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Impacts of Carbohydrate Depletion by Repeated Clipping on the Production of Subterranean Turions by Dioecious Hydrilla

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ABSTRACT

The production of subterranean turions (tubers) by hydrilla (*Hydrilla verticillata* (L.f.) Royle) is understood to be dependent upon the production, translocation, and storage of excess total non-structural carbohydrates (TNC). In a greenhouse experiment, dioecious hydrilla plants were cut to heights of 30 cm or 8 cm or were left un-clipped; clipping occurred either continuously or after 90% of the stems had reached the water surface. Plants were harvested 4 months after clipping was begun and above-ground tissues were analyzed for TNC, along with growth variables of tuber and rhizome production, and the biomass of all removed and remaining tissues. Clipped removal of above-ground tissue reduced the number of tubers produced, with complete inhibition and a significant reduction in TNC concentrations in plants continuously maintained at 8 cm. Clipping frequency had more influence on tuber numbers than did plant height, suggesting that formation of a surface canopy was important for tuber production. However, of the plants allowed to form a canopy, reduced plant height resulted in smaller tubers. There was an exponential correlation between TNC concentrations and tuber numbers, indicating that tuber inhibition may occur below a threshold TNC concentration.

Key words: biomass, aquatic weeds, mechanical control, tubers, *Hydrilla verticillata.*

INTRODUCTION

Two biotypes of hydrilla have been identified in the USA. The dioecious form predominates in Florida and most of the

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southern states, while the monoecious form is becoming more widespread across the northeastern states and has been identified in Washington State (Netherland 1997). Although the monoecious form is capable of producing seeds, vegetative reproduction from stem fragments, regeneration from root crowns, and the production of axillary turions and subterranean turions, is considered to be the principal reproductive mechanism (Harlan et al. 1985). For the dioecious form, which is exclusively female in the USA, regeneration from vegetative propagules is obligatory. Dioecious hydrilla only produces tubers under short-day conditions, specifically October to April (Haller et al. 1976, Van et al. 1978), but in Florida, the monoecious biotype can produce them throughout the year (Sutton et al. 1992, Van 1989).

Management strategies must be repeated, or persist, for a long enough period of time to deplete the tubers in the sediment, to provide long-term control of nuisance populations. The longevity of the tuber-bank for monoecious hydrilla was shown to be at least 4 years (Van and Steward 1990). This management objective can be achieved when hydrilla is continuously removed from the water column prior to producing new tubers. Hydrilla growth and tuber production can be greatly reduced or stopped by repeated use of herbicides or by grass carp (*Ctenopharygodon idella* Val.) (Sutton 1996).

Apart from an understanding of photoperiodic influences on tuber production, there is a paucity of information about environmental and physiological factors that influence tuber formation (Netherland 1997). Because tubers contain 46% starch (Miller et al. 1976) and 60 to 70% total non-structural carbohydrates (TNC) (Madsen and Owens 1998), it may be assumed that their production depends upon the translocation from photosynthetic tissues of TNC that have been produced in excess of respiratory requirements. However, there have been very few studies of TNC in hydrilla (Guha 1965, MacDonald et al. 1995, Madsen and Owens 1998) and no reports specifically relating TNC concentrations in the above-ground tissues to tuber production. Thus, it is not known whether the number and size of tubers produced vary in direct proportion with TNC concentrations, or if there is a threshold TNC concentration below which tubers are not formed.

It is known that the carbohydrate content of subterranean storage organs of plants can be reduced by removal of leaves and stems during the seasonal period of starch accumulation as in the rhizomes of *Zizaniopsis miliacea* (Michx.) Doell. & Asch. (Birch and Cooley 1983). However, there has been no systematic determination of how much and how often photosynthetic tissue of hydrilla needs to be removed in order to inhibit tuber production. The experimental determination of such damage thresholds, to allow the prediction of the inhibition of tuber formation from above-ground tissue TNC concentrations, would have three advantages: (a) experiments would only have to last for as long as it takes for TNC concentrations to be altered rather than for rhizomes and tubers to be initiated and formed; (b) special environmental conditions which induce tuber production such as photoperiod would not be needed; and (c) only above-ground tissue would have to be sampled.

Specific objectives of this study were to quantify the extent of hydrilla leaf and shoot loss that inhibited tuber production, and to relate impacts on tuber production to TNC concentrations in the above-ground tissue.

