

## Note

# Sprouting of subterranean turions in response to temperature differs between Virginia and Florida dioecious hydrilla populations

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### ABSTRACT

Hydrilla [*Hydrilla verticillata* (L.f.) Royle] is an invasive aquatic plant that is more commonly documented in southern U.S. states. Recently, a population of dioecious hydrilla was documented in Philpott Reservoir (Bassett, VA) at the northern end of its reported, introduced range. This study compared the sprouting dynamics of subterranean turions of this Virginia population of dioecious hydrilla with those of a Florida population along a water temperature gradient. The Florida population had a wider temperature range for sprouting and stem growth than the Virginia population, although the sprouting of both populations peaked at 29–30 C. Stem elongation was highest at 26 and 30 C for Florida and Virginia populations, respectively.

*Key words:* environmental cue, *Hydrilla verticillata*, plant response, vegetative reproduction.

### INTRODUCTION

Dioecious *Hydrilla verticillata* (L.f.) Royle (hydrilla) is an aquatic macrophyte native to India (Madeira et al. 1997). Hydrilla was introduced to waterways in the United States beginning in the 1950s and has become a widespread invasive aquatic weed species due to multiple competitive strategies. These include several mechanisms for reproduction and long-term dispersal, prolific growth rates, and utilization of unoccupied niches, such as areas in the water column that are void of sunlight (Langeland 1996, Haug et al. 2019). Because of these features, hydrilla can outcompete native aquatic flora, interfere with fauna, modify local ecological processes, and decrease potential for recreational opportunities on many waterbodies where it has been

introduced (Langeland 1996). To date, three recognized clades of hydrilla have been documented in the United States: a monoecious, dioecious, and genetically distinct but phenologically uncharacterized clade (Tipperty et al. 2020).

The production of turions as a mode of vegetative reproduction is a shared feature among all three hydrilla clades in the United States. In particular, the monoecious and dioecious biotypes of hydrilla produce both axillary turions (“turions”) and subterranean turions (sometimes referred to as “tubers”). The study of hydrilla subterranean-turion dynamics has become a major driving factor for hydrilla management because these structures can remain dormant through multiple growing seasons and aid in the long-term persistence of an established population.

Approximately 15 ha of dioecious hydrilla were first identified in Philpott Reservoir in August 2017 following a lake-wide submersed plant survey. Biotype confirmation was completed following methods from Madeira et al. (2004). Philpott Reservoir is located along the Smith River near Martinsville, VA, and is a part of the Roanoke River basin in the Piedmont ecoregion (USACE 2018). The 1,165-ha impoundment is owned and maintained by the United States Army Corps of Engineers and provides flood control and hydroelectric power generation (VDGIF 2023). Continued monitoring through 2023 has demonstrated persistence of dioecious hydrilla in Philpott Reservoir.

Philpott Reservoir supports the northernmost known and established dioecious hydrilla population among the Atlantic Coast states. Dioecious hydrilla populations have previously been recorded north of Virginia, with limited establishment success. In 1995 dioecious hydrilla was discovered in community with monoecious hydrilla in the Virginia portions of Lake Gaston, another Piedmont reservoir within the Roanoke Rapids watershed (Ryan et al. 1995). According to these prior reports, dioecious hydrilla populations discovered in these regions have not been able to persist in the same way that the population in Philpott Reservoir has; however, findings from these previous reports provide continued evidence of this species’ adaptable nature within the environment (Langeland 1996).

Water resource managers have utilized various methods for hydrilla control, which incorporate physical, biological, and chemical tactics. For best practice, managers formulate control decisions based upon the phenological stages of

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DOI: 10.57257/JAPM-D-24-00013

hydrilla to prescribe the most appropriate control method. Therefore, hydrilla phenology must be well understood to guide management practices that achieve maximum effectiveness and mitigate further spread.

