

## ABSTRACT

HENRY, AMY LOU. Monoecious Hydrilla (*Hydrilla verticillata* (L.f) Royle) Growth and Phenology in Two Dissimilar Climates. Under the direction of Dr. Robert J. Richardson.

*Hydrilla* (*Hydrilla verticillata* (L.f.) Royle) is a federal noxious submersed aquatic macrophyte that is expanding its range in North America. There are two biotypes, a triploid monoecious form and a triploid female dioecious form that have different growth habits, suitable environments, and physiology. The majority of research has been conducted on dioecious biotypes in warmer climates, which results in a large knowledge gap of how monoecious hydrilla will behave in cooler climates. To investigate the growth behavior of monoecious hydrilla in different climates, outdoor mesocosm trials were conducted at two separate research locations in Raleigh (35.7796° N, 78.6382° W; 96 m) and Laurel Springs (39.8201° N, 75.0063° W; 838 m), North Carolina. Phenology studies were completed, where timing of six life stages were recorded. All life stages occurred earlier in the warmer climate than the cooler climate. In the warm climate, turion and tuber sprouting, female and male floral initiation, turion formation, and plant senescence occurred at 12.4, 15.0, 27.7, 23.9, 24.2 and 8.5° C, respectively. In the cool climate, turion and tuber sprouting, female and male floral initiation, turion formation, and plant senescence occurred at 7.0, 10.1, 25.9, 23.1, 20.1 and 7.5° C, respectively. A year long competition study was also conducted in these two climates. Hydrilla tuber production ranged from 246 to 998 tubers m<sup>-2</sup> in the warm climate, and from 0 to 1000 tubers m<sup>-2</sup> in the cool climate. Dry weight of hydrilla ranged from 29.80 to 67.00 g in the warm climate and 0.34 to 72.17 g in the cool climate. Water temperatures affected the competitive abilities of both monoecious hydrilla and three competitor plants. In the cool climate, the competitor plants were able to suppress hydrilla growth compared to hydrilla grown alone. This suppression was not observed in the warm climate. This has major implications on the spread of hydrilla in northern

bodies of water that are known for their biodiversity and highly developed plant communities. Information about chilling requirements and behavior of these propagules in cooler climates is limited in the literature. Climatic conditions impact the production, viability, and sprouting of propagules were investigated. Temperatures during hydrilla development also affected the production and viability of asexual propagules. When overwintered at 4° C after being grown in the warm climate, tubers had an average viability rate of 63%, while when grown in cooler climates, the average viability rate was 89%. Tubers produced in the cooler climate were able to withstand 0° C temperatures for seven months, while tubers produced in the warmer climate did not remain viable at any of the harvests. Tubers were heavier when grown in the cool climate, averaging 0.113 g, while warm climate tubers averaged 0.096 g. Tuber densities also differed between climates, with cool climate hydrilla averaging 823 tubers m<sup>-2</sup>, while the warm climate averaged 2142 tubers m<sup>-2</sup>. Results from these studies demonstrates the difference in growth and phenology of monoecious hydrilla is impacted by ambient and water temperatures.

Monoecious Hydrilla (*Hydrilla verticillata* (L.f) Royle) Growth and Phenology in Two  
Dissimilar Climates.

by  
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## **DEDICATION**

To the best buddy a girl could have.

## **BIOGRAPHY**

Amy Lou Henry was born and raised in Kidron, Ohio. Drawing from her early experiences on her grandmother's farm, she attended The Ohio State University Agricultural Technical Institute, receiving an Associate's Degree in Greenhouse Production and Management. After working full time for a commercial perennial grower, she returned back to OSU to receive a Bachelor's Degree in Sustainable Plant Systems, specializing in Horticulture. Then, she moved to North Carolina to pursue a Master's Degree in Crop and Soil Science, focused on Aquatic Weed Control. She is looking forward to entering a rewarding research career in plant sciences after graduation.

