Selected Life Cycle Features Of Fanwort

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ABSTRACT

Some aspects of the life cycle of fanwort (*Cabomba caroliniana* Gray), a hydrophyte in the family Nymphaeaceae, were investigated. Germination of fanwort seeds in Louisiana contributes to its reproductive potential; however, optimum conditions for germination were not conclusively defined. Anthesis occurred from approximately 10 AM to 4 PM. Insects, primarily the introduced honey bee (*Apis mellifera* L.), were determined to be the pollinating agents for cross-pollination. Successfully pollinated flowers developed mature seeds within 28 to 31 days of anthesis. Non-insect pollinated flowers on emersed peduncles and flowers opening beneath the water surface failed to produce seeds. Seeds were produced on shoots not rooted in hydrosoil, indicating that the shoot does not require being rooted for successful seed production.

INTRODUCTION

Due to the increasing importance of fresh water for recreation and potable uses, influences which aquatic plant communities may exert on the aquatic ecosystem have now been recognized.

An excellent summary of the limnological role of higher aquatic plants is given with mixed blessings:

> "Higher plants make lakes more habitable for waterfowl and fishes, but . . . help to destroy the habitat for both themselves and their animal associates. They add oxygen . . . but cut down on the ability of the water to absorb it. They furnish ducks with essential food, but their contribution of decomposing material may periodically help to reduce oxygen to the point where botulism can develop and take its till. They support an abundance of fish food, but their dense growths may favor an increase of snails and other intermediate hosts of fish parasites." (4)

Fanwort, a submersed, dicotyledonous, aquatic perennial of the family Nymphaeaceae, is native throughout the southeastern states (3). Various species of *Cabomba* used as aquarium plants pose a threat to waterways when aquaria are carelessly emptied into canals and lakes. *C. caroliniana*, *C. pulcherrima* (Harper) Fass., and *C. aquatica* Aubl. are species sold by the aquarium industry in the United States (US).[3]

Fanwort is a problematic plant in waters throughout the Gulf Coast States. Louisiana, which has a fresh water to land ratio of 1:12 with 607,500 ha in lakes and reservoirs, has a severe aquatic weed problem composed of both emersed and submersed species (7). Fanwort has been and continues to be a major pest plant in many Louisiana waters.

The range of fanwort was found to extend from Massachusetts to Michigan, west to Kansas, south to south central Texas and east to southern Florida (3). Seed germination studies revealed that sexual reproduction does occur in Louisiana. Fresh weight increase and average stem elongation of seedlings were greatest in a pH range of 4 to 6. Alkaline conditions definitely inhibited growth and caused defoliation. Stem elongation of fanwort seedlings increased as turbidity increased, with the greatest seedling elongation occurring at a turbidity level of 70-110 Jackson Turbidity Units (JTU). Vegetative reproduction was possible from a stem section containing only one node and a pair of leaves (3). Due to the great potential reproductive capability of fanwort and its potential as an aquatic plant pest a study was funded by the US Army Corps of Engineers to elucidate selected life cycle features of this hydrophyte.

Objectives of this study were in part to determine: (1) optimum environmental conditions for seed germination, (2) flowering period of flowers opening above and beneath the water surface, (3) pollination mechanism, and (4) length of time for seed maturation.

METHODS AND MATERIALS

Seed Viability and Germination

Seeds used in seed viability and germination studies were collected by hand from a) Joyner's Pond, Bienville Parish, Saline, Louisiana, and b) Iatt Lake, Grant Parish, Louisiana. Dark-colored, swollen carpels were collected with peduncle attached and maintained under laboratory

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[3]Lazor, Robert L. 1975. Personal Communication.

conditions at 22 C with a 9-hr photoperiod in jars containing lake water. Seeds were allowed to mature and were considered ripened when the pericarp ruptured and seeds sank. Fifteen seeds from each lake were treated with 0.1% tetrazolium chloride solution to determine viability.

A total of 1,095 seeds were subjected to various environmental conditions. All seeds were placed under laboratory conditions with diffused light following each treatment, unless otherwise specified.

Experiment I. Seeds were collected from Joyner's Pond in November 1973. Two replications of 25 seeds per treatment were placed in plastic containers measuring 7.0 cm in height by 4.5 cm in width by 5.0 cm in length containing pond water when the treatment involved an aqueous solution. All seeds were exposed to 20 minutes of red light (650 nm) by use of a red filter box. Results were recorded as seeds germinated, and seedlings were removed from the plastic containers.

