*J. Aquat. Plant Manage.* 28: 11-15

# Production Of Axillary Turions By The Dioecious *Hydrilla verticillata*

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## ABSTRACT

Twelve different temperature ranges were examined to determine their role in turion production. Four other variables were also examined and evaluated to determine which were significant in the stimulation of turion formation. Variables which significantly influenced dioecious hydrilla [Hydrilla verticillata (L.f.) Royle] axillary turion production ($p < 0.05$) were temperature, the source of plant material, the length of time the plants were in the study, and aeration. Investigations in environmental growth chambers showed that dioecious hydrilla can produce up to 46 turions per 1.0 g dry weight plant material (equivalent to 2803 axillary turions per $m^3$). The temperature range which consistently produced the greatest number of turions was 17 to 27 C under an 8-hour photoperiod. Although the range of necessary environmental conditions for optimum turion production in hydrilla is narrow, each of hydrilla's many diverse reproductive strategies should be considered when designing a control program.


*Key words*: Hydrilla, reproductive strategies, propagules, temperature, turions, fragmentation.

## INTRODUCTION

Hydrilla has earned worldwide recognition as one of the most noxious weeds among submerged plant species (Haller, 1976; Robson, 1976; Swarbrick et al., 1981; and Pieterse, 1981). It has earned this reputation because of its rapid growth and its ability to adapt and proliferate even in what appear to be extremely unfavorable conditions. It can withstand low light intensities; suspended sediments; high conductivity waters; drawdown periods; warm, temperate to tropical water temperature ranges; numerous control techniques; and can outcompete many other aquatic weed species (Mitra, 1960; Robson, 1976; Pieterse, 1981; Swarbrick et al., 1981). Hydrilla can reproduce via fragments, subterranean turions (tubers), axillary turions (turions), seeds, stolons, and rhizomes. Tubers and turions are especially adapted to survive unfavorable conditions (Pieterse, 1981). Turions, called overwintering organs by Sculthorpe (1967) and Haller (1976), develop primarily on floating plant fragments (Haller et al., 1976).

It is important to know as much about the biology of hydrilla as possible to manage its growth effectively. While many articles have been written on the production of tubers, the production of turions is not as well documented. It has been reported that dioecious hydrilla produces many more tubers than turions when rooted (Haller and Sutton, 1975; Steward, 1984; Bowes et al., 1979; Spencer et al., 1987; and Sutton and Portier, 1985). Mitra (1955) observed that tubers outnumber turions by ten times in a given area of hydrilla. Anderson (1986)[2] stated that although monoecious plants produce 20 to 30 percent of their total number of turions aboveground (stem and axillary turions), dioecious plants produce few, if any, turions. Perhaps one reason few turions have been observed on dioecious hydrilla plants in the field is that few quantitative observations have been made of floating hydrilla, specifically unhealthy appearing, floating hydrilla mats in the fall.

The purpose of this study was 1) to determine if hydrilla could be induced to produce numerous turions by altering environmental conditions and 2) to determine what set of conditions was necessary to induce the production of turions. If those conditions are duplicated in the field, then the management of hydrilla could be much more difficult, particularly if those conditions are overlooked. Pinpointing the conditions of maximum turion production, and thus attempting to alter those conditions, could further help in the successful management of the weed.

## MATERIALS AND METHODS

All experiments were conducted for one or two six week periods and performed in Percival environmental growth chambers, except for two temperature studies performed outdoors. The growth chambers were on timers to control the daily photoperiod and daily air temperature. Light intensity was approximately 180$\mu$E $m^{-2}$ $sec^{-1}$ PPFD (Photosynthetic Photon Flux Density) inside the growth chambers. A photoperiod of 8 hours was used in all the studies performed in the growth chambers because it was the photoperiod which produced turions in preliminary studies. The growth chamber controls were set to achieve a simulated daily warming and cooling of the water. Temperature ranges used in the studies were the daily highs and lows of the water in each test container (described later). The water temperatures therefore formed regular diurnal curves when plotted over time. The water temperature ranges were chosen from preliminary temperature trials and from educated guesses. The temperature ranges studied in the growth chambers included (with the number of replications in parentheses): 19-29 C (4), 27-28 C (2), 19-28 C (6), 18-28 C (8), 20-27 C (2), 17-27 C (28), 17-26

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[2]Anderson, L. W. J. 1986. Summary of *Hydrilla* biology and control. Presented at the Joint Meeting of Weed Committees of U.S. Dept. of Agriculture and U.S. Dept. of Interior, Washington, D.C., unpublished, 4 pp.

