Impact of Management on the Sprouting of Dioecious Hydrilla Tubers

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ABSTRACT

The dioecious biotype of hydrilla (*Hydrilla verticillata* (L.f.) Royle) was established twice in mesocosm tanks (900 L) during September 1996 and 1997 in Gainesville, Florida. Hydrilla shoots were planted in 10-cm diameter PVC cores with a hydrosoil depth of 30 cm to allow for biomass establishment and tuber formation during the fall, winter, and spring. In late May 1997 and 1998, the dense hydrilla canopies were controlled by using the aquatic herbicide endothall (7-oxabicyclo [2.2.1]heptane-2,3-dicarboxylic acid), by mechanically clipping the canopy just above the hydrosoil, or by using endothall to remove biomass followed by replacement with healthy non-rooted hydrilla shoots to simulate the presence of a plant canopy. The sprouting of quiescent *in situ* tubers was then recorded at 4, 8, 12, and 20 weeks following treatment. Light availability at the sediment surface and sediment temperatures increased following canopy removal, whereas these values were similar for the control and artificial canopy treatments. Total tuber sprouting remained below 20%, and was not impacted over the 20-week study period by any of the treatments when compared to the controls. Results of these mesocosm trials suggest that chemical or mechanical management efforts have no discernible impact on the short-term sprouting of hydrilla tubers *in situ*.

Key words: Hydrilla verticillata, subterranean turion, aquatic propagules, aquatic plant management, aquatic herbicides.

INTRODUCTION

The influence of management techniques on sprouting of quiescent subterranean turions (hereafter called tubers) of hydrilla has received limited research attention, yet the dynamics of sprouting and subsequent plant establishment are especially relevant when considering hydrilla management strategies (Netherland 1999). Most research on hydrilla tuber sprouting has been conducted on propagules that have been removed from the sediment, thereby creating environmental conditions (described below) that favor a rapid sprouting response. Due to the significant stimulation of tuber sprouting imposed by lake drawdown and subsequent reflooding (Miller 1975, Haller and Shireman 1983) secondary control measures may be necessary to prevent rapid re-infestation. While multiple drawdowns have been proposed to disrupt tuber formation and provide long-term hydrilla control, rapid recovery has generally been noted once the site is reflooded (Haller and Shireman 1983, Doyle and Smart 2001).

The ability of dioecious hydrilla to maintain both a significant and viable population of quiescent tubers over a period of several years remains somewhat unique among submersed aquatic plant species. Although Sastruotomo (1982) suggests that aquatic propagules of several aquatic plants can remain dormant, his classification system of short and long-term dormancy is not quantitative. Bartley and Spence (1987) surveyed the literature and concluded that propagules of aquatic plants apparently do not display true dormancy and that a wide variety of environmental factors are responsible for release from quiescence. Due to the beneficial nature of many native submersed species, a paucity of data exists concerning short and long-term propagule dynamics following management techniques.

Some studies have suggested that sprouting of tubers is stimulated following various chemical and mechanical methods to control hydrilla (Mitra 1955, Van and Haller 1979, Joyce et al. 1992). Increased light penetration through the water column, changes in sediment temperature, and changes in the gaseous constituents of sediments have all been suggested as possible triggers to stimulate a subsequent sprouting response. While tubers are reported to be exposed to different and changing environmental gradients, e.g., temperature, light, oxygen, and CO_2 levels (Titus and Hoover 1991, Spencer and Ksander 1997), the role of aquatic plant management in changing these gradients and stimulating *in situ* tuber sprouting is unknown (Netherland 1999). Removal of tubers from the sediment, and subsequent sprouting rates that exceed 90% within days, does suggest that management activities could change an environmental gradient that would promote *in situ* sprouting (Netherland 1997).

In non-managed systems, tuber sprouting was reported to be random and non-seasonal following several years of field sampling in four south Florida lakes (Sutton and Portier 1985). Studies by Sutton (1996) and Fox and Haller (personal communication) suggest that intense chemical management designed to prevent tuber formation for 3 to 4 years can substantially deplete the tuber population. This would infer that the *in situ* tubers are sprouting and the plants were subsequently controlled, or the propagules may be subject to rotting. Sample methods for these studies did not allow determination of sprouting rates and plant survival following treatment. Data are lacking both in terms of the timing and magnitude of tuber sprouting following application of management techniques, as well as hydrilla tuber sprouting rates in comparison to untreated systems.