Experiment II. Seeds collected from Joyner's Pond and Iatt Lake in July 1974 were subjected to 12 treatments of red light when red light was part of the specified treatment. Seeds were held at the specified temperature for 4 days prior to red light treatment and during the treatment. Red light exposure was made inside modified refrigerators, which were holding seeds at specified temperatures, by inserting an 100-watt incandescent bulb wrapped in red plastic. Carbon dioxide (CO_2) was rapidly bubbled in the plastic dish with a small exchange port in order to develop a high CO_2 environment. Air was rapidly bubbled into a similar dish to establish a high oxygen (O_2) environment. A 60-watt incandescent light was placed 15 cm above treatment containers to establish high light intensity.

Methods of Pollination

A series of experiments was designed to determine the pollination mechanism of fanwort and whether seed development from an unrooted stem section was possible. Pollination mechanisms given consideration were: (1) pollen suspended in water, (2) self-pollination, (3) aquatic insects, (4) wind, and (5) airborne insects.

Stem section of fanwort containing inflorescences, six pairs of leaves and no roots were collected in June 1974, from Joyner's Pond, Sabine, Louisiana. Each inflorescence contained at least three flower buds. Stem sections with inflorescences were placed in the laboratory in aquaria containing lake water. No insects were observed visiting flowers in aquaria. Each flower was tagged with labelling tape attached to nylon monofilament fishing line and tied loosely to the peduncle in order to mark anthesis date. Daily observations of carpel development were conducted and recorded for each trail. Aquaria were supplied with air and were exposed to a photoperiod of 9 hr and initially fertilized with 2 g of Precise (6-12-9) fertilizer.

Experiment I. a) Two inflorescences in each of four aquaria were tied with monofilament line to a sunken small glass jar to prevent elongating peduncles from extending newly formed flowers above the water surface. If seeds developed, pollination would be attributed to either self-pollination or cross-pollination by suspended pollen in the water. Twenty-five flowers were tagged. The remaining two inflorescences in each aquarium were allowed to float at the water surface. Developing seeds would be attributed to self-pollination or wind pollination. Twenty-five flowers were tagged.

b) Stem sections containing inflorescences with a total of 25 flower buds were tagged and placed inside two insect-proof boxes on Joyner's Pond for 1 week. The boxes were positioned so the lower edge would remain beneath the water surface to prevent flying and crawling insects from entering. Seed development would be attributed to wind or self-pollination.

c) Twenty-five flowers were tagged which were open for the second day, and were observed to have been visited by flying insects. Styrofoam floats were attached to the stems with monofilament line in order to retrieve the inflorescences containing pollinated flowers.

d) Rooted plants containing newly forming buds were tied with monofilament line to stakes driven underwater in such a manner as to prevent flowering above the water surface. Seed development would be attributed to water pollination. All inflorescences used in b, c, and d remained on the lake 1 week.

e) Jars glued on boards were placed over erect peduncles on newly opening flowers in the laboratory. The jars extended below the water line to prevent pollination by wind or flying or crawling insects. Seed development would be attributed to self-pollination.

Experiment II. Flowers observed with insects crawling on stigmas and stamens under field conditions were used in this study. Ten flowers open for the 1st day (1-day flowers) and ten flowers open for the 2nd day (2-day flowers) were tagged after insects were seen visiting the flowers. Insects observed on the flowers were allowed to leave without being disturbed.

Fifteen flowers which opened under laboratory conditions were not treated and were tagged as control specimens. A total of 10, 1-day flowers in the laboratory were artificially cross-pollinated. Stamens were removed from these by forceps and mature stamens from 2-day-old flowers were brushed against stigmas of 1-day flowers. Visible pollen remained on the stigmas of cross-pollinated flowers. This procedure was also performed on 18, 2-day flowers.

Flowering Period and Carpel Development

In conjunction with experiments designed to determine the pollination mechanism of fanwort, dates and times of flowers opening and closing were recorded.

RESULTS AND DISCUSSION

No seeds were found to be viable in seed viability tests using tetrazolium chloride. These results, however, are possibly not a true indication of seed viability because conventional staining tests are less favorable for dicotyledonous seeds such as fanwort than for seeds of monocotyledonous plants (1).

Experiment I. Fanwort produces viable seeds in Louisiana. Of the 450 test seeds studied, 5.5% germinated. This con-

firmed Gregory's statement that some seeds do not require a period of after-ripening (3).