C (27), 15-25 C (6), 13-25 C (2), and 6-16 C(2). The outdoor studies were conducted during May 28, 1987 to July 9, 1987 (using 5 replicates) and August 19, 1987 to September 30, 1987 (5 replicates), and performed in 190-liter Nalgene baths covered with 13 mm wire mesh screens. The daily warming and cooling formed diurnal water temperature curves but the daily high and low temperatures varied with the weather. The water temperature extremes for each study were 9-20 C during the first six weeks, and 12-27 C during the second.

Hydrilla for the temperature studies was obtained from the Imperial irrigation canal, in the Imperial Valley of southern California (32.47N, 115.33W) and from Long Lake, near Austin, Texas (30N, 97W). Hydrilla for the source studies, length of time studies, and gas studies was obtained from the Imperial irrigation canal; Long Lake, Bastrop Lake and Fayette Lake, near Austin, Texas (30N, 97W); Lake Texana between Houston and Corpus Cristi, Texas (29.00N, 96.41W); and the monoecious hydrilla was obtained from the Potomac River near Washington D.C. (38.37N, 77.11W). Numbers of replications are listed in Tables 1 and 2. The plant material arrived by air express from each field site. It was used in studies fresh from the field or it was cultured in greenhouses in 95-liter plastic barrels containing a Colorado clay loam soil to a depth of approximately 100 mm. During the greenhouse culturing period, photoperiod was natural (ranging from about 9 to 15 hours of daylight) and temperatures fluctuated (ranging from 32 to 10 C, with a maximum daily fluctuation of 10 C) according to the prevailing climatic conditions. When the cultured plant material was used in a study the conditions were similar for all source material at the same time. When fresh field plant material was used in a study, material from all areas was collected at approximately the same time. Harvest dates are listed in Tables 1 and 2.

Hydrilla stem fragments were used in the studies. Whether the plant material was from the field or the greenhouse it was rinsed in tap water, the healthiest fragments were selected, and their fresh weight was measured. Approximately 50 g of hydrilla was used in each replication unless there was a shortage of material from a particular source. In case of a shortage, the total amount available from that source was split among the replications.

The containers were either 13.2-liter glass chromatography jars (diameter = 210mm, height = 450mm, volume $\approx$ 0.013 $m^3$) or semitranslucent Nalgene 41.6- liter rectangular baths (457 X 305 X 305mm, volume $\approx$ 0.042 $m^3$). The temperature studies had no additional air added to the containers, but later studies were aerated using an aquarium air pump (Second Nature Whisper) attached to 28 X 15mm airstones via Penn Plax flexible plastic tubing (4mm inside diameter). The air flow into each container of 15 to 56 $cm^3$/min was regulated by Penn Plax plastic and brass gang valves. Other studies had nitrogen gas ($N_2$) bubbled through the airstones and gang valves into each container from a compressed $N_2$ gas cylinder.

The containers were flushed with fresh water, which was tempered to match the original water temperature, 3 times a week. The water was filtered by running it through a charcoal canister and a 1.0 $\mu$ Filterite filter to remove chlorine, chloramines, and other organics (Mike Perez, 1989. pers. comm.). Analysis of the water was performed by the Soils Laboratory of Colorado State University, Fort Collins, Colorado.

After six weeks, turions were harvested from each container and the plant material weighed. Fresh weight was measured after blotting off all adherent water. Dry weights were measured after the plant material was dried at 105 C for 24 hours (Westlake, 1974). The data obtained during the second 6 week period was not cumulative over the entire 12 weeks but reflected only the turions which were produced during the second 6 weeks. The average dry weight of the hydrilla plant material was 13.0 percent of the fresh weight (n = 182, s.d. = 0.029).