Mesocosm studies were conducted in order to determine the short-term impact of various management techniques on

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the sprouting of dioecious hydrilla tubers. The objective of these studies was to quantify the short-term sprouting response of quiescent *in situ* tubers following application of various management techniques that result in the rapid loss of plant biomass.

MATERIALS AND METHODS

An outdoor mesocosm system containing concrete vaults (900 L volume and 219 cm long × 76 cm wide × 64 cm deep) located in Gainesville, Florida, was used to conduct studies during 1996 through 1998. Polyvinyl chloride (PVC) cores with a 10-cm diameter and 20-cm height were filled with commercial potting soil (Vitahume) and amended with 5 g of Osmocote (20-5-5) per kg of soil. Each core was covered with a 2 to 4-cm layer of builders sand to minimize sediment suspension during initial flooding. Five shoot apices of hydrilla were planted in each unit. A total of 24 cores were placed in the concrete vaults in early September of 1996 (first study) and 1997 (second study). Water levels were maintained by adding water to the 64-cm depth on a weekly basis and tubers were allowed to form from late September through May (Van et al. 1978). The source water had a pH of 7.8, alkalinity of 1.4 meq L^{-1}, and conductivity of 350 $\mu S\ cm^{-1}$.

A Campbell CR10X Datalogger (Campbell Data Systems, Logan Utah) was programmed to record temperature at 4-hour intervals. Thermocouple probes were placed 10 cm below the water surface and at a depth of 0, 4, and 10 cm in the sediment for each treatment. In addition, redox probes were deployed at sediment depths of 0, 2, 8, and 12 cm in PVC cores of each treatment vault. Redox potential was recorded by attaching a hand-held pH/mV meter to the marked probes on a daily basis for 21 days post-treatment and weekly thereafter for the remainder of the study. Methods for redox determination were similar to those described by Faulkner et al. (1989). Light measurements at the water and sediment surface were recorded with a LiCor quantum scalar irradiance meter weekly following the application of management techniques.

Hydrilla was established in 18 vaults during the first week of September 1996. On May 26th, 1997, a total of 48 PVC cores in two of the vaults were harvested to quantify pretreatment biomass, as well as the total number of quiescent and sprouting tubers that had formed during the previous fall, winter, and spring.

Treatments applied on May 27, 1997, included the following: 1) untreated controls, 2) potassium salt of endothall applied at 4.0 mg acid equivalent (ae) L^{-1}, 3) potassium salt of endothall at 4.0 mg ae L^{-1} followed by placement of detached hydrilla shoots at the water surface to simulate the untreated control plant canopy (see description below), and 4) mechanical clipping of hydrilla to < 15 cm above the sediment surface every other week for the duration of the study. Clipping resulted in the removal of the canopy and greater than 90 percent of the shoot biomass. Each treatment was replicated four times in a completely randomized design.

The study was repeated in 1997 and 1998 using the same methods. Hydrilla was established during the first week of September in 1997 and the pretreatment harvest of two vaults was conducted on May 28, 1998. Treatments were applied on June 1, 1998.

The objective of the static endothall applications was to rapidly remove all of the surface vegetation. The endothall treatment followed by the use of the detached artificial canopy was conducted to control the established rooted hydrilla (control of both shoot and root mass), yet simulate canopy conditions and maintain sediment temperatures and light levels that occur under surfaced vegetative mats. This treatment was included to test the hypothesis that changes in light penetration or sediment temperature stimulate tuber sprouting. The vegetative canopy of hydrilla was constructed with a fine mesh fabric (2 mm) secured in the vaults 15 cm below the water surface and covered with detached healthy hydrilla stems. Hydrilla stems fared quite well on the mesh barriers and were only replaced infrequently as the detached plants formed extensive aqueous rooting systems through the mesh and remained viable.

The objective of the mechanical clipping treatments was to rapidly remove the surface canopy and yet maintain a greatly reduced but viable hydrilla shoot and root system. This treatment was included to test the hypothesis that increased light penetration and sediment temperature influenced sprouting of quiescent tubers. Hydrilla was clipped back every 2 weeks to maintain plants between mid-depth and the sediment surface.

Following treatment, six cores were harvested from each vault at 4, 8, 12, and 20 weeks, and contents washed through a fine mesh screen. Total numbers of quiescent, sprouting, and rotting tubers were quantified. A tuber was denoted as sprouting if there was any indication of shoot elongation. In addition, shoot and root biomass (dry weight) were also determined. Following removal from *in situ* conditions, non-sprouted tubers were placed in petri dishes for a 10-day period and the number sprouting was quantified.