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## **Chapter 1 Monoecious Hydrilla Literature Review**

### **Abstract**

Invasive aquatic macrophytes may have a negative effects on aquatic and terrestrial ecosystems and impact human health and recreation. Hydrilla (*Hydrilla verticillata* (L.f.) Royle) is a federal noxious, submersed aquatic macrophyte that is expanding its range in the United States (US). There are two biotypes found in the US, a triploid monoecious form and a triploid female dioecious form that have different growth habits, suitable environments, and physiology. The majority of research has been conducted on the dioecious biotype in warm climates, which results in a large knowledge gap of how monoecious hydrilla will behave in cool climates.

### **Species Overview**

Hydrilla is an obligate submersed noxious aquatic weed in the family Hydrocharitaceae (Figure 1-1). This family is believed to have originated 70-100 million years ago (Sculthorpe 1967) with fossil evidence of the genus *Hydrilla* dating back to 40 million years ago (Bowes et al. 2002). All species in this family exhibit a perennial lifecycle, and are especially adapted to aquatic environments, with aerenchyma and uniform internal structures (Ancibor 1979). Hydrilla has been found on every continent except for Antarctica, and is aggressively expanding its range (Langeland 1996, Netherland and Greer 2014). The plant has been discovered growing in Harbin, China, which is at the same latitude as Portland, Oregon and Montreal, Canada. This demonstrates that hydrilla would be able to grow in most of the continental US, including Alaska and southern Canada (Balciunas and Chen 1993). Along with the ability to grow in a variety of climates, hydrilla has developed many adaptations to survive and thrive in diverse aquatic environments. Small ponds, irrigation canals, rivers, lakes, and large reservoirs have all been found to support hydrilla populations (Dayan and Netherland 2005).

As a species, hydrilla contains highly variable genetics, and exhibits different morphological and physiological growth depending on its environment (Cook and Lüönd 1982). Genetically identical plants can exhibit different phenotypes based on water velocity, light penetration, and depth (Madsen 1991). The growth and appearance of hydrilla is impacted by water quality, temperature, and light availability within an aquatic environment (Carter et al. 1994). These characteristics are different throughout the same body of water, so the variability found between bodies of water can be extreme. Correct identification of the species is very difficult, as hydrilla exhibits a plastic phenotype, and appears similar to several other species, such as *Elodea canadensis* (Michx.) and *Egeria densa* (Planch.) (Blackburn et al. 1969, Carter et al. 1994).

Hydrilla grows submersed in the water column, with stems growing towards the water surface. The plant is rooted in the hydrosol with thin, white or reddish roots and produces several rhizomes and stolons to remain embedded in the sediment (Langeland 1996). Both biotypes grow to the water surface and form thick mats that cause diel temperature and oxygen fluctuations (Dayan and Netherland 2005). Stem fragments break off due to natural water movement and human activities, and are able to survive in the floating state. These fragments sink to the sediment within four days, and are able to root and grow in the new environment (Sutton et al. 1980). Along with the roots anchoring the plant to the hydrosol, adventitious roots are formed, and can contain chlorophyll to undergo photosynthesis if exposed to sunlight (Langeland 1996).

Leaves are typically in whorls containing four to seven leaves, and are approximately 2-4 mm wide and 6-20 mm long with serrated edges (Langeland 1996), which are influenced by the alkalinity of the water. Plants growing in hard, alkaline water have more distinct edges than

those growing in soft, acidic water (Kay 1992). The lamina of the leaf is extremely thin, only consisting of two cells, decreasing protection from the sun, leading to more UV-driven mutations (Dayan and Netherland 2005).

The monoecious biotype produces both female and male flowers on the same plant. Flowering appears to be concurrent with tuber formation, and is influenced by environmental conditions, such as photoperiod (Steward 1993). Flower initiation is different for monoecious hydrilla based on location. For example, North Carolina monoecious hydrilla began flowering earlier and lasted longer than monoecious hydrilla in Delaware. In North Carolina, flowering began in summer and extended into the fall, while in Delaware, flowering began in September and only lasted until October (Harlan et al. 1985; Miller 1988).