Numbers of turions per 1.0 g of fresh plant material were analyzed using GLM (general linear model) factorial

TABLE 1. HYDRILLA AXILLARY TURION PRODUCTION DATA BY TEMPERATUE RANGE PLUS PREINDUCTION INFORMATION. ALL PLANT MATERIAL ORIGINALLY CAME FROM THE IMPERIAL VALLEY OF SOUTHERN CALIFORNIA, EXCEPT FOR THE FRESH MATERIAL USED IN THE 17-27 C TESTS WHICH CAME FROM LONG LAKE NEAR AUSTIN, TEXAS.

| Temperature ranges (Celsius) | Reps | Culture | Dates harvested | Turions/gram fresh weight Mean ± S.D. | |
|---|---|---|---|---|---|
| 19-29 | 4 | gh[a] | 08-27-87 | 0.00 (0.00) | AB[b] |
| 27-28 | 2 | gh | 08-27-87 | 0.00 (0.00) | AB |
| 19-28 | 6 | gh | 05-28-87 | 1.52 (1.29) | AB |
| 18-28 | 8 | gh | 08-27-87 | 0.08 (0.17) | B |
| 20-27 | 2 | gh | 08-27-87 | 0.07 (0.04) | AB |
| 17-27 | 28 = | 24 fresh, 4 gh | 11-22-87, 12-07-87 | 2.26 (1.79) | A |
| 12-27 | 5 | gh | 05-28-87 | 0.05 (0.08) | AB |
| 17-26 | 27 = | 9 gc, 18 gh | 1,2,3, 5-87, 1-88 | 1.07 (1.56) | AB |
| 15-25 | 6 | gh | 05-28-87 | 0.00 (0.00) | B |
| 13-25 | 2 | gh | 08-10-87 | 0.00 (0.00) | AB |
| 9-20 | 5 | gh | 05-28-87 | <0.01 <(0.01) | B |
| 6-16 | 2 | gh | 08-10-87 | 0.00 (0.00) | AB |

[a]Fresh denotes plant material harvested from the field and used in the study shortly after its arrival to the laboratory. Gh denotes plant material which has been cultured in the greenhouse for at least 3 months. Gc denotes plant material which was grown in an environmental growth chamber under an 8-hour photoperiod and a 17-26 C temperature range.
[b]Means with the same letter are not significantly different ($p < 0.05$) using Tukey's Studentized Range test.

TABLE 2. HYDRILLA AXILLARY TURION PRODUCTION DATA BY SOURCE MATERIAL, LENGTH OF TIME THE PLANTS WERE IN THE STUDY, AND THE GAS ADDED, PLUS PREINDUCTION INFORMATION. SEVERAL SOURCES OF PLANT MATERIAL WERE USED IN THE LENGTH OF TIME AND THE GAS ADDED STUDIES.

| Source of plant material | Culture | Reps | Turions/gram fresh weight Mean ± S.D. | | Date harvested |
|---|---|---|---|---|---|
| Test 1: | | | | | |
| Texas, Long | fresh[a] | 24 | 2.60 (1.70) | A[b] | 11-22-87 |
| California | gh | 26 | 0.48 (0.93) | B | 12-07-87 |
| Test 2: | | | | | |
| Texas, Texana | fresh | 40 | 0.80 (1.04) | A | 6-13-88 |
| California | gh | 40 | 0.64 (0.59) | AB | 6-14-88 |
| California | fresh | 40 | 0.40 (0.38) | B | 6-28-88 |
| Test 3: | | | | | |
| Texas, Texana | gh | 34 | 0.08 (0.12) | A | 10-11-88 |
| California | gh | 34 | 0.09 (0.11) | A | 10-11-88 |
| D.C. (monoecious) | fresh | 6 | 3.27 (1.70) | | 10-17-88 |
| Test 4: | | | | | |
| Texas, Texana | gh | 18 | 0.13 (0.10) | C | 1-19-89 |
| Texas, Texana-Simon | fresh | 12 | 0.47 (0.42) | BC | 12-16-88 |
| Texas, Long | gh | 12 | 1.38 (1.55) | B | 1-19-89 |
| Texas, Bastrop | fresh | 12 | 2.94 (2.56) | A | 12-13-88 |
| Texas, Fayette | gh | 6 | 1.46 (1.01) | ABC | 1-19-89 |
| Texas, Fayette | fresh | 6 | 1.18 (1.06) | BC | 12-14-88 |
| California | gh | 18 | 0.11 (0.10) | C | 1-19-89 |
| D.C. (monoecious) | gh | 18 | 8.83 (14.57) | | 1-19-89 |
| Length of time the plants were in the study | | | | | |
| 12 weeks | | 135 | 2.06 (5.95) | A | 11-87 to 1-89 |
| 6 weeks | | 137 | 0.78 (1.22) | B | 11-87 to 1-89 |
| Gas added | | | | | |
| no air | | 60 | 0.21 (0.27) | A | 11-87 to 9-88 |
| air | | 92 | 0.70 (0.79) | B | 6-88 to 1-89 |
| $N^2$ | | 36 | 0.05 (0.07) | A | 10-88 to 1-89 |