Germination of fanwort seeds occurred from 5 to 10 weeks after treatments but germination percentages were insufficient to allow for statistical analysis. Twenty seeds held in darkness did not germinate while 20% of the seeds placed under greenhouse conditions germinated. (Table 1).

Fanwort seeds received exposure to natural light and a daily temperature fluctuation of 8 to 16 C in the greenhouse. Seeds in the laboratory were kept at a constant temperature. Interaction of natural light and fluctuating temperature could be an important factor in seed germination.

TABLE 1. GERMINATION (%) OF FANWORT SEEDS COLLECTED IN THE FALL OF 1973 FROM JOYNER'S POND, SALINE, LOUISIANA.

Treatment[a]	Germination (%)	
	Red light	No red light
1. Placed in greenhouse	16	20
2. Placed in laboratory	8	4
3. Air dried 14 days	8	0
4. Cold treatment, 14 days	8	0
5. Dilute HCl, 70 sec	4	8
6. Cold treatment, 30 days	4	8
7. Frozen dry, 14 days	4	4
8. Frozen, thawed, re-frozen	0	4
9. Placed in darkness	0	0

[a] Seeds, except those in treatments 1 and 9, were placed in the laboratory at 22 C with a 9-hr photoperiod following treatment.

Experiment II. This test was primarily designed to determine the germination percentage for seeds at specific temperatures exposed to red light. Gregory, (3) hypothesized that interaction of red light (650 nm) with temperature could be an important factor in germination of fanwort seeds. However, no significant difference was detected.

A 1.8% germination occurred for the 600 seeds (Table 2). The number of seeds which germinated differed by only one seed in the range of 10 to 45 C. These data for greenhouse germination differ sharply from those of previous studies. A low percentage of viable seeds among those tested offers a possible explanation of these findings. Neither high O_2 nor CO_2 were found to influence seed germination.

TABLE 2. GERMINATION (%) FROM TWO SUMMER 1974 COLLECTIONS OF FANWORT SEEDS.

Treatment	Germination (%)	
	Joyner's Pond[a]	Iatt Lake[a]
1. 10 C, red light, placed in lab	8	0
2. 20 C, red light, placed in lab	8	0
3. 25 C, red light, placed in lab	4	0
4. 35 C, red light, placed in lab	4	0
5. 45 C, red light, placed in lab	4	0
6. Placed in lab	4	4
7. Red light, high oxygen	4	0
8. Red light, placed in lab	0	4
9. Red light, greenhouse	0	0
10. Greenhouse	0	0
11. Red light, high CO_2	0	0
12. Red light, high light intensity	0	0

[a] Germination based on 25 seeds.

Methods of Pollination

Many fanwort flowers were observed to open underwater and were never exposed to wind or flying insects; therefore, a seemingly logical initial hypothesis was that the pollination mechanism involved either self-pollination or pollen transport by water. Inflorescences held underwater in both laboratory and lake conditions failed to develop seeds or show carpel development (Table 3). These flowers displayed a 2-day flowering period, but usually decayed within 1 week after anthesis. Because no fruits developed from submersed flowers, it was assumed that submersed flowers were not successfully pollinated.

TABLE 3. SEED DEVELOPMENT (%) OF INSECT AND NON-INSECT POLLINATED FANWORT FLOWERS FROM JOYNER'S POND.

Method of pollination	Flowers tagged (number)	Seeds developed (9/19)	Seed development (%)
Non-insect pollinated in Joyner's Pond 8/21/74	25[a]	0	0
Insect pollinated in Joyner's Pond 8/21/74	25[a]	14	56
Inflorescence held under water in Joyner's Pond 8/21/74	25[a]	0	0
Non-insect pollinated in lab 6/13/75	25	0	0
Inflorescence held under water in lab 6/13/75	25	0	0

[a] Plants were placed under laboratory conditions 10 days after flowering.

Flowers which were open above the water surface on erect peduncles and screened from insects by netting were exposed to wind and other natural elements. It was assumed that wind pollination could occur due to the relative size of the mesh to pollen grains. No mites or other small organisms were observed on the emersed flowers. These flowers displayed the same flowering period as did others on the pond, but also failed to produce seeds. Carpel development failed to occur under laboratory conditions in which no insects were observed (Table 4).