Data were analyzed via analysis of variance (ANOVA). No differences were noted for tuber sprouting data between study years, therefore, tuber data were pooled for analysis. Data within a sample date were subjected to Duncan's Multiple Range Test (0.05) to determine if differences between treatments were significant. Hydrilla shoot biomass data were subjected to ANOVA and means were separated via Duncan's Multiple Range Test. Environmental data from the treatment tanks were compared to untreated control tanks through the use of a Dunnett's Test (0.05).

RESULTS AND DISCUSSION

Initial hydrilla growth was vigorous during both studies, and formation of a solid surface canopy was noted by early October. Pretreatment data collected for both study years showed that biomass and tuber production were similar, and greater than 94% of all tubers remained quiescent prior to the treatments (Table 1). At all sampling times (4, 8, 12, and 20 weeks after treatments (WAT)), removing the tubers from the sediment and placing them in petri dishes stimulated >90% sprouting within 10 days. This rapid sprouting response following removal from the sediment confirmed that the vast majority of the propagules were viable and under an environmentally imposed quiescence. The increasing water temperatures experienced through the spring season did not significantly stimulate sprouting, and this lack of seasonal re-

TABLE 1. PRETREATMENT SHOOT BIOMASS AND TUBER PRODUCTION OF HYDRILLA THAT HAD BEEN PLANTED DURING EARLY SEPTEMBER OF 1996 AND 1997. VALUES ARE PRESENTED AS THE MEAN ±1 SD. THE NUMBER OF QUIESCENT AND SPROUTING TUBERS WAS QUANTIFIED.

Harvest Date	Shoot biomass[1] (g dry wt/core)	Tubers (number)	Quiescent (%)	Sprouting (%)
May 27, 1997	41 ± 9	57 ± 9	97 ± 1.3	1.1 ± 0.9
June 1, 1998	47 ± 12	49 ± 8	95 ± 1.2	2.1 ± 1.0

sponse is in contrast to reports for sprouting dynamics of monoecious hydrilla tubers in more temperate climates (Carter et al. 1987, Rybicki and Carter 2002).

Following the endothall treatments, most of the hydrilla stems were brown, waterlogged, and laying on the bottom sediments within 2 WAT. Due to the high use rates and static exposures, treatments resulted in near complete control of vegetative biomass in the water column. Mechanically clipped plants continued to produce new shoot growth. Untreated plants maintained a dense surface canopy and biomass was generally consistent throughout the 20-week studies (Table 2). Shoot biomass in the endothall-treated, artificial canopy, and mechanically clipped tanks were significantly different from untreated controls throughout the study. By 20 WAT significant differences in hydrilla biomass were noted between the endothall treatments and the endothall treatments that included a canopy (Table 2). This provides evidence that tubers sprouting under a canopy face environmental conditions (namely low light) that are unfavorable for plant establishment.

After the vegetative canopy was removed by herbicide and mechanical clipping treatments, temperature and light intensities measured at the sediment surface increased significantly above untreated controls (Tables 3 and 4). In contrast, the artificial canopy provided similar sediment temperatures and light profiles as the untreated controls. Both canopies greatly reduced light penetration and served to trap the warm water at the surface during the day. This allowed sediment temperatures to remain up to 11 C cooler during the daytime compared to treatments that resulted in canopy removal. Water temperatures in the plant canopy often approached 40 C during the heat of the day, whereas surface water temperatures measured in vaults without a canopy rarely exceeded 30 C. While tuber sprouting occurs across a

TABLE 2. HYDRILLA SHOOT BIOMASS (G DRY WT/CORE) AT HARVEST DATES FOLLOWING VARIOUS HERBICIDE AND MECHANICAL CONTROL TREATMENTS. DATA FROM HARVESTS CONDUCTED IN 1997 AND 1998 WERE COMBINED.

Treatment	Weeks after treatment			
	4	8	12	20
Untreated	55.1 a	57.2 a	63.4 a	67.1 a
Endothall	1.1 c	0.3 c	4.8 c	17.1b
Mechanical Clip	16.3 b	13.0 b	11.5 b	21.4 b
Endothall + Art. Canopy	2.4 c	1.6 c	0.9 d	1.9 c

[1]Letters represent significant differences between treatments at each sample period according to Duncan's multiple range test (n = 4).

TABLE 3. DAYTIME TEMPERATURE PROFILES (C) RECORDED IN THE SEDIMENT (4-CM DEPTH) AT 1500 HRS ON DESIGNATED HARVEST DATES FOLLOWING TREATMENT OF MESOCOSM TANKS IN 1997 AND 1998.

