED ON SEED GERMINATION DATA OF *MYRIOPHYLLUM SPICATUM* GENERATED BY EXPOSURE OF SEEDS TO SIX DIFFERENT LIGHT QUALITIES.

Kruskal-Wallace	Dunnet's 2-Tailed Multiple Range
$X^2 = 16.68$	[520 nm, 700 nm, 725 nm, White]
$p = 0.005$	[445 nm]
$\alpha = 0.05$	[dark]

TABLE 2. PERCENT GERMINATION OF *MYRIOPHYLLUM SPICATUM* SEED UNDER 50 cm OF WATER TAKEN FROM PAT MAYSE lake. SEE TEXT FOR DETAILS.

	Trial			
	1	2	3	4
Light Intensity ($\mu E \cdot m^{-2} \cdot sec^{-1}$)	4.5	4.5	4.5	9.0
Percentage of Seed Germination	8.3	0.0	0.0	42.0

Light quality measurements using an International Light 300 Research Radiometer were taken in Pat Mayse Lake at the site of milfoil infestation. The readings indicated a loss of 50 to 60% of light in the 695-705 nm range between 0.1 and 0.4 m of water near the shore. The extinction coefficient for this light was calculated to be 1.74. Longer wavelengths suffered an even greater rate of absorption. Light in the 445-455 nm range had an extinction coefficient of 2.55 m.

These measurements explain the relation between the results of the water column and filter experiments. The longer wavelengths of light shown to induce germination in the filter experiments are substantially reduced after passing through the first 0.5 m of water. The inhibitive blue light persists further through the water column. At 0.5 m, a greater percentage of blue light than red reaches the seeds, thereby inhibiting germination. However, it was also demonstrated in the water column experiments that an increase in light intensity at the same depth produced an increase in percent germination, presumable due to a higher incidence of red light.

That light intensity is also a factor in seed germination rate is shown in Figure 2. An extreme reduction or increase in intensity beyond the range under which the other experiments were conducted was shown to decrease the amount of germination. The percentage of seed germination under a light intensity of 3.5 and 7.0 $\mu E \cdot m^{-2} \cdot sec^{-1}$ was exactly equal to the results obtained under white light in the filter experiment, 83.3% (Fig. 1). As expected from the earlier poor response of seeds left in the dark, the seeds receiving the least light, 1.25 $\mu E \cdot^{-2} \cdot sec^{-1}$, showed a decline in germination rate from 83.3% to 42%.

The results of these experiments combine to show that specific light qualities and quantities both influence the germination rate of milfoil seed. The germination rate in the water column following the increase in light intensity (which represents a greater amount of the total spectrum reaching the given depth), plus the negative effects of an overall lowering of available light as seen in Figure 2, dem-

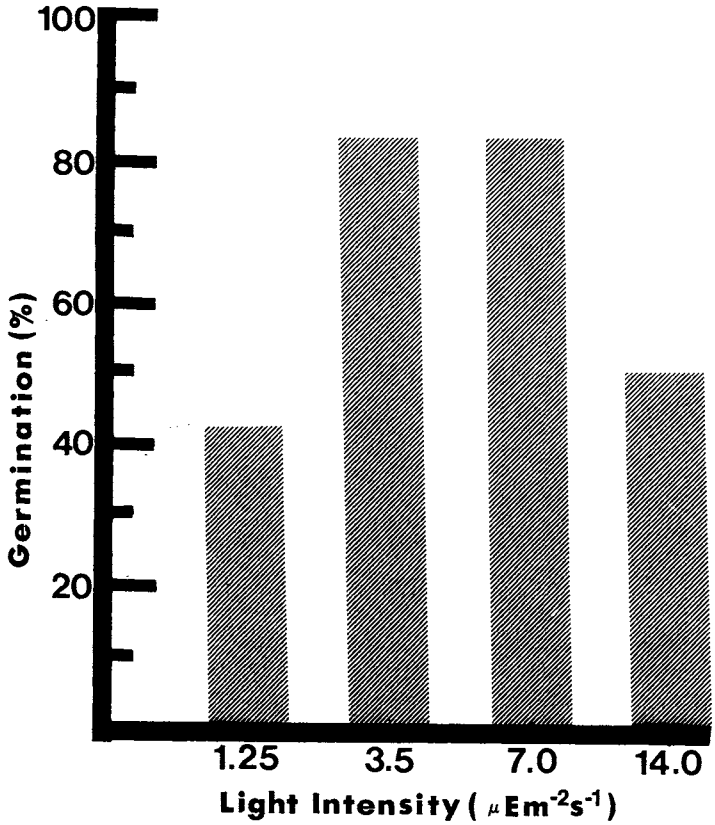

Figure 2. Mean percentages of seed germination of milfoil exposed to four different intensities of full white light at a temperature of 24 C.

onstrate the need for the presence of a given minimum quantity of red light to achieve good germination. The data from the water column experiments may also indicate the need for a specific proportion of red light vs. blue in order for the effect of the blue light to be overcome since the water column is changing the quantity of the different spectra at different rates. More work is needed to elucidate this point more clearly.

The extremely high germination rate in the red light range and the inhibition of germination in blue light both indicate the presence of an active phytochrome system (Hartmann, 1966; Malcoste *et al*, 1972; Rollin, 1970; Rollin and Maignan, 1966; Mitrakos and Shropshire, 1972 within milfoil seeds. Experiments are being done to explore this possibility.

Although the sexual reproductive capacity of milfoil is high in potential, nevertheless, the light environment which the seeds experience is limiting. Due to the light filtering which takes place in the lake water, the quick dispersal of red light and the persistence of blue light, the area of vertical distribution available for substantial rates of germination of milfoil seeds is restricted. The seeds would need to be in less than 0.5 m of water under a light intensity of more than 9 $\mu E \cdot m^{-2} \cdot sec^{-1}$ for a period of 2-3 days to germinate at a rate of about 50%. Even if germination occurred, seedling survival would be problematical and this aspect of milfoil reproduction should be investigated.

It appears that the milfoil stand which served as the resource for these experiments would benefit little, if any, from seed production. The bottom surface of the cove in which it is located quickly slopes to a depth exceeding 0.5 m. Almost the entire stand is rooted in water 1.5 to 2.5 m deep where most of the seeds will sink well below light needed for germination.

Therefore, efforts to control this weed in Pat Mayse and at other similar locations need not concentrate on inhibition of its sexual reproductive cycle. If anything, the energy which the stand expends on flowering and seed formation may simply be counted as wasted energy with respect to stand growth.

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