Previous studies have demonstrated that some phenological patterns of monoecious and dioecious hydrilla subterranean turions tend to be dependent on water temperature. These include Haller et al. (1975), which found that the optimal water temperature for dioecious hydrilla turion sprouting ranged from 15 to 35 C. In a similar study, Steward and Van (1987) found that monoecious hydrilla sprouts more successfully in 15 C water than dioecious hydrilla. The results of these studies agree with the geographic distribution of hydrilla biotypes in the United States as dioecious plants tend to be found in warmer climates when compared to monoecious plants (Jacono et al. 2024). McFarland and Barko (1987) determined the thermal optimum for biomass production in both monoecious and dioecious hydrilla occurred at 28 to 32 C. Based on the last 10 yr of record, the median water temperature at Philpott Lake has averaged 10.0 C (USGS 2024). Considering the aforementioned studies, dioecious hydrilla, as understood from studied populations based in the southern United States, should not be as persistent in Philpott Reservoir because of the annual temperature ranges and phenological patterns that the plant has been shown to display under mesocosm and field evaluations.

Based upon prior literature, we hypothesized that the hydrilla population at Philpott Reservoir has adapted to survive and develop reproductive characteristics that allow subterranean-turion sprouting and plant success in cooler water temperatures compared to biotypes found in warmer climates. The present study investigates variation in environmental response of a Virginia population compared with a Florida population under controlled temperature gradient conditions.

## MATERIALS AND METHODS

Dioecious hydrilla subterranean turions were collected from Philpott Reservoir (Patrick County, VA) and from the UF/IFAS Center for Aquatic and Invasive Plants outdoor research ponds (Alachua County, FL) in March 2018. Hydrilla subterranean turions from both populations were sprouted under greenhouse conditions (mean air temperature: 26.0 C; mean light: 3,000 lux) at the Aquatic Weed Control Labs at North Carolina State University (NCSU; Wake County, NC). Sprouted subterranean turions representing hydrilla cohorts from the distinct climatic locations were then transferred to an outdoor mesocosm facility at the NCSU Research Farm (Raleigh, NC) in May 2018. Each sprouted subterranean turion was individually planted in topsoil amended with (3 g kg<sup>-1</sup>) fertilizer<sup>1</sup> and topped with a thin layer of sand in a plastic 6-inch nursery pot (2.9 L; 17.8 by 16.5 cm). Planted pots were subsequently placed in a 1040-L mesocosm filled with pond water. To ensure separation between the Virginia and Florida hydrilla populations while providing identical conditions for growth, pots were planted in adjacent mesocosms but remained physically separate to avoid any movement of plant material between populations.

Following close monitoring throughout the growing season, subterranean-turion production was confirmed in both

populations. In December 2018, subterranean turions from respective propagation mesocosms were harvested, rinsed, and immediately refrigerated (4 C) for 72 h. The first-generation progeny from the source hydrilla populations (Virginia and Florida) were used for the subsequent research evaluations of temperature-dependent sprouting effects to reduce potential maternal environmental influences of each source population tested.

A temperature gradient was produced under laboratory settings using a stainless-steel table supplied with hot (50 C) and cold (5 C) water introduced underneath the tabletop at opposing ends (Mohapatra and Johnson 1986; Figure 1). A randomized complete block design was successfully achieved as the temperature treatments ( $n = 7$ ) remained constant within respective temperature columns evaluated. Atop the table, 500 ml beakers ( $n = 28$ ) were arranged in seven equidistant columns with each treatment having four replicates. Beakers were filled with 1.5 cm of pea gravel and 100 ml DI water. Within each respective beaker, six 125-ml test tubes filled with 100 ml DI water and 0.5 ml water conditioner<sup>2</sup> were placed. Water temperature settings were calibrated 1 wk prior to study initiation to ensure a constant gradient temperature was achieved. Once calibrated, water temperature treatments ( $n = 7$ ) within test tubes were 9, 15, 20, 24, 29, 33, and 36 C throughout the duration of the 14-d study.