Sample	Untreated	Endothall	Endothall + Art. canopy	Mechanical clip
2 WAT				
1997	20	30*	20	28*
1998	21	28*	22	28*
4 WAT				
1997	22	29*	21	30*
1998	23	31*	22	29*
8 WAT				
1997	21	31*	22	31*
1998	20	30*	22	30*
12 WAT				
1997	21	29*	21	29*
1998	22	31*	22	30*
20 WAT				
1997	18	21	19	22
1998	21	24	22	23

*Treatments were significantly different from controls according to a Dunnet's test$_{0.05}$ (n = 3).

broad range of water temperatures between 15 and 35 C (Haller et al. 1976, Steward and Van 1987), these studies were conducted to determine the impact of a rapid change of sediment temperature on tuber sprouting.

Measurement of redox at the sediment-water interface following removal of the vegetative canopy resulted in a wide variation in readings. Nonetheless, sediment redox readings at 2, 8, and 12-cm depths suggested that sediments remained highly reduced through 12 WAT regardless of the management technique (Table 5).

ANOVA conducted for each harvest date showed there were no differences in sprouting percentages between treatments (Figure 1). While the total percentage of tuber sprouting increased over time, it is important to note that these values were cumulative and did not represent an increased rate of sprouting. Once a tuber has sprouted, the shoot can remain physically attached to the sprouted tuber for two or more months (Netherland 1999). For example, the majority of tubers that had sprouted at 4 and 8 weeks would have been included in the 12 and 20-week data. The percentage of tubers found rotting was consistent and less than 3% of the population during the course of the study (Figure 1). The mesocosm sprouting data agreed with a subsequent field study that showed various management techniques had little impact on subsequent tuber sprouting over a 36-month period (Netherland 1999).

These observations also support Sutton and Portier (1985) findings that tuber sprouting in Florida is likely nonseasonal and random for unmanaged populations of hydrilla. For the dioecious biotype of hydrilla in Florida, significant seasonal tuber sprouting or sprouting based on degree-days

TABLE 4. LIGHT READINGS (μMOL M^{-2} SEC^{-1}) RECORDED AT THE SEDIMENT SURFACE AT APPROXIMATELY 1500 HRS ON DESIGNATED HARVEST DATES IN 1997 AND 1998 FOLLOWING TREATMENT OF HYDRILLA IN MESOCOSM TANKS.

Sample (Time)	Untreated	Endothall	Endothall + Art. Canopy	Mechanical Clipping	Air
Pretreat (1997)	19	280*[1]	31	185*	1904
2 WAT	15	309*	29	244*	2012
4 WAT	21	147*	16	221*	1786
8 WAT	26	283*	11*	173*	2046
12 WAT	29	176*	17	216*	1794
20 WAT	18	225*	21*	227*	1766
Pretreat (1998)	19	320*	11	294*	2033
2 WAT	11	211*	16	343*	2242
4 WAT	8	247*	15	191*	2147
8 WAT	15	193*	21	281*	2021
12 WAT	7	296*	14	303*	2006
20 WAT	22	214*	22	277*	1821

[1]Variation in endothall-treated and mechanically-clipped tanks is indicative of water quality changes due to phytoplankton blooms.
*Treatments were significantly different from untreated controls according to Dunnett's test$_{0.05}$ (n = 6).

is not supported by the data as has been noted for monoecious hydrilla (Spencer and Ksander 2001). The near continuous formation of tubers through the fall, winter, and spring, and the lack of an appreciable winter and spring chilling period in Florida, may contribute to the limited sprouting response compared to monoecious hydrilla. It remains

unknown if the sprouting dynamics of dioecious hydrilla tubers would be expected to increase with exposure to a more substantial chilling period in more northern climates.

TABLE 5. SEDIMENT REDOX POTENTIAL (MV) IN CORES CONTAINING POTTING SOIL (0, 2, 8, AND 12-CM DEPTHS) FOLLOWING VARIOUS HYDRILLA MANAGEMENT TECHNIQUES IN 1998.

