

Factors Affecting Germination of Seeds of Fragrant Waterlily (*Nymphaea odorata*)¹

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ABSTRACT

Fragrant waterlily (*Nymphaea odorata* Ait.) is an aquatic weed of wide distribution. Seeds from weed populations in southern New Jersey were studied to determine germination requirements. Seeds were dormant at time of release and no after-ripening requirement was observed. Mechanical puncturing of the seed coat had no effect upon germination. Seeds germinated when large numbers were crowded into a small container, and it was thought that the seeds themselves produced something that promoted their germination. Promotion of germination by 2-chloroethylphosphonic acid (ethephon) and inhibition of germination by aeration and CO₂ suggested that this substance was ethylene gas in solution. Germination under conditions of seed crowding was inhibited by darkness and promoted by stratification. Stratification at 4.4 C for 5 months resulted in germination of crowded seeds in excess of 90%. Germination was strongly inhibited by periods of freezing or drying as short as 1 day.

Key words: seed dormancy, ethylene, ethephon, stratification.

INTRODUCTION

Fragrant waterlily (*Nymphaea odorata* Ait.) is a floating-leaved, shallow-rooted, rhizomatous, perennial aquatic plant which inhabits lakes, ponds, and slow-moving streams throughout the eastern half of the United States. Dense infestations interfere with boating and fishing and may accelerate the natural siltation process in shallow bodies of water. In addition, fragrant waterlily can clog irrigation ditches, retarding water flow and accelerating water loss through transpiration. This is an especially serious problem for the cranberry (*Vaccinium macrocarpon*) growers of New Jersey's Pinelands, whose success depends on their ability to move large amounts of water through ditches on short notice. At the present time there are no herbicides registered for use against fragrant waterlily when it infests irrigation ditches in cranberry bogs, and control is achieved through manual or mechanical digging.

Dense stands of fragrant waterlily seedlings have been observed in these ditches following experimental control of adult plants with herbicides (9). The objective of this study was to determine the factors affecting seed germination in this species in order to help formulate better control measures.

MATERIALS AND METHODS

All seeds used in this study were collected from plants growing in an irrigation ditch in the Pine Barrens of Burlington County, N.J. Mature fruits were collected by hand from the ditch bottom in August and September. After collection, the fruits were placed in water and transported to a laboratory where they were allowed to dehisce. After the seeds had become free of their surrounding arils they were stored in fresh water on a laboratory bench at room temperature with alternating 12 hour periods of light and darkness. All experiments began within 3 weeks of the date of seed collection.

All germination experiments were conducted in 35 ml glass vials measuring approximately 2.5 cm in diameter and 10.5 cm in height. Seeds were counted and placed in these vials with either 20 or 25 ml tap water, depending on the experiment. Illumination was by Sylvania Gro-Lux® and Norelco® Cool White fluorescent light bulbs suspended above the vials, with alternating 12 hour periods of light and darkness. Opaque plastic caps were screwed loosely on the vials, and the vials were placed on a bench top at room temperature until germination appeared to be complete. All seeds with a protruding epicotyl were considered to have germinated.

Experiments were designed and analyzed as completely randomized designs in all cases except the experiments on gas effects, ethephon and stratification, which were designed and analyzed as factorial experiments.

In initial experiments, seeds collected in 1978 failed to germinate when small numbers were placed in petri dishes. Extra seeds which were left in a crowded condition in a glass dish on a laboratory bench, did germinate, however, at a rate of approximately 50%. It thus appeared that the seeds themselves were producing some substance which, when present in sufficient quantity, was capable of breaking dormancy and inducing germination.

Experiment 1. An experiment was designed to determine the degree of crowding necessary to enhance germination. Seeds were counted in increments of 20, ranging from 20 to 240, and placed in vials with 20 ml of tap water. There were four replications of each seed density treatment. Caps were screwed on loosely, and the percent germination was determined after three weeks.

