

Turion Production by Dioecious Hydrilla in North Florida¹

JANICE D. MILLER, W. T. HALLER AND M. S. GLENN²

ABSTRACT

A 14-month study was conducted to determine the effects of photoperiod, plant density, temperature, and herbicides on turion production by floating apical stems of dioecious hydrilla [*Hydrilla verticillata* (L.f.) Royle]. Turion production begins under short day conditions in September, decreases during the cold months of December and January, increases again in the late spring and essentially ceases during June through August. Free-floating (non-rooted) hydrilla stems produced more turions than stems planted in sand. Short daylength (<12 hr light) increased turion production and high plant density decreased production. Exposure to 2.5, 5.0 and 10.0 µg/l bensulfuron methyl or fluridone significantly reduced turion production.

Key words: bensulfuron methyl, fluridone, herbicide, photoperiod, plant density, temperature.

INTRODUCTION

Dioecious hydrilla is the most widespread submersed aquatic weed in Florida. Its spread and reproduction is limited to asexual means including fragmentation and the production of two specialized reproductive propagules, subterranean turions and axillary turions. Subterranean turions have also been called "tubers" in several papers (Haller *et al.* 1976, Van *et al.* 1978, Van and Steward 1990) and the term "tuber" will be used in this paper to avoid possible confusion. Axillary turions will be called simply, turions.

Tubers are produced in the soil at the ends of rhizomes in response to short day conditions (Van *et al.* 1978). Turions, or winter buds (Mittra 1955, 1964), are condensed shoots (axes) of 12 to 15 compact internodes surrounded by fleshy leaves arranged in alternating whorls (Lakashmanan 1951). They are oval to oblong in shape, 3 to 12 mm long and 2 to 3.5 mm wide, green in color, are produced in the axils of the leaves, and are filled with reserve food material in the form of starch (Lakashmanan 1951). Turions can be distinguished from vegetative buds by the lack of spines on the midrib of their leaves (Mittra 1964).

Tubers are thought to be the more important of the two propagules for reproduction (Haller *et al.* 1976, Van *et al.* 1978, Sutton and Portier 1985), consequently, much research into tuber production has occurred while the biology of turions is not well documented.

The objective of this study was to determine the influence of environmental conditions and herbicide exposure on turion production by hydrilla.

MATERIALS AND METHODS

Five experiments were conducted to examine aspects of annual turion production, production by floating or rooted stems, effects of photoperiod, influence of plant density, and the effects of treatment with selected aquatic herbicides. Apical stem sections collected for all the experiments were examined for the presence of turions, and any turions found were discarded. All experiments, except the production experiment, were begun in March 1992, and ended in May 1992 using hydrilla from Newnan's Lake, Florida. Results were expressed as numbers of turions per kilogram of hydrilla fresh weight for both initial and final weights. Hydrilla was weighed after all excess water was removed by blotting with paper towels.

The outdoor tanks used in most of the experiments were 217 cm long by 75 cm wide by 64 cm deep. Water depth was maintained at 56 cm using a standpipe, giving a water volume of 911 L.

All data were subjected to an analysis of variance (ANOVA). Numbers of turions per kilogram (kg) fresh weight were transformed by taking square roots in order to satisfy the homogeneity of variance assumption of ANOVA (Snedecor 1940).

Production. The production experiment was begun in the first week of August 1991 and continued until October 1992. Five hundred grams of hydrilla apical stem sections (approximately 20 cm long) from Jumper Creek, Florida, were allowed to float on the water surface in each of three sections of each of two tanks (tanks A and B) containing well water and exposed to ambient climatic conditions. Each tank was divided into thirds with window screen to allow free water movement between the sections but to contain the hydrilla within each section. During the first week of September (after 1 month) the hydrilla from tank B was harvested, processed, and 500 g of freshly collected hydrilla was put in each section

¹Published with the approval of the Florida Agricultural Experiment Station as Journal Series No. R-02910.

²Graduate Assistant, Professor, and Biologist, respectively. Center for Aquatic Plants and the Agronomy Department, Institute of Food and Agricultural Sciences, University of Florida, Gainesville, FL 32611.

of tank B. At the same time 500 g of hydrilla was started floating in each of the three sections in a third tank (tank C). During the first week of October, the hydrilla in tank A, into which hydrilla had been placed in August (2 months prior), and that in tank C, into which hydrilla had been placed in September (1 month prior), were harvested, the number of turions determined, and new hydrilla started. During the first week of November, hydrilla in tank B (2 months) and in either of tank A or C (1 month) were harvested, processed, and restarted with new floating hydrilla. Each month thereafter the hydrilla from two of the tanks was harvested, processed, and restarted. One of the tanks contained hydrilla that had been floating for 2 months and the other tank contained hydrilla that had been floating for 1 month. The pseudoreplication (tanks divided into thirds) was used in order to minimize the amount of hydrilla which had to be processed both initially (to be discarded) and at harvest for the collection of turions, yet maintain a plant density (1.0 kg fresh wt/m²) typical of a hydrilla population. At harvest each pseudoreplicate was weighed and the number of turions was counted. Beginning in April 1992, hydrilla from Wacissa River, Florida, was used to continue this experiment as Jumper Creek was inaccessible due to low water.

