Germination of *Ceratophyllum demersum* Seeds in Aseptic Liquid Culture

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Germination experiments were conducted in liquid culture using seeds of the submersed aquatic vascular plant *Ceratophyllum demersum* L. (Ceratophyllaceae; Hornwort). Several techniques for enhancement of germination and culture of seedlings (scarification, dark/cold pretreatment, surface disinfection, and addition of growth-enhancing chemical agents) were evaluated. Maximal germination rates did not exceed 10 percent, suggesting that culture of *Ceratophyllum* from seed may not be a viable means for axenic propagation of plants under laboratory conditions.

INTRODUCTION

Several aquatic vascular plant taxa have been grown axenically under laboratory conditions via vegetative propagation (1,2), but few species have been cultured successfully from seed. In the Naiadaceae, *Najas flexilis* (Willd.) Rostk & Schmidt has been grown in axenic liquid media culture with seed germination frequencies of up to 20 percent (3). Using similar protocol, Francko (4,5) has reported germination frequencies in excess of 98 percent in *Nelumbo lutea* (Willd.) Pers. (Nymphaeceae; American lotus). Riemer (6) reported germination percentages of from 0 to 18 percent for closely related *Nuphar advena* Ait., although these plants were not grown axenically. Similarly, *Nymphaea odorata* has been grown non-axenically from seed under laboratory conditions (7) with germination frequencies from 10 to 90 percent. hello

Physiological experimentation on aquatic plant taxa, including many nuisance species, has been hampered by the lack of sterile plant material. Contaminant organisms in seedling cultures can compromise the interpretation of experimental results (e.g., susceptibility to herbicides). *Ceratophyllum demersum* L. Ceratophyllaceae; Hornwort or Coontail) is a common submersed nuisance species of considerable economic importance in the United States, since its proliferation often clogs waterways. The only report of successful germination of *Ceratophyllum* seeds (8) was based on a study conducted in mud-water culture. That study suggested that *Ceratophyllum* seeds could be germinated with success (62 percent germination rates) after a year of dormancy.

Here, we report preliminary results of axenic liquid culture germination experiments, on *Ceratophyllum*, which were conducted using adaptations of modern, published methods.

MATERIALS AND METHODS

Seeds were collected during late summer of 1984 from a dense population of *Ceratophyllum* found in the littoral zone of Sangre Isle Lake, a small eutrophic impoundment located in Payne County (R2E, T18N, S17), Oklahoma. Limnological characteristics of this lake have been published elsewhere (9,10). As is the case with many aquatic plants which reproduce vegetatively (11), seed set in *Ceratophyllum* is infrequent, so that only about 300 seeds could be collected from over 100 kg (fresh wt) of plant material. Subsequent analyses were limited by this small sample size. An additional sample of seeds (30) was collected in October of 1985.

Seeds were separated into two groups on the basis of morphology. Slightly larger, harder seeds with three spine-like projections were designated as "mature" seeds, while smaller, softer seeds with a single projection were designated as "immature". Subsamples of each group of seeds were stored dry in contact with air in the dark at 4 °C or under fluorescent room lights at 22 °C.

Liquid culture techniques and seed sterilization methods employed have been

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shown to maximize seed germination in other aquatic vascular plant taxa (3,4,5). One subsample of mature and immature seeds were scarified lightly with a metal file and were inoculated directly into sterile artificial media. Forsberg's inorganic Medium II (12), adjusted to pH 8.2, was used in all cultures. In other experiments, seeds were rinsed for varying lengths of time in 70 percent ethanol, then in 4 to 6 percent sodium hypochlorite, and finally sterile distilled, deionized water (2 to 5 washes). All transfers were made in a laminar-flow hood. Following the final water rinse, seeds (10 per flask) were transferred aseptically to 250 ml of Medium II. Flasks were placed in a dark incubator (35 °C) for 24 h to two weeks, a procedure found necessary for the germination of *Najas flexilis* seeds (3). Cultures were then transferred to an illuminated growth chamber (60 μE $m^{-2}s^{-1}$; 23 °C; 12:12 LD cycle). Cultures were maintained in growth chambers for several months to ensure sufficient time for full germination.