Experiment 2. An experiment was performed to determine if some substance present in the water surrounding crowded, germinating seeds was capable of producing germination in uncrowded, dormant seeds. Crowded treatments consisted of 250 seeds in 20 ml water and uncrowded treatments contained 30 seeds in 20 ml water. There were five replications per treatment. After seeds in

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the crowded treatments began to germinate, the water in the uncrowded vials was replaced with water from the crowded vials. Care was taken to minimize agitation of the water during transfer. Five uncrowded vials served as controls.

Experiment 3. The effects of various dissolved gases upon germination were determined by continuously bubbling air, CO₂, or a mixture of 30% CO₂ and 70% air through vials containing 25 ml of water and either crowded seeds (250/vial) or uncrowded seeds (30/vial). The rate of gas delivery varied between 5 ml/min and 55 ml/min.

Experiment 4. The effect of ethylene on fragrant waterlily germination was determined by adding ethephon at six concentrations, increasing by factors of 10, from 0.01 to 100 ppm, to vials of crowded and uncrowded seeds. The use of ethephon to determine the response of seeds to ethylene is well established (4). Vials were tightly capped to prevent the escape of ethylene gas.

Experiment 5. To determine the effect of light on germination, 250 seeds were placed in vials containing 20 ml of water. Half of the vials were kept on a laboratory bench lighted as described in the general Materials and Methods section, above. The other half were kept in a darkened cabinet.

Experiment 6. A preliminary experiment had indicated that stratification periods as long as 9 weeks failed to produce germination of uncrowded seeds. In this experiment, both crowded seeds (250/vial), and uncrowded seeds (30/vial), were tested to determine their response to 5, 7, and 9 months of cold treatment. Seeds were stored in the dark at 4.4 C for the indicated time, after which the water was replaced with fresh water and the germination trials were begun.

Experiment 7. In this experiment, fragrant waterlily seeds were air-dried for 0, 1, 3, 10, 24, 58, and 96 hours. At the end of each prescribed drying period, 250 seeds and 20 ml tap water were added to each vial. Percent germination was determined 16 days after adding water to the vials.

Experiment 8. The effect of freezing fragrant waterlily seeds (in water), for periods of 0, 1, 4, 7, 10, 13, and 16 days was determined in this experiment. Only crowded seeds (250/vial) were used.

Experiment 9. The seed coats of a number of fragrant waterlily seeds were punctured with a needle at the micropylar end to determine if the dormancy was seed-coat imposed. The pierced seeds were placed in 5 vials in an uncrowded condition (20 seeds/vial), with an additional 5 vials of unpierced seeds serving as controls.

RESULTS AND DISCUSSION

In three seasons of experimental work not a single fragrant waterlily seed germinated when the number of seeds per vial was 20 or less. Under these conditions dormancy was complete. Germination did not increase with time, indicating that there is no after-ripening requirement. Approximately 60% of the seeds rotted after a year's storage in water at room temperature.

Experiment 1. Germination increased significantly with increasing concentrations of seeds, starting at 60 seeds/vial

and reaching a maximum at 100 seeds/vial (Figure 1). Densities greater than 100 seeds/vial (5 seeds/ml) had no further effect on germination.

Experiment 2. Transfer of water from a container of actively germinating seeds to a container of uncrowded seeds which were not germinating initiated germination in the uncrowded seeds. Germination in the uncrowded and crowded vials was 2.7% and 47% respectively, at the time of transfer. After the transfer, germination of the treated, uncrowded seeds averaged 42.0%, whereas germination in the uncrowded controls never exceeded 3.4%. There was a highly significant difference between the uncrowded controls and the uncrowded seeds treated with water from crowded vials ($P < 0.0005$).