Fresh weight and length of hydrilla subterranean turions were recorded before experiment initiation. Three subterranean turions from the Virginia population and three subterranean turions from the Florida population were randomly assigned to each beaker. Individual subterranean turions were kept in separate experimental test tubes (mesocosms) within each beaker to effectively avoid any potential physical or chemical interactions between subterranean turions that could influence their individual response to temperature treatments. Test tubes were capped with aluminum foil to reduce water evaporation, and when water loss did occur, test tubes were filled to the initial water volume. Water within each test tube was aerated daily with compressed air for 30 s, and water temperature in each test tube was recorded every 24 h with a digital thermometer.<sup>3</sup> Stem lengths of each sprouted subterranean turion were recorded for 14 consecutive days and were measured with electronic calipers.<sup>4</sup>

The study was conducted in December 2018 and then repeated in January 2019. Data were analyzed in JMP Pro 14.2.0<sup>5</sup> ( $\alpha = 0.05$ ). Because no temperature by run interaction was detected for either stem length or subterranean-turion sprouting ( $P = 0.68$  and  $P = 0.081$ , respectively), the data from both runs were combined for subsequent analysis. Data were regressed using JMP software and graphed in SigmaPlot 14.0.<sup>6</sup> After testing multiple models for regression analysis, subterranean-turion response to temperature was described with a Gaussian three-parameter equation that best fit the data:

$$f = a \times \exp \left[ -0.5 \left( \frac{x - x_0}{b} \right)^2 \right],$$

where  $a$  represented the maximum sprouting value,  $x_0$  was the critical temperature at  $a$ ,  $b$  was the sprouting rate,  $x$  was