In some experiments, the germination enhancer ethephon (2-chloroethylphosphonic acid; 100 mg $liter^{-1}$) was added to cultures after seed inoculation or after cultures had been in the growth chamber for several weeks (7). All experiments were conducted after seed collection in 1984, then were repeated one year later (late 1985) on subsamples of seeds collected in 1984.

The presence of contaminant organisms in seedling cultures was assayed by plating 0.5-ml aliquots of culture media onto Medium II and nutrient agar plates. Plates were then incubated in the dark at 35 °C for two weeks prior to analysis. Aliquots of media were also examined by phase contrast microscopy.

Forsberg (12) has suggested that seeds of aquatic vascular plants germinate best under the reduced oxygen tension associated with sediment-water slurries at the bottom of a lake. As already noted, Guppy's experiments on *Ceratophyllum* (8) were also conducted in mud-water culture using seeds that had been stored for one year prior to inoculation. Accordingly, four subsamples of mature seeds (15 seeds each) collected in 1984 were placed in lake sediment-water slurry cultures (1 cm below sediment surface) and allowed to germinate without further treatment. Another series of four subsamples of seeds were placed in filtered (0.45-μm Millipore filtration) lake water rather than artificial media. These experiments were conducted early in 1986, so that seeds had been stored dry for about 18 months prior to experimentation. Seeds collected in 1985 were incubated in the dark at 4 °C for four months and were then placed into mud: lake water slurry or filtered lake water culture (1 of each, 15 seeds per flask).

RESULTS AND DISCUSSION

Table 1 summarizes results of various treatments on *Ceratophyllum* seed germina-

TABLE 1. Germination frequencies of mature seeds of *Ceratophyllum demersum*.

EXPERIMENT	N[a]	TREATMENTS				% GERMINATION
		Cold/Dark Pretreatment at 4 °C.	Scarification	Sterilization Period (min), EtOH: Hypochlorite	Dark Incubation Period (35 °C)	
Medium II - 1	3	none	–	15:30	48 h	7
Medium II - 2	2	none	+	15:15	24 h	0
Medium II - 3	1	1 month	+	–	24 h	10
Medium II - 4	2	70 days	–	15:15	2 weeks	5
Medium II - 5	3	5 months	+	15:10	72 h	0
mud:water	1	4 months	–	–	–	7
lake water	1	4 months	–	–	–	7
mud:water	4	18 months	–	–	–	0
lake water	4	18 months	–	–	–	0

[a]Denotes number of cultures, 10 seeds each, except for mud:water/lake water experiments, where 15 seeds per culture were used.

tion. Only the seeds designated as "mature" exhibited any germination. The highest germination rates (ca. 10 percent) were noted in a culture that was not subjected to any ethanol/hypochlorite treatment. While this result suggests that sterilization procedures may harm the embryo, ca. 7 percent germination was noted in a series of three cultures which had been treated with ethanol and hypochlorite for 15 and 30 min, respectively. Ethephon was added to each of the Medium II cultures shown in Table 1 after cultures had been in growth chambers for two months. No additional seed germination occurred in ethephon-amended cultures. Although Guppy (8) reported that maximal germination in *Ceratophyllum* seeds occurred only during the second year after seed set, we could not confirm this observation. All artificial medium treatment variations shown in Table 1 were repeated on seeds stored for 1 year, and none of the inoculated seeds germinated. Germination was not enhanced by incubation of seeds in mud-water media or in lake water; seeds added to these media after dry storage for 18 months did not germinate, while seeds collected in 1985 that were placed in mud-water or lake water after 4 months of dry storage in the dark at 4 °C each exhibited 7 percent germination frequencies.

We found that ca. one-third of cultures containing seeds treated with ethanol/hypochlorite for at least 15 min each remained free of contaminant organisms that would grow on either nutrient agar or Medium II agar or were visible under phase contrast microscopy. This percentage is consistent with previous sterility assays on aquatic plant seed cultures in the literature (3,4,5).

Collectively, our analyses suggest that seed germination in *Ceratophyllum* is quite low, approaching the lowest percentages reported by workers using other aquatic plant taxa. We report no success in enhancing germination through modifications of culture conditions. Thus, while we can report limited success in producing a few individual plants in culture with no visible or plateable contaminants, further advances are required before *Ceratophyllum* seed culture can be expected to provide sufficient material for experimental studies.

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