Female flowers have three white sepals and three clear petals, and arise from a single spathe (Cook and Lüönd 1982). The sepals are 10-50 mm long, and 4-8 mm wide. The flowers, attached at leaf axils close to the tip of the stems, float on the surface of the water when mature. The female flowers are resistant to wetting, as they are encapsulated in a bubble when submerged, and will immediately return to the surface of the water after being submerged (Langeland 1996). Male flowers have three sepals that are white or brown, are smaller than female flowers, and develop before female flowers (Cook and Lüönd 1982). The male flowers float freely on the surface of the water when mature, and there can be thousands floating in the water at one time (Langeland 1996). Monoecious hydrilla is able to produce viable seeds, which could complicate management plans (Netherland and Greer 2014). However, seed production is more common in tropical regions, and no seed production has been observed in North Carolina (Harlan et al. 1985).

## **Adaptations**

Hydrilla has several adaptations which make it highly competitive, and have led to it being referred to as “the perfect aquatic weed” (Langeland 1996). These adaptations include having several ploidy levels, numerous reproductive strategies, rapid vegetative growth, and the ability to survive in a wide range of environments, including up to 13 ppt salinity (Steward and Van 1987). Additional adaptations include the ability to form thick monospecific mats, alternate from C3 to C4 photosynthetic pathways, and grow in extremely low light and carbon dioxide (CO<sub>2</sub>) conditions (Dayan and Netherland 2005, Michel et al. 2004, True-Meadows et al. 2016).

In the same population of hydrilla, there can be several levels of ploidy exhibited- diploid, triploid, and tetraploid (Arias et al. 2005). Variability in ploidy levels may lead to beneficial adaptive features being exhibited by the plant, and also enables genetically identical plants to behave differently in the same environment (Verkleij et al. 1983). Due to increased levels of chromosome sets, the rate of natural mutation in hydrilla DNA is high, along with the increased number of UV light-driven mutations (Dayan and Netherland 2005).

Reproduction of hydrilla is primarily asexual, through the production of propagules and vegetative fragmentation. However, sexual reproduction through seed production has also been observed (Langeland 1996). Aquatic herbaceous perennials have been found to allocate 25% of their resources to asexual reproduction, and less than 5% to seeds, illustrating the importance of asexual reproduction to the plant (Madsen 1991). The two distinct vegetative propagules that are produced are called axillary turions (Figure 1-2) and subterranean turions (Figure 1-3), often called tubers for simplicity.

The green axillary turions are produced in the above-ground biomass leaf axils, and consist of overlapping leaf scales surrounding a single dormant plant meristem (Netherland

1997). Tubers also are covered in leaf scales, and are produced on the terminal ends of rhizomes in the hydrosol, and come in a variety of colors that depend on soil composition and maturity (Netherland 1997). Monoecious turions are smaller than tubers, and therefore, have a shorter longevity. Turions are viable for one year as compared to tubers, which can remain viable in undisturbed hydrosol for greater than five years (Nawrocki et al. 2016). Turions are released from the plant by an abscission layer when fully mature, while tubers are separated from the plant when the rhizomes decompose (Netherland 1997).

Mature tubers are resistant to desiccation, herbicides, and freezing when in the hydrosol, making the management of hydrilla extremely difficult (Langeland 1996). These propagules appear to be the most important mechanism of survival and movement for the plant (Van and Steward 1990), especially tubers, whose control has been described as the most important part of a successful management plan (Netherland 1997). Environmental conditions affect the amount of resources allocated to form turions or tubers. In tropical and cool temperate regions, the majority of the resources go towards producing tubers, while in warm temperate regions, turions are produced at a greater rate (Sastroutomo 1982). A single monoecious hydrilla plant is able to produce more than 5,000 tubers per m<sup>2</sup>, leading to a tuber bank, equivalent to a seed bank. This complicates management, as larger meristem banks enable hydrilla to be a more aggressive competitor (Spencer and Rejmánek 2016). Nawrocki et al. (2016) discovered that densities of tubers as low as 11 tubers per m<sup>2</sup> were enough for re-infestation, and could result in an 11.36 fold increase in the amount of tubers found after just one season.