No seed development or carpel swelling occurred among 28 flowers which were cross-pollinated by hand in the laboratory. Flowers and peduncles began to decay 1 week after anthesis. However, 8 of 20 flowers which were insect-pollinated at Joyner's Pond developed mature seed in approximately 1 month (Table 4). Twenty percent of flowers visited by insects during the 1st day of flowering developed seeds; however, 60% of the flowers visited by insects on the 2nd day of flowering developed mature seeds. Anthers did not dehisce until the 2nd day; therefore, fanwort flowers were found to be protogynous. Fourteen of the 25, 2-day flowers which were visited by insects developed seeds within a month (Table 3). An average of 58% of the 2-day flowers visited by insects on Joyner's Pond produced

TABLE 4. SEED DEVELOPMENT (%) OF FANWORT EXPOSED TO VARIOUS POLLINATION METHODS.

Method of pollination[a] (7/2/74)	Flowers tagged (number)	Seed maturation date 7/26	7/29	7/31	8/3	Seed development (%)
D1	10					0
D2	18					0
N	15					0
1	10			1	1	20
2	10	1	1	2	2	60

[a] D1—Flower open for 1st day with stamens removed and pollinated by stamens from 2nd day flower under laboratory conditions.
D2—Flower open for 2nd day with stamens removed and pollinated by stamens from 2nd day flower under laboratory conditions.
N—Control flower; open in the laboratory, no special treatment.
1—Flower open for 1st day and insect pollinated at Joyner's Pond, then brought into the laboratory..
2—Flower open for 2nd day and insect pollinated at Joyner's Pond, then brought into the laboratory.

mature seeds. These results provide evidence that the pollination mechanism for fanwort is natural cross-pollination by insects, and seed development on detached inflorescences can occur.

Principal pollinators of fanwort were the dragon fly (*Anax* spp.), damselfly (*Enallagma* spp.), sweat bee (*Halictus* spp.), and the introduced honey bee (*Apis mellifera* L.). Honey bee, the most frequently observed pollinator, usually landed on petals and crawled over the anthers, thereby gathering pollen on its legs and body. The cause of this attraction for insects is probably the warmth and odor of the opening flowers (5).

Presently insects have not been utilized for biological control of fanwort. It is most important that insects selected for biological control not only be safe and effective from the feeding and development standpoint, but should not contribute toward improving reproduction or weediness of the pest species. Insects which are attracted to fanwort flowers but do not damage the reproductive organs should not be considered for plant management.

Flowering Period and Carpel Development

Anthesis for flowers above the water surface and for those opening beneath the surface displayed very similar opening and closing times (Table 5). Flowering periods for 1-day flowers above and below the water surface ranged from 3 hr and 40 minutes to 6 hr and 5 minutes. Earliest opening time was 10:15 AM for flowers above the water surface and 10:10 AM for those below the surface. All 24 observed flowers closed between 4:00 PM and 4:20 PM.

All tagged fanwort flowers displayed dianthesis, opening by 10:45 AM on the 2nd day of flowering and closing by 4:20 PM. Dollar bonnet (*Brasenia schreberi* J. F. Gmel.) flowers open about 9 AM and descend beneath the water surface, rising and opening again the following morning when anthers shed their pollen (8,9).

Flower buds were observed on peduncles near the water surface 3 hr before anthesis the 1st day. Flower buds were raised on erect peduncles from 2 to 5 cm above the water surface 2 hr before anthesis. The peduncle appeared to lose turgor pressure and returned the flower beneath the water surface after petal closure. Rising and falling of peduncle and flower occurred each day of flowering. Flowering period lengths for fanwort above and below water were not significantly different. The flowering period of fanwort, as that of other angiosperms, has probably evolved to permit access by specific insects which were most active at this time in order to insure maximum pollination.

Carpel position changed during the flowering period (Figure 1). The distal stigmatic portion of the carpels turned outward and away from other carpels during the 1st day of flowering. Stamens were approximately one-half the carpel length on the 1st day. Stamens elongated to a height level with the stigmas and nearly touched them on the 2nd day. Carpels were erect and continguous. The anthers dehisce from 1 to 3 hr after the flower is fully open the 2nd day. Pollen at this time is in a sticky mass,

TABLE 5. DAILY FLOWERING PERIOD OF FANWORT FLOWERS BELOW AND ABOVE THE WATER SURFACE.