MATERIALS AND METHODS

Plant material and general growth conditions: In September 1995, dioecious hydrilla was collected from Silver Glenn Springs, Florida. On September 12, four apical stem sections approximately 15 cm long were planted in each container that was 14 cm tall, and had base and top diameters of 10 cm and 12 cm, respectively. There was topsoil (supplied by Vitahume, Haynes City, FL) in each container that was covered with a 1 cm layer of sand for a total sediment depth of 12 cm. The soil and sand weighed approximately 1,500 g per pot and 4 g of Osmocote® N:P:K 14:14:14 slow-release fertilizer (Scotts-Sierra Horticultural Products Co., Marysville, OH) was mixed throughout the top half of the soil. These pots were placed in two 1,000 liter fiberglass tanks that contained water to a depth of 72 cm. Each tank was divided into 8 sections by unsealed plexiglass partitions so that there was a total of 16 sections between the two tanks. Six containers with plants were placed within each of 15 of these sections.

To ensure that these plants were not subject to insect damage, the insecticide temephos [O,O'-(thiodi-4,1-phenylene) O,O,O',O'-tetramethyl phosphorothioate; 5% Skeeter Abate®, American Cyanamid, Wayne, NJ] was added to the tanks every 30 days as pellets at a rate of 0.93 mg l^{-1} active ingredient. Air bubblers were placed in each tank section to facilitate the circulation of carbon dioxide in the water.

Air temperature was maintained at approximately 25C in the greenhouse and fluorescent lights were used to create long-day conditions of 16 hours of light to inhibit tuber formation. The light cycle was changed to short days with 8 hours of light on January 23, 1996.

Clipping treatments: Aspects of clipping height and frequency were varied to provide a range of stresses to the plants. Three replicate sections, randomly assigned within the two tanks, were allotted to each of five clipping treatments:

Long-Surface: Clipped to stem height of 30 cm above the soil surface such that the tops of the plants were 30 cm below the water surface. This treatment was repeated only when at least 22 of the regrowing stems reached the water surface in all replicates, with 22 stems representing 90% of the original number of 24 plants.

Long-Continuous: Clipped to stem height of 30 cm repeated on a continuous basis so that no stems reached the water surface.

Short-Surface: Clipped to stem height of 8 cm above the soil surface such that the tops of the plants were 52 cm below water surface. This treatment was repeated only when 90% of regrowing stems reached the water surface.

Short-Continuous: Clipped to stem height of 8 cm repeated on a continuous basis.

Control: Control treatment with no clipping.

The hydrilla was initially clipped on January 11, 1996. Treatments *Long-Continuous* and *Short-Continuous* were reclipped on: February 1, 13, and 26; March 14, April 3; and May 3. The intervals increased as the plants progressively produced less regrowth. Treatment *Long-Surface* was reclipped on: February 13; March 14; and May 3 and treatment *Short-Surface* was repeated on February 26 and May 3. The biomass of hydrilla removed from each replicate at each clipping was dried to constant weight at 60C.

Harvest: All plants were harvested on May 24, 1996, and the number of tubers and rhizomes in each pot recorded. For each treatment replicate, the dry biomass of above-ground material of stems and leaves, roots, rhizomes, and tubers was pooled from the six pots. These data were not collected per pot because of the difficulties of separating the shoots of unclipped plants. After drying and weighing, the above-ground tissues were ground using a Wylie mill (Thomas Scientific, Miami, FL) with a 0.5 mm mesh filter. Samples were analyzed for TNC using the enzymatic incubation of plant tissues with the Nelson-Samogyi color reaction (Smith 1981, as modified by Christiansen et al. 1988). All TNC data were reported in mg g^{-1} of dry weight.

Statistical analysis: Most variables were compared between treatments with analysis of variance (ANOVA) using SAS Software (Littell et al. 1991). A one-way ANOVA with Dunnett's test was used to see if any of the treatments differed from the control. A two-way ANOVA was conducted to compare the influence of the factors of clipping height (long and short) and clipping frequency (surface and continuous). The control data were excluded from this comparison because it was the types of clipping regimes, not absence of one, that was being analyzed. The numbers of tubers produced were analyzed as functions of the plant biomass removed by clipping and TNC, using linear and exponential regression models, respectively. All reported differences are significant at $P \leq 0.05$.