Floating/Rooted Production. Twenty apical sections were allowed to float in each of five 24-L buckets submerged in a tank and covered with 20 cm of water. The buckets had cylinders of plastic screen placed in them to contain the hydrilla. Another 20 apical sections were planted in washed builders sand (no nutrients added) in each of another five 24-L buckets with screens extending above the water surface to contain the hydrilla. In addition to containing the hydrilla, the purpose of the buckets and the screens was to collect any turions that would fall from the free floating or rooted hydrilla.

Photoperiod. Six 100-g replicates of hydrilla apical sections were floated in 24-L buckets and kept outdoors under ambient light conditions of a gradually lengthening day (from approximately 11 hr in March to 13 hr in May). Another six 100-g replicates of hydrilla were floated in 24-L buckets outdoors using 2 floodlamps and a timer to provide a 16-hr photoperiod.

Density. Four weights (100 g, 250 g, 500 g and 1000 g) of hydrilla apical stem sections were placed in 24-L buckets with cylinders of plastic screen in each and submerged in tanks of water. The calculated densities of the hydrilla in the buckets were 2.5, 6.25, 12.5 and 25.0 kg fresh weight/m³. Two tanks were used with three replicates of each density per tank. In addition to the ANOVA, regression analysis was used to determine the relationship between the square root of the number of turions/kg and the number of kg/m³ of hydrilla.

Herbicides. The 2-month herbicide study was performed twice, once beginning in December 1991 and ending in Feb-

ruary 1992 and again beginning in March 1992 and ending in May 1992. A total of seven tanks were used in this experiment to determine the effect of bensulfuron methyl (methyl 2-[[[(4,6 dimethoxy pyrimidin-2-yl)amino]-carbonyl]-amino]sulfonyl]methyl]benzoate) and fluridone (1-methyl-3-phenyl-5-[3-(trifluoromethyl)phenyl]-4(1H)-pyridinone) on turion production. Bensulfuron methyl was chosen for this experiment because previous studies showed a reduction in tuber formation in hydrilla (Haller *et al.* 1992). Fluridone has been successfully used to control hydrilla in many locations but its effects on hydrilla turion production are unknown. Each tank was divided into three sections (pseudoreplicates) and three rates of herbicide (one per tank) were used: 2.5, 5 and 10 µg a.i./L with one tank as the control. Pseudoreplication in this experiment was used for the reasons previously stated for the production experiment as well as to minimize measurement error in the herbicide application.

Five hundred grams of hydrilla apical stem sections were placed in each of the divided areas of the tanks immediately after applying and thoroughly mixing the herbicides with the water.

An orthogonal polynomial contrast was used to compare the control to all rates for each herbicide after the ANOVA was done.

RESULTS AND DISCUSSION

Production. Turion production (Table 1) in the hydrilla held for 1 month was highest in October and November, declined from December to February, and increased again in March. Turion production in the hydrilla held for 2 months showed the same general trend with a significant increase in March-April followed by a large decline in April-May. This bimodal activity is very similar to that reported for tuber production during cold winters (Van *et al.* 1978). The decline in turion production through the winter months coincides with the decline in the average temperature and photoperiod which occurs naturally with the change of seasons. Turions were not produced in significant numbers in the fall until October when daylength was 12 hr or less. Although daylengths were less than 12 hr in December through February, few turions were formed, most likely because of low temperatures ($\leq 15^{\circ}\text{C}$). A 14°C temperature was previously noted as the minimum temperature at which this tropical species becomes physiologically active (Haller *et al.* 1976). In the spring, turions formed under daylengths greater than 12 hr in March and April. Plants at this time of the year are exposed to gradually lengthening days. Photoperiods ≤ 12 hr probably induce turion formation and turion production continues for a period of time beyond the spring equinox (March 21). Relatively few turions were produced by plants floated April 1; consequently, the natural induction

TABLE 1. TURION PRODUCTION BY HYDRILLA APICAL STEM SECTIONS (turions/kg freshwt/month) FLOATED FOR 1-MONTH AND 2-MONTH DURATIONS WITH AVERAGE DAYLENGTH AND TEMPERATURE FOR THE PERIOD.