Experiment 3. All gas treatments of crowded seeds resulted in significant decreases in germination compared to untreated controls (Figure 2). In uncrowded seeds there were significant differences among all gas treatments; while in the crowded seeds there was no significant difference between the treatments with CO₂ plus air and air alone. Crowded seeds, however, did exhibit a significantly lower

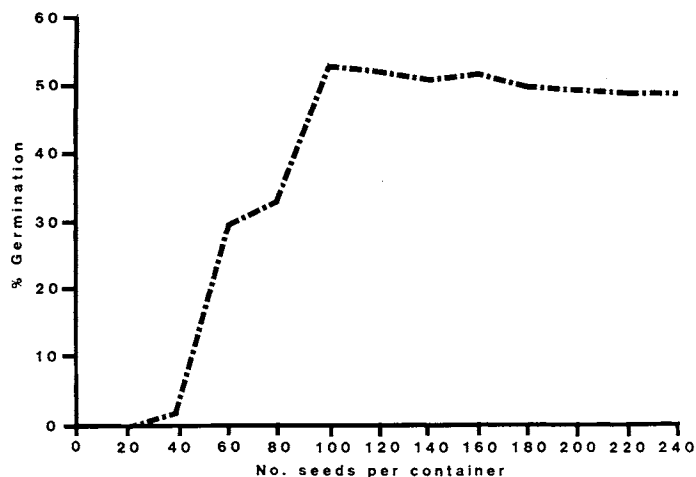


Figure 1. Germination percentage of fragrant waterlily seeds with increasing number of seeds per container.

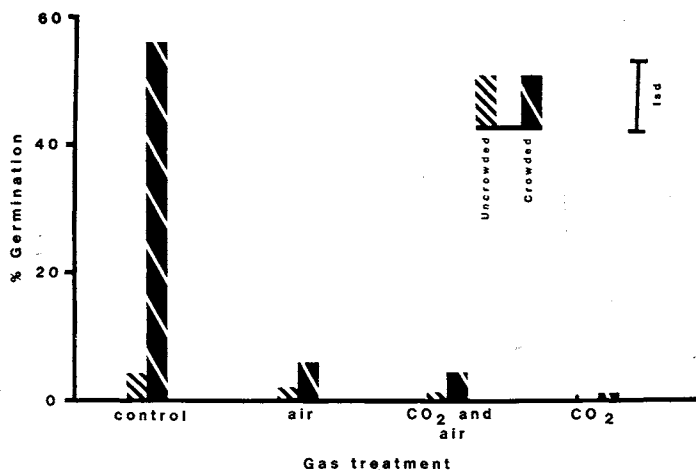


Figure 2. The effects of bubbled air, CO₂, and a 30% CO₂ in air mixture on the germination of crowded and uncrowded seeds of fragrant waterlily.

germination percentage when treated with CO₂ than when treated with air.

Since the bubbling of gases through the water decreased germination in all cases, we suspected that the endogenously-produced substance which stimulated germination was a gas and that this gas was being replaced by the introduced gases.

Experiment 4. Treatment with ethephon stimulated germination in all cases when the ethephon concentration was 1.0 ppm or more in the uncrowded seeds and 0.01 ppm or more in the crowded seeds (Figure 3). A concentration of 100 ppm produced the greatest germination in the uncrowded seeds. In the crowded seeds, all ethephon concentrations resulted in increased germination over the controls. Few germination percentages exceeded 20% in this experiment, which is lower than those which occurred in the other experiments. It is possible that the tight vial closure, necessary in this experiment to keep the ethylene gas from escaping, resulted in the buildup of toxic respiratory products or products antagonistic to the function of ethylene. Carbon dioxide has been reported to have an antagonistic role to ethylene in many physiological functions, possibly including germination (1). The results of this experiment support the hypothesis that the germination-inducing substance is ethylene.

Endogenously-produced ethylene has been found to stimulate germination in peanut (*Arachis hypogaea*) and has been suggested as having a role in germination in subterranean clover (*Trifolium subterraneum*), cocklebur (*Xanthium pennsylvanicum*), redroot pigweed (*Amaranthus retroflexus*), witchweed (*Striga asiatica*), medic (*Medicago trunculata*), and a number of other species (1, 2, 3, 4, 6, 8). It thus seems possible that ethylene could have a role in the germination of fragrant waterlily seeds.