RESULTS AND DISCUSSION

Compared to the control, hydrilla in both continuously clipped treatments produced fewer tubers, and clipping frequency was the only significant factor for this variable (Tables 1 and 2). The weight per tuber in the *Short-Surface* treatment was less than in the control and long clipped treatments. The factor of height was significant for this variable with short clipped plants having smaller or non-existent tubers. The product of these two variables, total biomass of tubers, was less for each treatment than for the control, but neither factor was significant. The total biomasses of tubers for the *Long-Continuous* and *Short-Surface* treatments were identical because the former treatment had fewer, full-sized tubers while the latter treatment had a larger number of tubers that were less than half the weight of those produced by the control plants (Table 1).

The number and total biomass of rhizomes were lower than the control values for all treatments but only the factor of clipping frequency was significant, with continuously clipped treatments producing less rhizomes. No rhizomes or tubers were produced by any of the plants in the *Short-Continuous* treatment (Table 1).

The sum of biomass of all plant material removed from each treatment replicate (after the initial cut to the appropriate plant height) varied with the factor of clipping frequency, with more material removed from plants growing to the surface than from the continuously clipped plants (Table 2). There was a linear relationship between the biomass of removed material and tuber number (Figure 1).

When all treatments were compared with ANOVA, the control always had greater final standing above-ground biomass at time of harvest and total production of all above- and below-ground biomass including initial cut and clippings than the other treatments. In the absence of any tissue removal, total biomass production of the control plants was about twice that of any of the clipped treatments (Table 1). Height was the only significant factor for these variables, with short-clipped plants having less biomass than the long ones (Table 2).

There was an exponential relationship between the amount of TNC in the above-ground biomass at final harvest and the average number of tubers produced per replicate (Figure 2). The ANOVA indicated that the TNC concentrations in only the *Short-Continuous* treatment was less than in the control plants (Table 1). The frequency of clipping was significant, with lower TNC concentrations in plants that were continuously clipped (Table 2). There was also a significant height by frequency interaction because for the continuously clipped plants, TNC concentrations were higher in the long clipped treatment but for plants allowed to grow to the surface the short clipped treatment resulted in greater TNC concentrations (Table 1).

The complete prevention of rhizome and tuber production under the harshest clipping regime shows that the production of these reproductive organs can be prevented in

TABLE 1. AVERAGE VALUES OF VARIABLES EVALUATED DURING CLIPPING STUDY. AVERAGES OF THREE REPLICATES PER TREATMENT. ALL BIOMASS DATA ARE DRY WEIGHT IN G.

Variable	Control	LongSur[1]	LongCon	ShortSur	ShortCon
Number of tubers	14.3	7.3	3.3*[2]	6.3	0*
Weight per tuber	0.18	0.14	0.14	0.08*	0*
Total biomass of tubers	2.69	0.84*	0.49*	0.49*	0*
Number of rhizomes	19.0	8.7*	2.3*	8.0*	0*
Rhizome biomass[3]	0.57	0.11*	0.02*	0.15*	0*
Biomass removed	0	11.5*	7.6*	13.8*	4.5*
Final above-ground standing biomass	121	19*	21*	6*	6*
Total biomass production	128	62*	62*	52*	48*
TNC (mg g^{-1} dry wt)	558	443	401	481	234*

[1]LongSur = Long height, cut after reaching the Surface; LongCon = Long height, cut continuously; ShorSur = Short height, cut after reaching the Surface; ShortCon = Short height, cut continuously.
[2]* = Value is significantly different from the control in ANOVA with Dunnett's test ($P \leq 0.05$).
[3]Rhizome biomass excluding tubers.

TABLE 2. AVERAGE VALUES OF VARIABLES EVALUATED DURING CLIPPING STUDY PER FACTOR OF CLIPPING FREQUENCY OR HEIGHT. CONTROL DATA OMITTED; AVERAGES OF THREE REPLICATES PER TREATMENT AND TWO LEVELS PER FACTOR. ALL BIOMASS DATA ARE DRY WEIGHT IN G.