1-month duration					2-month duration				
Month of growth	No. turions		Daylength (hr)	Temp (C)	Month(s) of growth	No. turions		Daylength (hr)	Temp (C)
	Mean	SD				Mean	SD		
Aug ¹	0	0	13.3	27	Aug-Sep ¹	2	0.3	12.9	26
Sep	0.3	0.6	12.4	26	Sep-Oct	179	48	12.0	23
Oct	179	16	11.5	21	Oct-Nov	221	39	11.2	18
Nov	141	87	10.9	15	Nov-Dec	33	7	10.7	15
Dec	3	3	10.5	15	Dec-Jan	18	10	10.8	13
Jan	32	6	10.9	11	Jan-Feb	4	2	11.3	13
Feb	32	6	11.6	15	Feb-Mar	83	40	12.0	16
Mar	157	66	12.5	16	Mar-Apr	400	100	12.9	18
Apr	1	1	13.4	19	Apr-May	16	15	13.7	20
May	11	7	14.1	22	May-Jun	7	4	14.2	24
Jun	13	3	14.4	26	Jun-Jul	4	2	14.2	27
Jul	0.7	1	14.1	27	Jul-Aug	0.4	0.6	13.7	27
Aug	0.7	1	13.3	26	Aug-Sep	2	0.7	12.9	26
Sep	0	0	12.4	26	Sep-Oct	73	76	12.0	23
Oct	861	280	11.5	20					

¹Hydrilla apical stem sections were floatede the first week of the month indicated and harvested the first week of the following month, or second month as indicated. Each value is the mean of three replicates followed by the standard deviation (SD).

that occurs in March does not continue for a long period of time. These data are very similar to tuber formation data reported previously (Haller *et al.* 1976). These data also suggest that there is an interaction between temperature and photoperiod on turion production which has been reported on tuber formation by hydrilla (McFarland and Barko 1990).

Floating/Rooted. Turions (± 1 SD) were found to be significantly more likely to be produced on floating plants (75.6 ± 37.4 turions/kg) than on rooted plants (28.6 ± 7.8 turions/kg). Haller *et al.* (1976) postulated that turions were produced more often on floating plants and Anderson (1985) showed that tubers were formed more readily on rooted plants than were turions. This is also consistent with speculation that turions are produced by plants which have broken away from a stand, an adaptation allowing hydrilla to become established in new areas (Haller *et al.* 1976, Thullen 1990). Tubers (± 1 SD) were produced (33 ± 34 tubers/kg) on some of the rooted plants in this study, but none were produced on the floating plants.

Photoperiod. Significantly greater ($p < 0.0007$) numbers of turions (± 1 SD) were produced by hydrilla which was maintained in an ambient (11.8 to 12.9 hr) photoperiod (512 ± 277 turions/kg) compared to the number produced under long day (16 hr) photoperiods (63 ± 63 turions/kg). Production of the 63 turions/kg fresh wt in the 16-hr photoperiod likely results from the pre-induction of the plants that were collected during March with short day ambient conditions and

subsequently placed in the artificial long day conditions. Van der Zweerde (1982) found that short days favored turion production under laboratory conditions in accordance with Haller *et al.* (1976) and Van *et al.* (1978) and also suggested that light intensity may increase turion production. Mitra (1955) also postulated that high light intensity promotes the formation of turions, which would seem reasonable, as detached floating stems would receive high light intensities at the water surface.

Density. Hydrilla density had an inverse linear relationship with turion production (Figure 1). Regression of all six replicates of turion production against density indicated that 70% of the variation in the production of turions can be explained by the variation in the density of hydrilla (Figure 1). As density increased it is likely that self-shading reduced light intensity to the plants that were closer to the bottom of the buckets, thereby reducing turion production.

Herbicide. Both bensulfuron methyl and fluridone were shown to suppress turion production in both studies conducted in December to February and in March to May. At harvest in May, the average numbers of turions in the control were much higher than in February. This is consistent with the findings of the production experiment where turion production is low from November and December to February and increases in March and April. Figure 2 shows the results of the March to May study. When the orthogonal polynomial contrast was used to compare the control against all rates, each rate was