Tubers appear to have variable dormancy periods to disperse the hydrilla population over time (Netherland 1997). Environmentally induced dormancy is thought to control sprouting of propagules, though the exact mechanisms and environmental conditions are not understood at



this time. Dormancy of aquatic plants has not been studied as extensively as terrestrial plants. However, it is hypothesized that this dormancy inhibits the propagules from sprouting during the same year they were formed, improving the chance of survival over time (Carter et al. 1987).

When tubers are removed from anoxic conditions where they were formed for research purposes, they are exposed to higher levels of light and oxygen as well as reduced levels of CO<sub>2</sub>, and dormancy is broken (Netherland 1997). In the laboratory, monoecious hydrilla has a tuber germination rate of 95-100%, regardless of season, while dioecious hydrilla exhibits tuber germination rates of 88%, and is affected by seasonality. The high rates of sprouting in laboratory studies indicates that tubers have an environmentally-induced quiescence instead of an internal dormancy (Netherland 1997).

More tubers are produced by monoecious hydrilla than dioecious hydrilla, due to the higher proportion of resources allocated toward tuber formation (Van 1989). Monoecious hydrilla tubers weigh less and are smaller (Spencer et al 1987). The average monoecious hydrilla tuber is 35.55 mg dry weight while dioecious hydrilla tuber dry weights average 66.66 mg (Madsen 1991).

Hydrilla propagules require a chilling period to sprout. Carter et al. (1987) examined the dormancy of hydrilla propagules, and found after a 7° C chilling period of 42 days, 92% of monoecious propagules sprouted, while 0% of propagules that were not chilled sprouted. Sastroutomo (1980) found that dormancy was broken after a 2° C chilling period for 33 days, while different lengths of photoperiods without a chilling period did not break dormancy. In the same study, photoperiod did not affect the sprouting of the propagules after exposure to a chilling period, signifying the importance of a chilling period.

A single method of completely killing or removing propagules has not been developed and a comprehensive, consistent long term management strategy is essential (Dayan and Netherland 2005). It took 7-10 years of chemical treatment to deplete the tuber bank by 99.5% in North Carolina (Nawrocki 2011). However, if a single year of treatment was missed, tuber densities did recover to 74% of the original amount in one year (Nawrocki 2011). Due to the great abundance of tubers produced, their resistance to unfavorable environmental conditions, and the inability to develop method of control, they are a considerable challenge in management plans.

Along with being able to survive and thrive in many different bodies of water, hydrilla can grow in a wide range of temperatures, sediment types, and pH (Madsen and Owens 2000). For hydrilla turions to sprout, the water temperature must be within 13-35° C, though hydrilla prefers higher temperatures, with an optimum temperature for photosynthesis of 36.5° C (Van et al. 1976). In warm climates, such as in Florida, hydrilla is able to sprout early in the season, when there is little competition for space, light, or carbon. Hydrilla is able to grow in a variety of sediment types, including sand, loam, and marl, again increasing the range of potential infestation (Steward 1984). The aquatic systems where hydrilla is found often experience a diel swing in pH, ranging from 7.1 in the morning to 10.2 in the afternoon as a direct result of hydrilla (Van et al. 1976). Due to the extremely high pH in the afternoon, the availability of inorganic carbon to plants is limited to only bicarbonate ( $\text{HCO}_3^-$ ), which several plants cannot utilize. However, hydrilla is able to utilize  $\text{HCO}_3^-$ , allowing the plant to survive in basic environments (Van et al. 1976).

Hydrilla has rapid vegetative growth, and with leaves composed of 90% water, a great deal of biomass can be produced with minimal mineral plant nutrient inputs (Langeland 1996).

Hydrilla's ability to quickly produce vegetative material is evidenced by the fact that a single dioecious hydrilla plant increased its biomass over 1,500 times in just sixteen weeks in laboratory conditions (Sutton et al. 1980). Stems are able to grow up to 15 meters in length, elongating to intercept any available light (Arias et al. 2005). Dioecious hydrilla produce nodes approximately every 12 millimeters, which each node being able to produce a new plant (Langeland 1996). This leads to a huge potential for new sources of plant material, as 40% of fragments consisting of one or two nodes were able to regrow in both greenhouse and field studies (Langeland and Sutton 1980). In the same study, 68% of fragments consisting of three to five nodes were able to regrow. The high potential for plant replication from vegetative fragmentation is an additional challenge in a management system.