[a]Fresh denotes plant material harvested from the field and used in the study shortly after its arrival at the laboratory. Gh denotes plant material which had been cultured in the greenhouse for at least 3 months.
[b]Means with the same letter are not significantly different ($p < 0.05$) using Tukey's Studentized Range test.

ANOVA (analysis of variance) procedures in SAS (Statistical Analysis System) software (SAS Institute, Inc., 1985). Mean turion production data of the monoecious hydrilla from Washington D. C. is included in Table 2 for comparison but is not included in the tests for statistical differences since its high turion production would mask the differences among the dioecious sources.

## RESULTS AND DISCUSSION

The results of these studies indicate that dioecious hydrilla plants produce great numbers of turions under suitable conditions on floating plant fragments and that turion development is influenced ($p < 0.05$) by four variables: daily temperature range, the source of hydrilla, the length of time the plants were in the study, and aeration. The mean number of turions produced per 1.0 g fresh plant material for each temperature range is given in Table 1. The most productive ranges were 17 to 26 C, 17 to 27 C, and 19 to 29 C. The highest density of turions produced in a single container was 7.12 per 1.0 g fresh plant material, or 46 turions per 1.0 g dry plant material (equivalent to 2803 turions per $m^3$ (total number of turions in the container X the conversion to $m^3$)), in the 17 to 27 C range. Temperature ranges with a minimum temperature other than 17-19 C produced few turions in these studies. It can be concluded therefore, that a range of temperatures around 17-19 C would be the minimum for optimal turion production. Likewise, the maximum temperature is about 26-28 C.

Hydrilla from different sources produced significantly different numbers of turions. However, in four separate studies no patterns emerged as to a particular source or condition which consistently produced more turions (Table 2). Although plant material from Texas produced significantly more turions per dry weight of plant material than California in two of the four tests, tests three and four had very different results. The plant material from Texas was not significantly different from that of California in the third test (although the addition of $N_2$ gas into the growth chambers appeared to inhibit turion production in all the containers), and in the fourth test no pattern was evident (Table 2). Because of the variability in turion production between the sources, there was a question whether or not different genotypes were causing the variability. However, isoenzymic analyses of collections of tur-

ions from Texas indicated no differences between these plants and those from California (Ryan, Thullen, and Holmberg, 1989. pers. comm.). Instead, the difference in numbers of turions produced must be due to the different environmental factors prior to their removal from the field as suggested by Verkleij and Pieterse (1986).

The length of time the plants remained in the study had a significant effect on the production of turions, according to the data (Table 2). Turion production increased significantly with additional time, from six weeks to 12 weeks. Increased turion production may require older, more mature plant material, but from visual observations it was often the more chlorotic and unhealthy fragments (plant material which was past its mature condition) which produced the turions. Pieterse et al. (1984) suggested that the formation of turions may be a means of surviving nutrient stress. In these experiments, chlorotic stem fragments less than 20 mm long produced healthy, viable, and often robust turions. It appeared that hydrilla was allocating the last of its reserves to produce progeny.