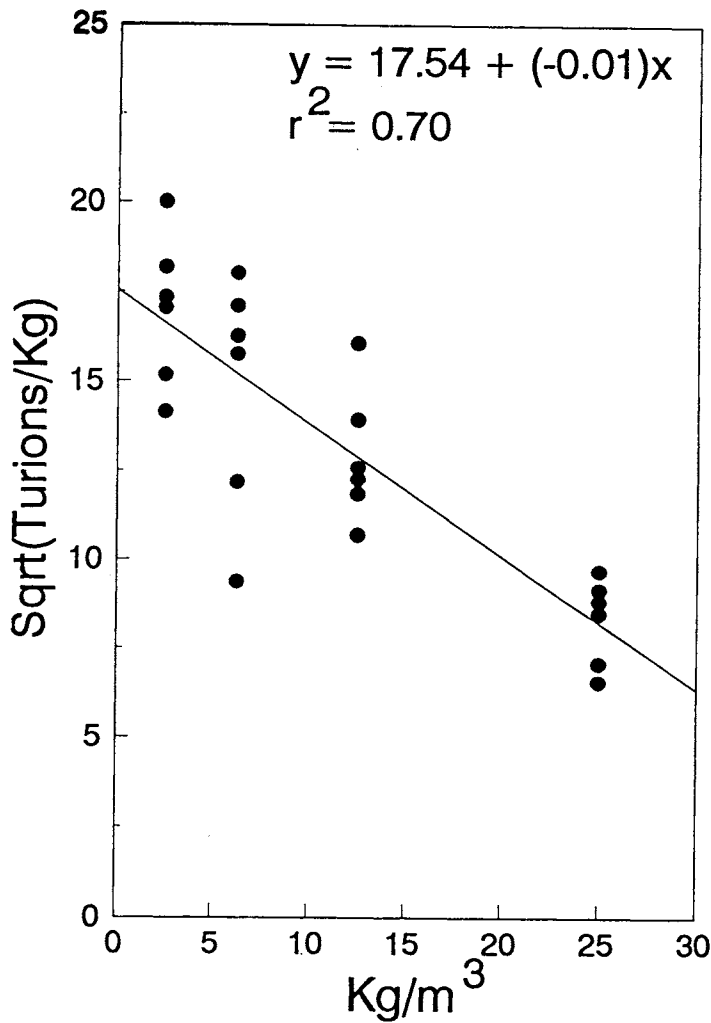


Figure 1. The effects of hydrilla density (kg/m^3) on turion production. Data for turions are expressed as the square root of the numbers produced per kilogram fresh weight.

significantly different from the control indicating that turion production was reduced across all rates. There were no significant differences in turion production between the rates. There was a significant decrease in turions produced across all rates of fluridone when compared to the control, but fluridone does not seem as effective as bensulfuron methyl as a turion inhibitor at the lower rates. The contrast showed that the concentration of fluridone has a definite linear effect. Had these plants not been pre-induced by short day conditions in the field, it is likely that turion production would have been much less, particularly by the hydrilla treated with bensulfuron methyl.

These studies have documented or verified previous hypotheses or experimental results that hydrilla reproduction by turions occurs primarily under short day conditions (pho-

toperiod ≤ 12 hr, September 21 through March 21) and is much more likely to occur on floating plants than on rooted plants. Turion production is greater when temperatures are 15C or higher and decreases as plant density increases. Bensulfuron methyl and fluridone both reduce turion production at application rates of 2.5, 5 and 10 $\mu\text{g/L}$. Further research is needed to determine the length of pre-induction period required to initiate turion production and to examine the possible interaction between temperature and photoperiod. The importance of light intensity also needs to be determined.