Rapid growth leads to issues when the plant forms thick, monospecific mats, which allows hydrilla to increase its competitive advantage by effectively shading out all other submersed plants (Van et al. 1976). This results in a drastic change in community structure, which could lead to a trophic cascade felt throughout the entire aquatic and surrounding terrestrial ecosystems (Grajczyk 2009). Underneath the surface mats, daily temperature fluctuations of up to 20° C have been observed, along with drastic pH and dissolved oxygen changes (Bradshaw et al. 2002; Dayan and Netherland 2005). These environmental conditions cause the majority of photosynthesis to occur in the morning, when there is a greater abundance of carbon and lower irradiance. Underneath a mat of hydrilla, the amount of photosynthetically available radiation (PAR) is reduced 95% in the top 0.3 m of water, while in open waters, there is only a 29% reduction of light (Barko and Smart 1981). It is stated that hydrilla "creates a harsh environment, and then proceeds to thrive there" (Dayan and Netherland 2005). The changes in the environmental conditions of the water body alter which organisms can survive.

Hydrilla has the ability to concentrate carbon around the carboxylation enzyme Rubisco through an inducible C4-type photosynthesis. This pathway uses phosphoenolpyruvate carboxylase (PEPC) instead of Rubisco as the initial fixation enzyme in photosynthesis (Bowes et al. 2002). This adaptation allows hydrilla to eliminate photorespiration, increasing the efficiency of photosynthesis. The evolution of this ability was brought about by unfavorable photosynthetic conditions found in water during the day: supersaturation of oxygen, temperatures above 35° C, pH greater than 10, and low levels of available inorganic carbon (Van et al. 1976).

Out of the 7,600 species of plants that have been found to undergo C4 photosynthesis, there are only a dozen aquatic species that have been identified. Photosynthetic pathway is determined by the pH, oxygen and CO<sub>2</sub> availability in the water (Bowes et al. 2002). In hydrilla, the pathway can change over the course of the day, or over the course of a growing season. Biochemical and molecular changes lead to the switch from C3-C4 pathways without any anatomical alterations, meaning that the plant does not have to grow new leaves when conditions change (Rao et al. 2006). In fact, leaves that were discovered to have C4-like CO<sub>2</sub> compensation points had previously exhibited higher C3 compensation points (Bowes et al. 1977).

Due to these many adaptations, hydrilla has been shown to be a strong competitor with many submersed aquatic plants, both native and invasive. In competition studies and the observation of natural displacement, *Vallisneria americana* (Michx.), *E. densa*, *Myriophyllum spicatum* (L.), and *Ceratophyllum demersum* (L.) have all been seen to be inferior competitors to hydrilla (Hofstra et al. 1999, Mony et al. 2007, Wang et al. 2008). Even though there have been many competition studies between hydrilla and other species of plants, most of the research has been completed on the dioecious biotype in warm climates.

Staminate dioecious hydrilla in New Zealand has been shown to be able to compete and overpower many other species in Hydrocharitaceae (Hofstra et al. 1999). In this study, hydrilla demonstrated the ability to suppress these species, indicating that it has potential to be more invasive than other invasive members in Hydrocharitaceae found in New Zealand. In another competition study, Wang et al. (2008) found that hydrilla was able to compete with *M. spicatum*, accumulating more aboveground biomass and also reducing *M. spicatum* root formation. This shading took place due to quick elongation of hydrilla stems to the water surface, where it was observed that it grew on top of the *M. spicatum* mat (Wang et al. 2008).

In field observations, hydrilla has been recorded to outcompete other submersed plants present (Carter et al. 1994; de Kozlowski 1991). In the Potomac River, monoecious hydrilla occupied greater than seventy percent of the plant community in just five years after being discovered in 1982 (Carter et al. 1994). Before the hydrilla infestation, *V. americana* and *M. spicatum* were the most dominant plant species present. Historically, this river supported a rich diversity of submersed macrophytes until 1972, when tropical storm Agnes hit (Serafy et al. 1994). After this year, the dominant macrophyte was hydrilla.