Above Water Surface Opening Time (A.M.)	Above Water Surface Closing Time (P.M.)	Above Water Surface Flowering Period Hr.	Minute	Below Water Surface Opening Time (A.M.)	Below Water Surface Closing Time (P.M.)	Below Water Surface Flowering Period Length Hr.	Minute
10:10	4:15	6	05	10:10	4:15	6	05
10:15	4:20	6	05	10:10	4:15	6	05
10:30	4:00	5	30	10:15	4:10	5	55
10:30	4:15	5	45	10:30	4:10	5	40
11:00	4:10	5	10	10:30	4:20	5	50
11:30	4:00	4	30	10:35	4:20	5	45
11:30	4:15	4	45	11:20	4:10	4	50
11:30	4:15	4	45	11:30	4:10	4	40
11:30	4:15	4	45	11:30	4:15	4	45
11:35	4:15	4	40	11:30	4:15	4	45
12:15	4:15	4	00	11:35	4:15	4	40
12:15	4:15	4	00	12:35	4:15	3	40
	Mean	5	00		Mean	5	16

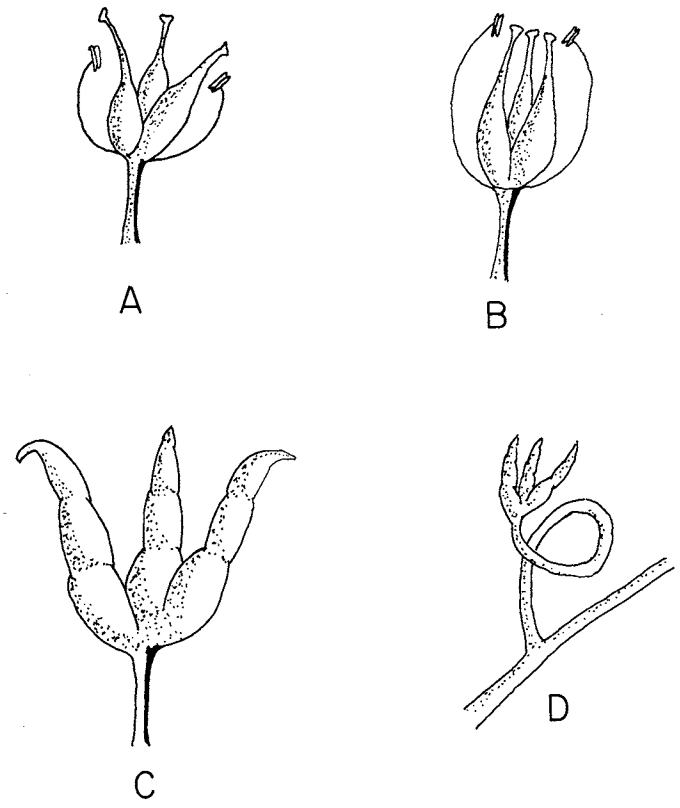

Figure 1. Carpel development of fanwort. A. First day of Flowering; B. Second Day of Flowering; C. Mature Carpel; D. Peduncle Coiling (D.P.T.).

but dries and becomes powdery soon after opening. Flower age can easily be determined by observing carpels and stamen positions.

Floral axis movements were observed for flowers which were fertilized and later developed fruit. Peduncle curvature downward, a phenomenon common in fanwort and other submersed aquatic plants, places developing fruits below the water surface (6) (Figure 1). Peduncle movement in this manner was observed only for fertilized flowers, as noted by swollen carpels. Carpel swelling was evident 1 week after pollination. Auxins released in the ovary are transported down the peduncle, preventing abscission of the fertilized flower. Natural coiling may be regulated by auxin liberated from the developing fruits near the stem and is thought to help protect carpels from being broken off the peduncle by fish and severe wave action (2).

The major growth of fanwort is due to vegetative production; however, germination of seeds contribute to the reproductive potential of this pest plant. (3) Management practices aimed at inhibiting insect pollination should include insect retardants and water fluctuation. Increased water levels to prevent anthesis of flowers above the water surface would inhibit insect pollination and of course seed production. Maintaining high water levels throughout the extended flowering period of fanwort, spring through fall in southern and southeastern states, may involve difficulties.

Water level fluctuation has proven to be a very effective tool in reduction of seed germination and vegetative regrowth. Consecutive fall-winter drawdowns yielded a 99% reduction of fanwort in Black Lake, Lousiana. High water levels during the early spring and late summer months existed after each drawdown.[4]

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