Experiment 5. Seeds kept in the dark exhibited only 2% germination, even when crowded, while seeds kept in the light exhibited nearly 30% germination. Such a light requirement might explain a phenomenon observed repeatedly in the field. Where adult waterlily populations are high, seedlings are rarely observed and those which do appear rarely survive the early summer. Where chemical control of adult plants is undertaken, however, large numbers of seeds germinate the following spring and many persist to

become adult plants. It is possible that this profuse germination is due to the removal, with the adult plants, of a dark-induced inhibition of germination.

Experiment 6. Seeds of a number of plant species are released from dormancy after storage in the imbibed condition at temperatures slightly above freezing. Early experiments indicated that fragrant waterlily seeds do not respond to stratification periods of 9 weeks or less. In this experiment, a chilling period of 5 months produced extremely high germination in the crowded containers but germination was not enhanced in the uncrowded containers. Stratification for 7 and 9 months enhanced germination in the uncrowded containers and resulted in germination percentages of nearly 100% in the crowded containers (Figure 4). An interaction between stratification and crowding is evident and it is possible that the effects of chilling and the germination-promoting substance found in the crowded vials were synergistic. Cold treatment has been found to increase ethylene production (7).

Experiment 7. Short periods of drying had a significant inhibitive effect on germination (Figure 5). A drying time of only 3 hours resulted in significant inhibition of germination and the percentage declined rapidly up to drying times of 24 hours. As Hutchinson (5) observed, the failure

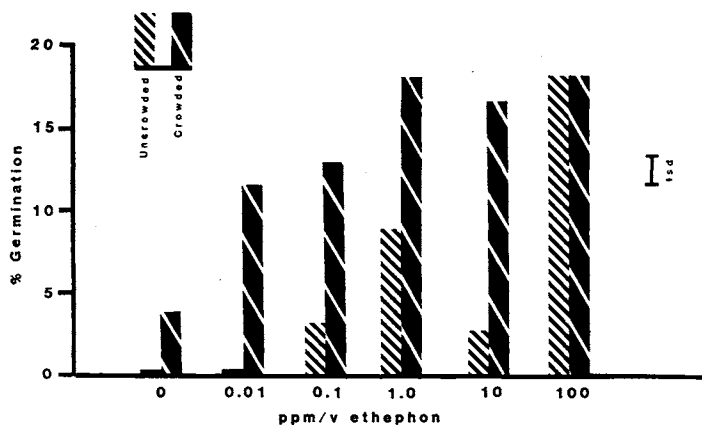


Figure 3. The effects of varying concentrations of ethephon on the germination of crowded and uncrowded seeds of fragrant waterlily.

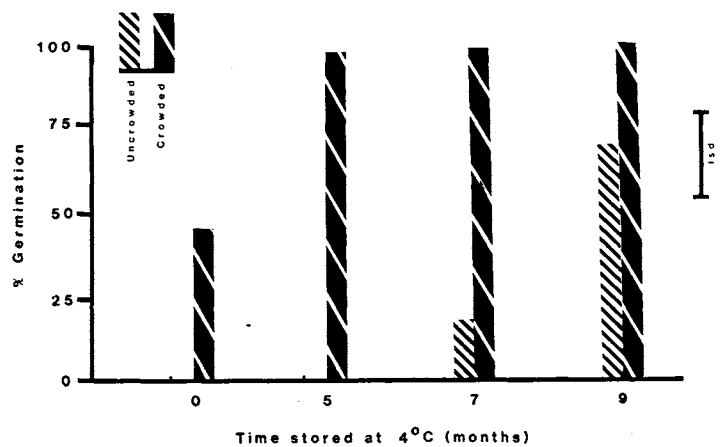


Figure 4. Germination percentage of fragrant waterlily seeds after storage in water at 4°C for 0, 5, 7, and 9 months.