	Height			Clipping frequency		
Variable	Long	Short	Significance[1]	Surface	Continuous	Significance
Number of tubers	5.3	3.2	—	6.8	1.7	*
Weight per tuber	0.14	0.04	*	0.11	0.07	—
Total biomass of tubers	0.66	0.25	—	0.67	0.24	—
Number of rhizomes	5.5	4.0	—	8.3	1.2	*
Rhizome biomass[2]	0.06	0.08	—	0.13	0.01	*
Biomass removed	9.5	9.2	—	12.6	6.1	*
Final above-ground standing biomass	20	6	*	12	14	—
Total biomass production	62	50	*	57	55	—
TNC (mg g^{-1} dry wt)	422	357	—	462	317	*

[1]* = Factor is significant in ANOVA ($P \leq 0.05$).
[2]Rhizome biomass excluding tubers.

established dioecious hydrilla plants by the removal of sufficient above-ground biomass. The observation that above-ground tissue concentrations of TNC were only reduced in this *Short-Continuous* treatment, and the appearance of a significant positive correlation between tuber number and TNC concentrations, support the hypothesis that there may be a predictable relationship between tuber inhibition and TNC concentrations. The exponential model of this correlation

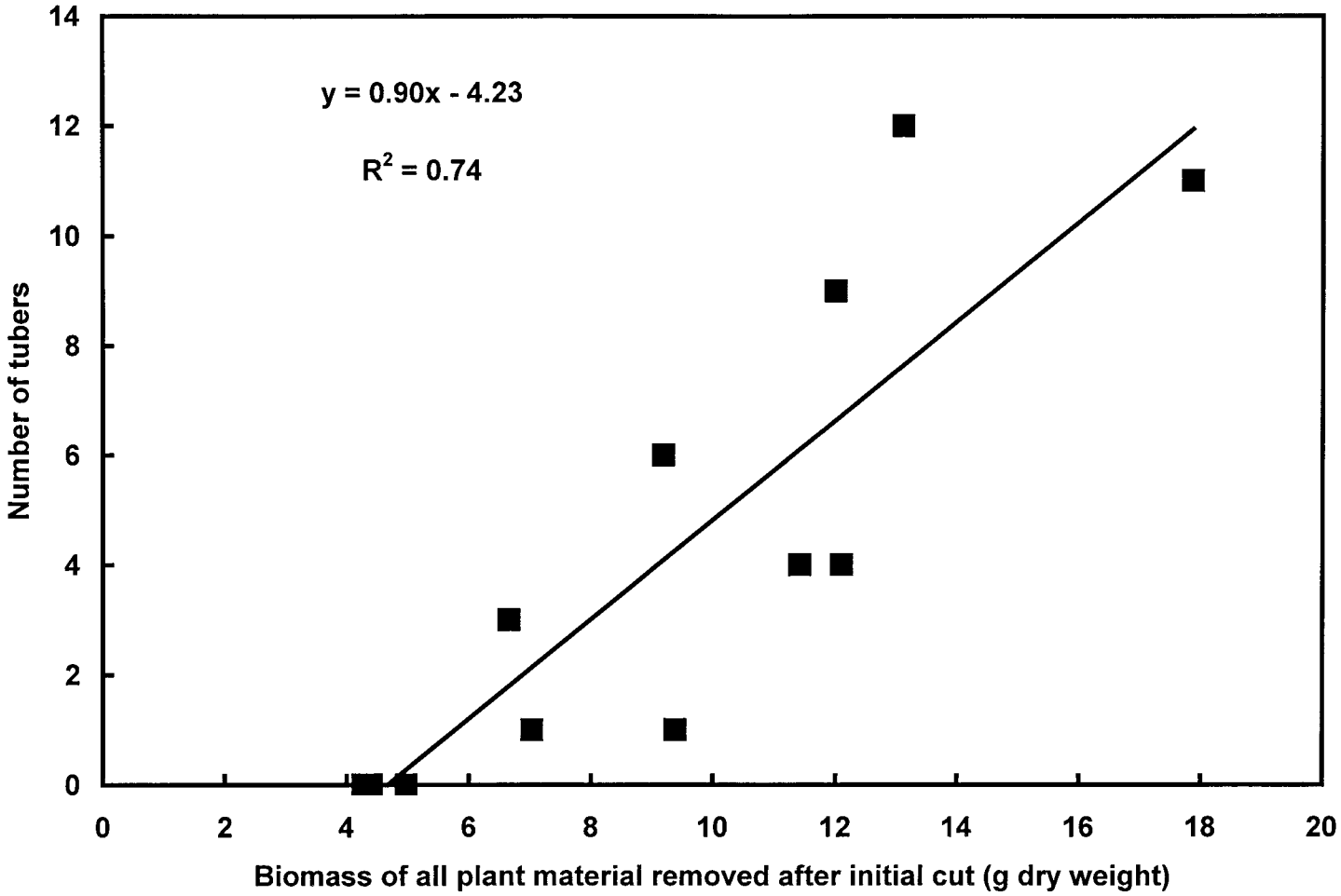

Figure 1. Number of tubers produced per replicate as a function of the biomass of all plant material removed after the initial cut to short or long plants. The control treatment is not included.