Pieterse et al. (1984) suggested that turion formation is stimulated by low levels of nitrogen and phosphorus in the water. The levels of 0.005 mg/L $NO_3$-N and 0.049 mg/L inorganic phosphorus, which Pieterse et al. used, produced 29 turions per 1.0 g dry weight plant material within 8 weeks. The levels present in the water during this study were <0.1 mg/L $NO_3$-N, ≤0.2 mg/L $NH_4$-N, and <0.01 mg/L inorganic phosphorus. The $NO_3$-N levels and phosphorus levels used in this study were similar to the levels used by Pieterse et al., however, the nitrogen available to the plants in the form of $NH_4$-N was considerably higher. The studies reported here (which produced a maximum of 1.6 times the number of turions produced by Pieterse et al.), as well as earlier unpublished studies conducted previously in this laboratory, demonstrated that turion production was not stimulated solely by low levels of nitrogen and phosphorus. Turions were not readily produced in other temperature ranges using the same water with low nutrient levels (as described earlier). Additionally, plants grown in the higher level of 0.2 mg/L $NH_4$-N (but a level which did not encourage turion production according to Pieterse et al.) produced up to 46 turions per 1.0 g dry weight of hydrilla. These data lead to the conclusion that a daily temperature range of about 17 to 27 C with an 8 hour photoperiod, play a substantial role in meeting requirements for turion production.

Aeration also increased turion production significantly in these studies. The data suggest that turion production requires a certain amount of air to be available to the plant material. Whether it is oxygen that is necessary, or a composite of gases that is necessary to induce turion production, is not known. The addition of $N_2$ into the containers inhibited the mean turion production but not significantly more than adding no air (Table 2). The agitation of the water and plant material did not seem to affect turion production since the agitation from the $N_2$ was similar to the agitation from the air. Therefore, the gas mixture and gas exchange was important but further research is necessary to pinpoint the relationship to turion production.

Sutton and Portier (1985) stated that tubers and turions in a steady state condition may form at the rate which equals the maximum number which can germinate in a particular sediment, water quality, etc. In these laboratory studies, performed without soil and very few nutrients in 13.2-liter containers and in a steady state for 12 weeks, it is unlikely that 168 turions (the maximum number produced in one jar) would be able to germinate and thrive.

Spencer et al. (1987) pointed out that turions and tubers represent different reproductive strategies. They theorize that turions have been subjected to selective pressures for greater dispersal ability since they are small and produced above ground. Because turions were so readily produced on the floating fragments in these studies, it is probable that utilizing floating fragments for dispersal makes this strategy even more effective. Alternatively, turion formation may occur primarily when plants are stressed and lack the vigor or the structures to form tubers or new shoots. Perhaps the total strategy combines these two by forming turions when the plant is stressed and supplying it with the means to disperse itself into a more habitable area. Newly formed turions still attached to plant fragments can then float with the water currents into new areas where they may sink to the hydrosoil and grow, forming new infestations.

From a management standpoint, these investigations show that hydrilla management programs which use techniques that fragment the plants, such as chaining or disking, should only be used if all fragments are collected and destroyed. In addition to spreading the plant downstream, the potential for formation of turions is greatly increased. Since turions can survive unsuitable conditions, they create an even greater threat (from their subsequent plants) to the possibility of clogging water conveyance systems than by fragments alone. These data should be considered before implementing any hydrilla control program.

## ACKNOWLEDGMENTS

I would like to thank David Sisneros, John Boutwell, and Victor Miyahara for their assistance in maintaining the water baths when I was out of the office, Sharon Campbell for her assistance with personal computer software, John Pringle, Richard Roline, Frederick Ryan, and three anonymous reviewers for critically reviewing this manuscript, and Elmer Remmenga for helping with all the statistical analyses. I would also like to thank those who collected plant material, tubers, and/or turions from the field and sent them express mail so they would be fresh for the studies. This includes J. Henry Neason, Michael Remington, Charles Dvorski, and Dianne Ingram.

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