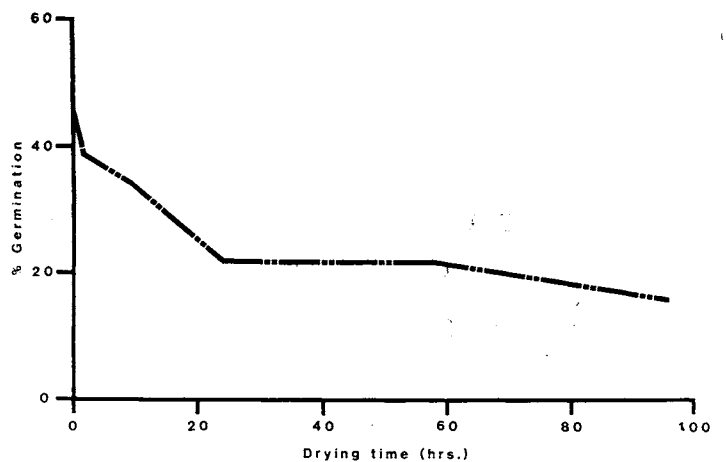


Figure 5. The effects of drying times on the germination of fragrant waterlily seeds.

of seeds to germinate after only short periods of drying would prevent the establishment of plants in all but the most consistently aquatic environments. Field observations of fragrant waterlily habitats support his conclusions.

Experiment 8. Freezing severely inhibited germination. In an initial experiment, no germination occurred after 2 or 4 weeks of freezing. In a second experiment using shorter freezing periods, a significant inhibition of germination resulted from freezing the seeds for only 1 day. Four days of freezing resulted in germination of only 4.9%, and one week of freezing prevented germination entirely.

Experiment 9. Mechanical rupture of seed coats failed to induce germination in fragrant waterlily. Thus, it does not seem likely that seed coat impermeability and/or mechanical restriction are causes of dormancy in this species.

This work presents some interesting opportunities for further research. Chemical analyses should be performed to determine the ethylene concentrations in waters containing crowded and uncrowded seeds. Possible synergistic relationships between factors affecting germination should be investigated. The seed environment of aquatic plants differs markedly from that of terrestrial plants in a number of

respects, and the differences in gas relations, in particular, have not been adequately explored.

LITERATURE CITED

1. Egley, G. H. and J. E. Dale. 1970. Ethylene 1-chloroethylphosphoric acid and witchweed germination. *Weed Sci.* 18:586-589.
2. Egley, G. H. 1980. Stimulation of common cocklebur (*Xanthium pennsylvanicum*) and redroot pigweed (*Amaranthus retroflexus*) seed germination by injections of ethylene into the soil. *Weed Sci.* 28:510-514.
3. Esashi, Y. and A. C. Leopold. 1964. Dormancy regulation in subterranean clover seed by ethylene. *Plant Physiol.* 43:1470-1472.
4. Globerson, D. 1978. Germination and dormancy breaking by ethphon in mature and immature seeds of *Medicago trunculata* and *Trifolium subterraneum*. *Austr. J. of Agric. Res.* 29:43-49.
5. Hutchinson, G. E. 1975. *A Treatise on Limnology*. Vol. III, Limnological Botany. John Wiley and Sons, N.Y. 660 pp.
6. Ketring, D. L. and P. W. Morgan. 1969. Ethylene as a component of the emanations from peanut seeds and its effect on Virginia-type peanuts. *Plant Physiol.* 42:333-337.
7. Letham, D. S., P. B. Goodwin, and T. J. V. Higgins. 1978. *Phytohormones and Related Compounds—A Comprehensive Treatise*. Elsevier/North Holland Biomedical Press, Amsterdam. 648 pp.
8. Toole, V. K., W. K. Bailey, and E. H. Toole. 1964. Factors influencing dormancy of peanut seeds. *Plant Physiol.* 39:822-832.
9. Welker, W. V. and D. N. Riemer. 1982. Fragrant waterlily (*Nymphaea odorata*) control with multiple applications of glyphosate. *Weed Sci.* 30:145-146.