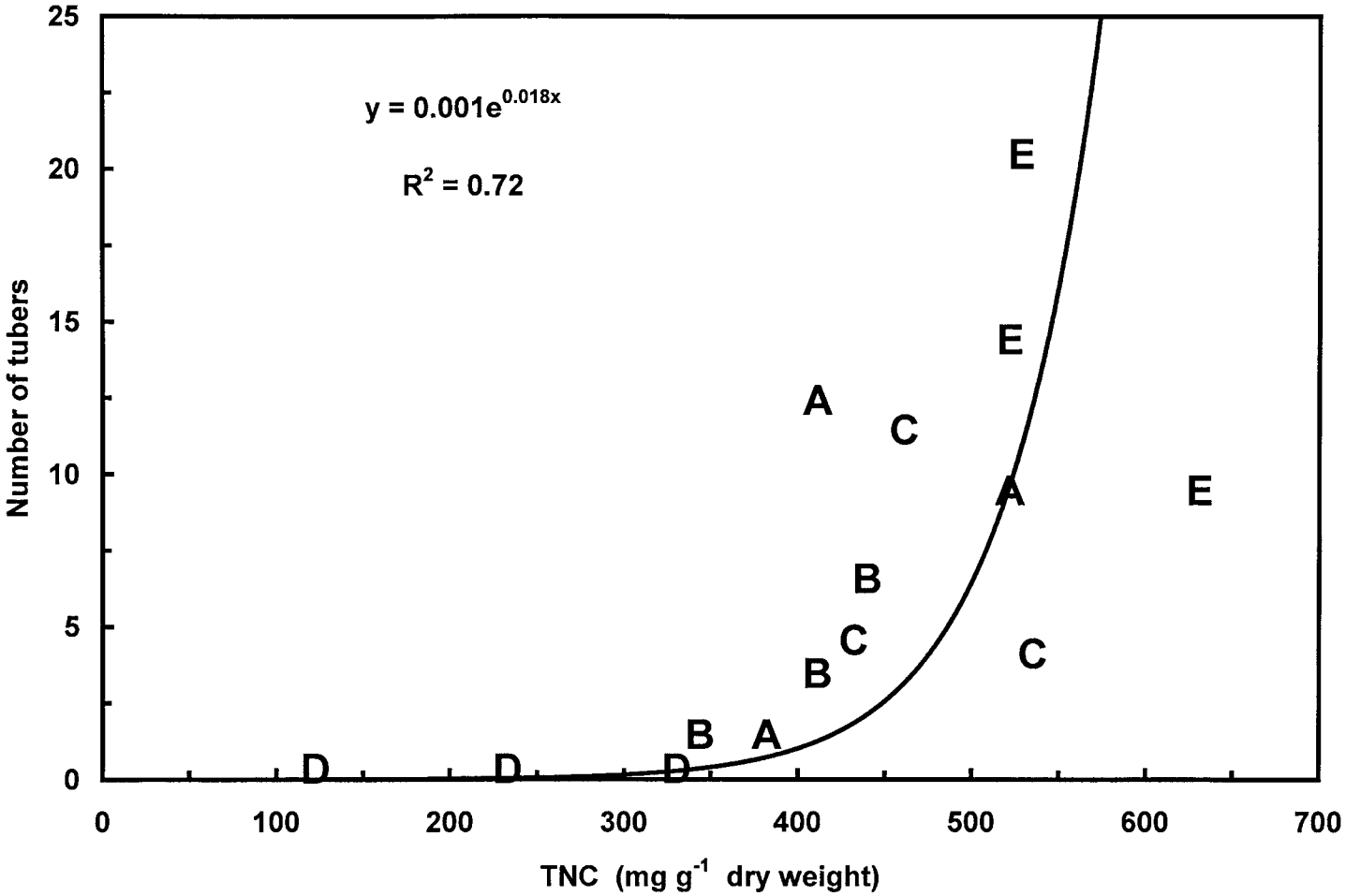

Figure 2. Number of tubers produced per replicate as a function of the total non-structural carbohydrate content of above-ground biomass at final harvest of clipping study. Clipping treatments: A = *Long-Surface*, B = *Long-Continuous*, C = *Short-Surface*, D = *Short-Continuous*, E = *Control*.