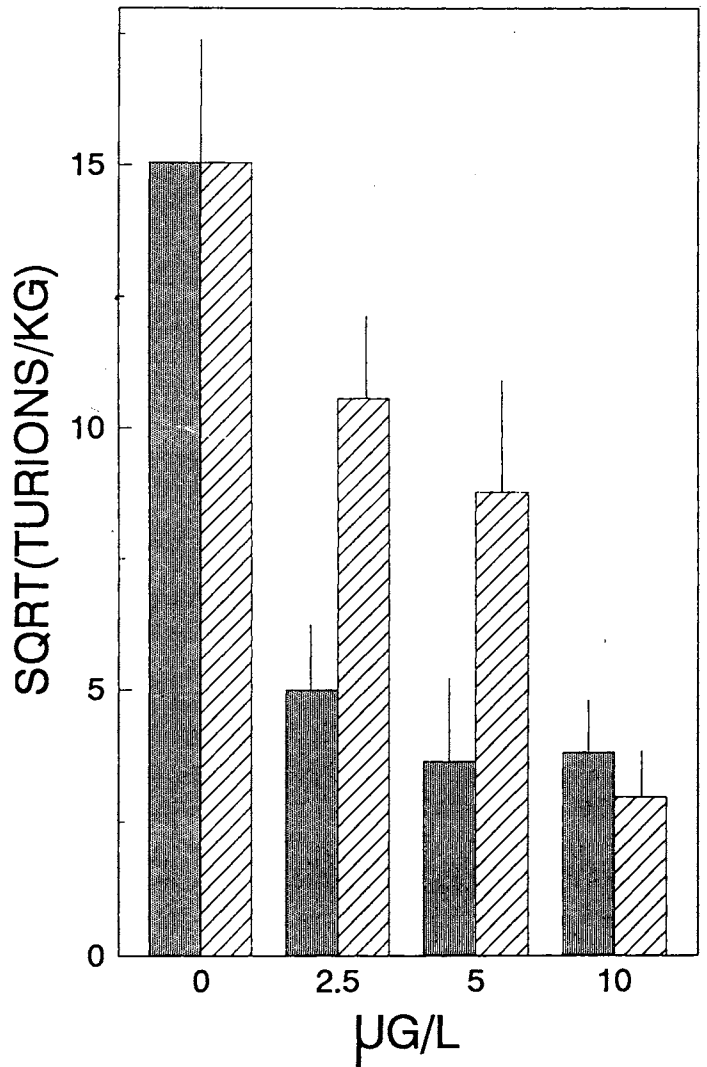


Figure 2. The effect of bensulfuron methyl (solid bar) and fluridone (hatched bar) on turion production by hydrilla. Data, from the March-May study, are expressed as the square root of the numbers of turions produced per kilogram fresh weight of hydrilla. Extended lines are one standard deviation.

ACKNOWLEDGMENTS

Partial funding for this project was provided by the U.S. Department of Agriculture and University of Florida Institute of Food and Agricultural Sciences Center for Aquatic Plants Cooperative Agreement No. ARS 58-43YK-9-0001.

The authors would like to thank Drs. Marija Arsenovic, Alison Fox and Paul Thayer for their kind assistance with the technical aspect of this study. We also thank Ms. Patty Mikell for her help on the manuscript.

LITERATURE CITED

- Anderson, L. W. J. 1985. The Potomac River hydrilla project. Preliminary research on monoecious hydrilla. Proc. 19th Annual Meeting, Aquatic Plant Control Research Program. U.S. Army Engineer Waterways Experiment Station, Misc. Paper A-85-4, pp 185-189.
- Haller, W. T., A. M. Fox and C. A. Hanlon. 1992. Inhibition of hydrilla tuber formation by bensulfuron methyl. *J. Aquat. Plant Manage.* 30:48-49.
- Haller, W. T., J. L. Miller and L. A. Garrard. 1976. Seasonal production and germination of hydrilla vegetative propagules. *J. Aquat. Plant Manage.* 14:26-29.
- Lakashmanan, C. 1951. A note on the occurrence of turions in *Hydrilla verticillata* Presl. *J. Bomb. Nat. Hist. Soc.*, 49(4):802-804.
- McFarland, D. G. and J. W. Barko. 1990. Temperature and daylength effects on growth and tuber formation in hydrilla. *J. Aquat. Plant Manage.* 28:15-19.
- Mitra, E. 1955. Contributions to our knowledge of Indian freshwater plants. I. On some aspects of the structure and life history of *Hydrilla verticillata* Presl. with notes on its autecology. *J. Asiatic Soc.* 2 (1):1-16.
- Mitra, E. 1964. Contributions to our knowledge of Indian freshwater plants. IV. On some aspects of the morphological and anatomical studies of turions of *Hydrilla verticillata* (L.F.) Royle. *J. Asiatic Soc.* 6:17-27.
- Snedecor, G. W. 1940. *Statistical Methods*. The Iowa State College Press. Ames, IA. 422 pp.
- Sutton, D. L. and K. M. Portier. 1985. Density of tubers and turions of hydrilla in South Florida. *J. Aquat. Plant Manage.* 23:64-67.
- Thullen, J. S. 1990. Production of axillary turions by the dioecious *Hydrilla verticillata*. *J. Aquat. Plant Manage.* 28:11-15.
- Van, T. K. and K. K. Steward. 1990. Longevity of monoecious hydrilla propagules. *J. Aquat. Plant Manage.* 28:74-76.
- Van, T. K., W. T. Haller and L. A. Garrard. 1978. The effect of daylength and temperature on hydrilla growth and tuber production. *J. Aquat. Plant Manage.* 16:57-59.
- Van der Zweerde, W. 1982. Some introductory experiments on the influence of day-length, light intensity, and temperature on turion formation and flowering in two strains of *Hydrilla verticillata* (L.F.) Royle. EWRS Symposium on Aquatic Weeds 6:71-75.