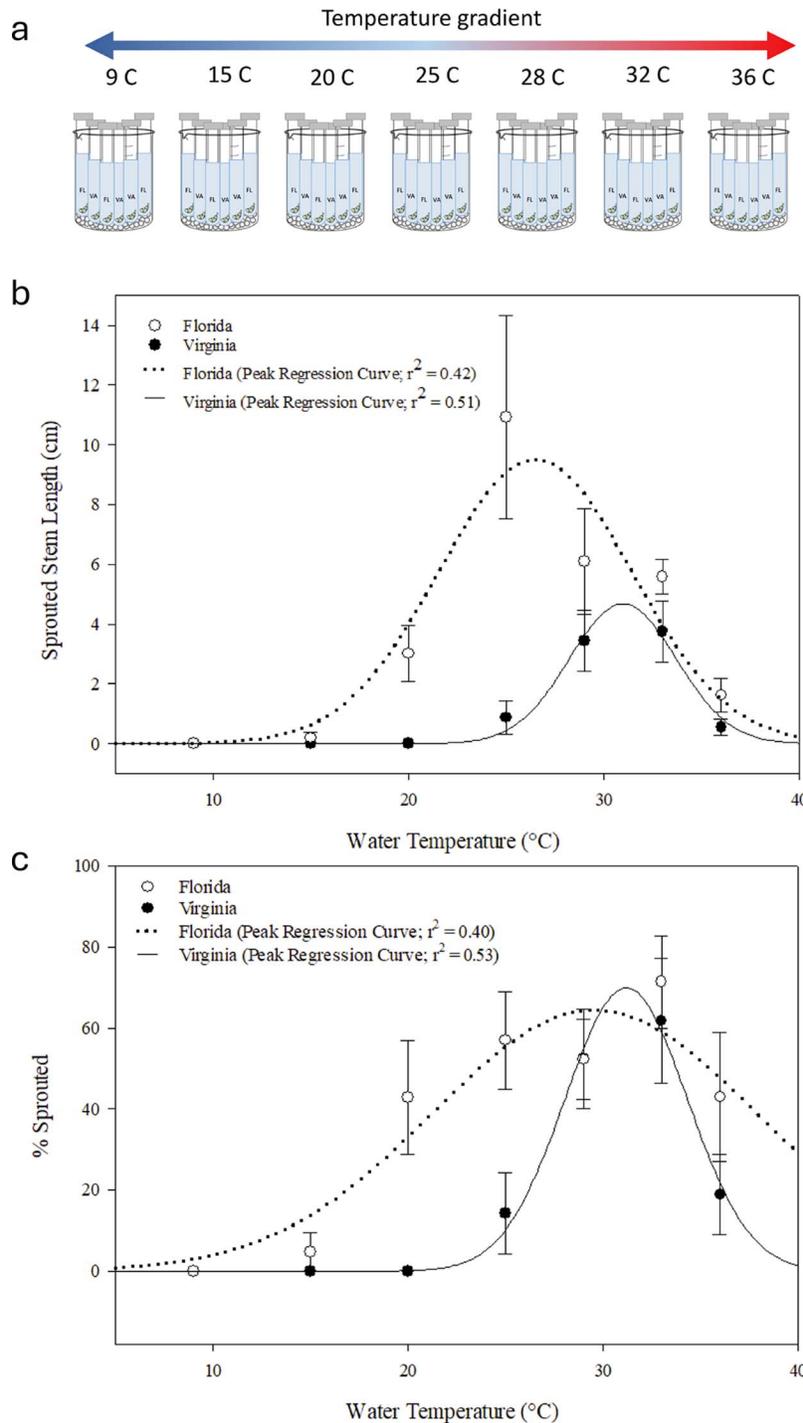


Figure 1. (a) Diagram of temperature gradient in which individual hydrilla tubers from Florida and Virginia populations were maintained in separate test tubes. (b) Hydrilla stem lengths from sprouted subterranean turions following the 14-d temperature study period. The dotted line is the best-fitted curve for tested Florida dioecious hydrilla subterranean turions ( $y = 9.5 \exp[-0.5(\frac{x-26.5}{4.9})^2]$ ). The solid line is the best-fitted curve for tested Virginia dioecious hydrilla subterranean turions ( $y = 4.6 \exp[-0.5(\frac{x-31.0}{2.7})^2]$ ). (c) Percent of sprouted subterranean turions by day 14 of the study. Bars indicate  $\pm$  SE. The dotted line is the best-fitted curve for tested Florida dioecious hydrilla subterranean turions ( $y = 64.5 \exp[-0.5(\frac{x-29.6}{8.3})^2]$ ). The solid line is the best-fitted curve for tested Virginia dioecious hydrilla subterranean turions ( $y = 69.9 \exp[-0.5(\frac{x-31.2}{3.2})^2]$ ). Bars indicate mean length  $\pm$  SE.

water temperature (C), and  $f$  was the proportion of hydrilla subterranean-turion sprouting based on  $a$ .

## RESULTS AND DISCUSSION

At the start of the study, Florida subterranean turions were 30% heavier ( $P < 0.0001$ ) than those from Virginia ( $0.30 \pm 0.01$  g and  $0.23 \pm 0.06$  g, respectively). The stem length of Florida individuals was influenced by both water temperature ( $P = 0.004$ ) and starting weight ( $P < 0.001$ ), but no interaction between these two factors was detected ( $P = 0.07$ ). The stem lengths of Virginia individuals were associated with water temperature ( $P < 0.001$ ), starting weight ( $P = 0.008$ ), and their interaction ( $P = 0.021$ ).

Regression analysis indicated that the peak of stem length for Florida individuals occurred at  $26.4 \pm 0.8$  C and was  $9.5 \pm 1.3$  cm, while for the Virginia population the peak occurred at  $31.0 \pm 0.4$  C with stem lengths of  $4.7 \pm 0.8$  cm by day 14 (Figure 1). Both Virginia and Florida subterranean turions exhibited similar optimal sprouting temperatures with  $69.9 \pm 9.9\%$  sprouting occurring at  $31.2 \pm 0.4$  C in the Virginia population and  $64.4 \pm 7.5\%$  sprouting occurring at  $29.6 \pm 1.4$  C in the Florida population. However, Virginia subterranean turions sprouted in a narrower temperature range when compared to those from Florida (Figure 1).