(Figure 2) indicates that this relationship may be more of a concentration threshold than of a linear proportionality between TNC concentrations and tuber numbers. In the *Short-Continuous* treatment, the high respiratory demand for TNC for shoot regrowth, combined with a low photosynthetic surface area and reduced exposure to light 52 cm below the water surface, reduced TNC concentrations below such a threshold of between 234 and 401 mg g^{-1}. In all other treatments the increased photosynthetic area and exposure to light, and/or a reduced need for regrowth allowed TNC concentrations to remain above the threshold.

The aspect of the clipping regimes that was most influential on tuber number, rhizome production, and TNC concentration, was not the size of the remaining plant but the frequency of clipping. The amount of plant material removed by clipping showed the same response to the frequency of clips as tuber and rhizome production and TNC concentration, with greater total regrowth (material removed by clipping) correlated with greater tuber production (Figure 1) and TNC concentrations (Table 1). Final above-ground standing biomass and total plant production were more influenced by the height to which the plants were clipped and, hence, these variables did not correlate with tuber numbers in this study.

An explanation for these observations is that the most crucial factor in determining the ability of dioecious hydrilla to produce enough photosynthates to allow the excess to be translocated below-ground to form rhizomes and tubers, is whether the hydrilla stems are able to reach the water surface. Stems of continuously clipped plants never approached the water surface and so were prohibited from forming a canopy. This difference in canopy production is indicated by there only being half as much biomass removed by the clippings from the continuously clipped plants compared to plants that grew to the surface (Table 2).

Changes in light quality with water depth influence the growth pattern of hydrilla, with stem elongation favored in deeper water, and a stimulation to branch near the water surface (Van et al. 1977). Thus, the continuously clipped plants did not branch to produce many new stems, despite the removal of any apical dominance, while stems in the treatments that were allowed to grow longer were able to branch and start forming a canopy as soon as they reached the water surface. The similarity of final above-ground standing biomasses of plants clipped at different frequency regimens but equal heights (e.g., *Long-Surface* and *Long-Continuous* treatments in Table 1), supports the hypothesis that repeated clipping did not result in profuse branching on stems below the clipping heights.

For plants that were unable to reach the water surface, the height of clipping did have some effect on tuber production. Plants in the *Long-Continuous* treatment did produce a few tubers whereas there was a lack of any tuber production in *Short-Continuous* treatments. Conversely, for plant able to form a canopy, the height of clipping did not affect the tuber number, but it significantly affected the size of individual tubers. Thus, canopy production was positively correlated with more rhizomes, greater TNC concentrations, and greater tuber numbers but not with total biomass of tubers.

For the *Short-Surface* treatments, it appears that the initiation of rhizome and tuber production did not differ from the control or *Long-Surface* treatment. As compared to the continuously clipped treatments, this initiation was possibly stimulated by either increases in TNC concentrations or other plant or environmental changes associated with canopy production. In the *Short-Surface* treatments, it appears that once initiated, tuber production did not continue to the stage of completion typical of control plants, resulting in smaller tubers. This production of small tubers was not positively correlated with TNC concentrations at the time of harvest (Table 1). Thus, it is not evident what aspect of additional stress caused by the short- compared to long-clipped treatments resulted in smaller tubers for the *Short-Surface* versus *Long-Surface* treatments. Whether this reduction in tuber size diminished the likelihood of successful propagation was not tested in this study, but there is evidence that small dioecious hydrilla tubers do not persist (Netherland 1999), germinate (Miller et al. 1976), or survive after germination in prolonged darkness (Bowes et al. 1977) as well as large tubers.

High spatial variation in densities of tubers in the field (Haller et al. 1976, Netherland 1997) make it difficult to compare the effects of operational management methods on tuber production directly. The implication that there may be a threshold relationship between TNC concentrations and tuber inhibition presents possibilities of testing management methods, such as harvesting, grass carp, and herbicide treatments, to find ways to reduce TNC concentrations of dioecious hydrilla below this threshold during periods of tuber induction.

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