Sample time	Untreated	Endothall	Endothall + Art. canopy	Mechanical clip
2 WAT				
sed/water[1]	-300	-265	-251	-211*
2 cm	-322	-310	-266	-269
8 cm	-281	-277	-298	-298
12 cm	-277	-311	-259	-245
4 WAT				
sed/water	-330	15*	-87*	100*
2 cm	-342	-290	-266*	-319
8 cm	-311	-295	-263	-299
12 cm	-297	-311	-309	-275
8 WAT				
sed/water	-304	109*	-127*	150*
2 cm	-312	-267	-331*	-234*
8 cm	-322	-288	-312	-309
12 cm	-309	-331	-342*	-325
12 WAT				
sed/water	-284	141*	-77*	144*
2 cm	-282	-299	-301	-298
8 cm	-302	-305	-277	-284
12 cm	-291	-329*	-323	-309

[1]Redox probes were placed just above the sediment surface for these readings.
*Treatments were significantly different from untreated controls at each sample depth and date according to a Dunnett's test$_{0.05}$ (n = 3).
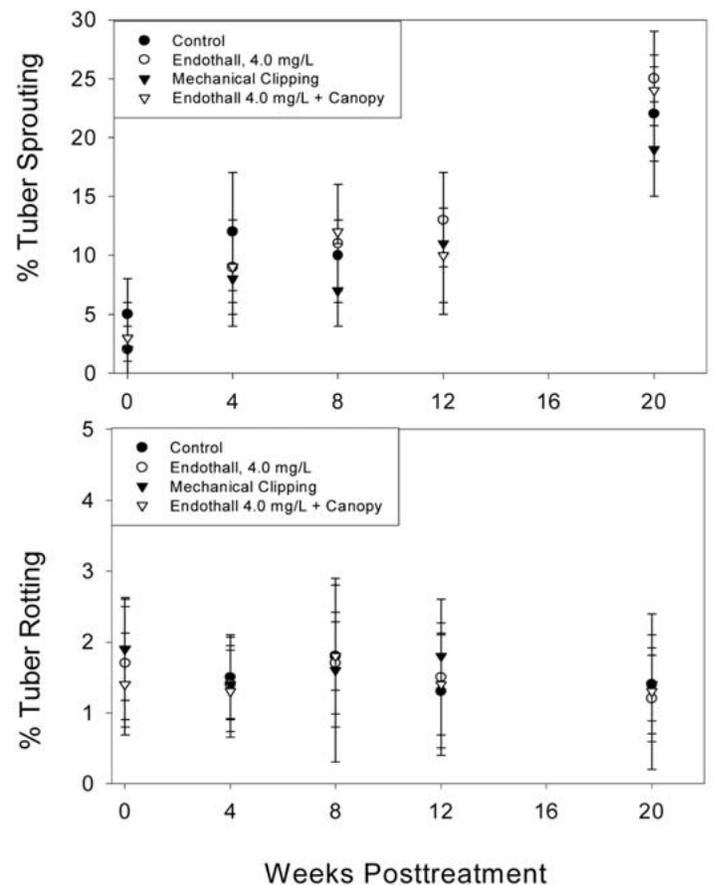

Figure 1. Mesocosm evaluation of the impact of three treatment methods on the sprouting and rotting of hydrilla tubers grown in PVC cores containing sediment. Based on ANOVA, no differences in percent tuber sprouting or rotting were noted in the PVC cores at each sample time following various management techniques. Each data point (+ 1 SD) represents the value of six cores collected from four replicate tanks.

The lack of an immediate response by a large percentage of the tuber population suggests that rapid re-infestation due to mass tuber sprouting is unlikely following management. The current study did not address the role of turions in rapid recovery following field management, but we did confirm that turion production was minimal during these studies as suggested by Miller et al. (1993).

The results provide evidence to contest earlier proposed hypotheses on factors influencing *in situ* hydrilla tuber sprouting. For example, while significant changes in sediment temperature and light penetration were detected, they did not stimulate sprouting. Removal of the hydrilla canopy by either chemical or mechanical means had no impact on sprouting. Likewise, the hypothesis that the presence of a canopy prevents sprouting (e.g. in the untreated control) was also refuted.

In summary, chemical or mechanical management techniques had no short-term impact on the overall *in situ* sprouting rates of dioecious hydrilla tubers. While tubers play a role in the ability of hydrilla to become established following removal of the vegetative biomass, it is unlikely that rapid recovery of hydrilla in a treatment area is due to a high proportion of the tubers sprouting following a management effort. Based on this study and the work of others (Sutton and Portier 1985), the sprouting dynamics of hydrilla tubers suggests that a low but consistent percentage of tubers continue to sprout if undisturbed in sediment over time. This sprouting strategy suggests that other than drawdown, various management techniques will have a limited impact on hydrilla tuber sprouting.

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