Overall, results do not support the hypothesis that subterranean turions from Virginia dioecious hydrilla populations sprout at cooler temperatures compared to subterranean turions from Florida dioecious hydrilla populations. Instead, we found that Florida subterranean turions exhibited the highest sprouting percentage and produced the longest stem lengths at cooler temperatures when compared to the Virginia population. One possible explanation for these results could be the dioecious hydrilla in Virginia has adapted to survive in temperate environments by delaying sprouting until the water reaches temperatures during the spring when the risk of freezing damage is low (Spindelbock et al. 2013). If this is the case, previously understood control techniques used for dioecious hydrilla (e.g., herbicide application timing) may need to be reevaluated for later-germinating subterranean turions in more northern populations. In other words, control timing could be determined based on environmental monitoring. Thus, identifying the temperature when a large proportion of the tubers has sprouted but before new tubers are produced will maximize hydrilla control. Future research must identify environmental variables that can be used to predict tuber production timing.

The sprouting temperature range exhibited by Florida dioecious hydrilla falls within the range reported by Haller et al. (1975). In that study, peak subterranean-turion sprouting was observed in water temperatures between 18 and 33 C. Similarly, McFarland and Barko (1987) found that peak biomass production for both monoecious and dioecious biotypes occurred between 28 and 32 C. Findings from the present study suggest that there is sprouting variation among geographically distinct dioecious hydrilla populations, which partially explains the wide range of conditions in which this species can grow.

It is important to consider that fresh weights of the dioecious hydrilla subterranean turions from Virginia were lower

than those from Florida. Because larger propagule size is considered to result in a competitive advantage in plants, this result may be a confounding factor in this study. For example, Bowes et al. (1979) found that hydrilla subterranean turions in a large size class (300–400 mg fresh weight) produced longer stems that survived for an extended period when held in dark conditions when compared to subterranean turions of smaller size classes. However, hydrilla subterranean-turion sprouting response to temperature may not be dependent on initial subterranean-turion size. In terrestrial species such as *Cyperus rotundus* (L.), bud dormancy release in tubers is mainly dependent on environmental signals and apical dominance over other buds (Kawabata and Nishimoto 2003). Additionally, the results of this study and the genetic differentiation reported by Tippery et al. (2020) suggest that evolutionary changes may be impacting the ecophysiology, growth, and success of dioecious hydrilla in Virginia, but a more detailed comparison of their genetic differences would help test this hypothesis.

In conclusion, the results of the present research confirmed that sprouting requirements differ between Florida and Virginia dioecious hydrilla populations. Future research should evaluate these laboratory-scale findings with observational and quantitative survey data under field conditions. Assuming that sprouting dynamics of dioecious hydrilla subterranean turions within Philpott Reservoir respond similarly to the laboratory trial, then management efforts must appropriately reflect the phenology of this population of hydrilla to maximize the efficacy of management efforts.

## SOURCES OF MATERIALS

<sup>1</sup>Osmocote® Smart-Release® Plant Food Plus, The Scotts Company, 14111 Scottslawn Road, Marysville, OH 43041.

<sup>2</sup>API® Tap Water Conditioner, Mars Fishcare North America Inc., 50 E. Hamilton Street, Chalfont, PA 18914.

<sup>3</sup>Fisher Scientific® Traceable Digital Thermometer, Thermo Fisher Scientific, 168 Third Avenue, Waltham, MA 02451.

<sup>4</sup>General® Stainless Steel Digital Fractional Caliper, General Tools & Instruments LLC, 75 Seaview Drive, Secaucus, NJ 07094.

<sup>5</sup>JMP Pro 14.2., SAS Institute Inc., 101 SAS Campus Drive, Cary, NC 27513.

<sup>6</sup>SigmaPlot 14.0, Systat Software Inc., 2107 North First Street, Suite 360, San Jose, CA 95131.

## ACKNOWLEDGEMENTS

We acknowledge the U.S. Army Corps of Engineers, Wilmington District, and the Northeast Aquatic Plant Management Society for providing funding for this work.

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