### **Introduction and Spread**

Around the world, there are twenty-three different hydrilla biotypes, increasing the genetic diversity and geographical range of hydrilla (Verkleij et al. 1983). In the US, there are two biotypes found: a triploid monoecious form and a triploid female dioecious form (Cook and Lüönd 1982). The distribution of both biotypes in the US is shown in (Figure 1-4).

Dioecious hydrilla was initially discovered in Florida in the 1960s, after it was intentionally introduced for aquarium plant trade. It was initially misidentified as Florida elodea, and no management was taken for seven years (Blackburn et al. 1969). This lengthy period

between discovery and treatment allowed the plants to establish in the area and quickly expand its range in Florida and be introduced into southern Alabama and Georgia (Blackburn et al. 1969). In just a decade, dioecious hydrilla was found in major bodies of water in every watershed of Florida (Langeland 1996).

Monoecious hydrilla was misidentified as American elodea when it was first discovered in 1976 in the Potomac River in Delaware (Madeira et al. 1997). By 1983, over 162 hectares in the river were infested with the plant (Rybicki and Carter 1986), comprising over 70% of the submersed aquatic vegetation population (Carter et al. 1994). Soon after 1976, other populations of monoecious hydrilla were found in North Carolina, Maryland, and Virginia (Steward and Van 1987). It is believed that at least one of the introductions was accidental, being mixed in with waterlilies in Kenilworth Aquatic Gardens in Washington DC (Vandiver et al. 1982). In North Carolina, monoecious hydrilla was first correctly identified in the 1980s in Umstead Park in Wake County, years after the first discovery of the plant (Harlan et al. 1985). Korea is the likely center of origin for the biotype of monoecious hydrilla that is found in the United States (Madeira et al. 1997).

From 2000-2011, dioecious hydrilla was discovered in three new states, while monoecious hydrilla has been identified in fifteen new states (Netherland and Greer 2014). Eleven of these states are in the northern US that had not experienced previous hydrilla infestations, and the other four are southern states that had only dioecious infestations. Even though monoecious hydrilla is aggressively expanding its range, research on this biotype has not kept pace with the expansion. In a recent search of all published literature on hydrilla, there were 5,011 records. Only 197 published papers mentioned “monoecious” or “biotype” (Netherland

and Greer 2014). That is less than 4% of papers that refer to monoecious hydrilla or even mention biotype.

The monoecious biotype is more adapted to temperate conditions in the US, and has naturalized in areas along the east coast, from North Carolina to Connecticut (True-Meadows et al. 2016). The dioecious biotype is found in tropical regions of the US, as in Florida, Georgia, Alabama, and California (Madeira et al. 1997), and requires a higher temperature for completing its lifecycle. Monoecious hydrilla above ground biomass dies back completely in the winter, exhibiting a herbaceous perennial lifecycle (Madsen 1991), where dioecious hydrilla biomass can overwinter in warmer areas, lengthening the growing season.

States that have both dioecious and monoecious hydrilla include Alabama, South Carolina, Georgia, and Tennessee (Netherland and Greer 2014). Much research has gone into understanding the plant's growth in the warmer, southern climates of the US, but more research is needed about the plant in cooler climates. Understanding the growth and spread and being able to develop appropriate management strategies in northern climates is the most important issue that needs to be tackled in regards to hydrilla control (Netherland and Greer 2014). It is assumed that longer photoperiod lengths and cooler water affect the growth, spread, and reproductive timeline of hydrilla, though research has been lacking in these aspects.

### **Comparisons between Biotypes**

Madeira et al. (2000) reported that dioecious and monoecious biotypes of hydrilla have different life strategies even though they are similar in appearance. These differences need to be understood for appropriate and effective management strategies. These life strategies include differences in physiology, reproduction, and responses to environmental conditions. Experiments have been completed to quantify the differences between monoecious and dioecious hydrilla,

looking at tuber formation (Steward and Van 1987, Sutton et al. 1992), and plant biomass accumulation (Grodowitz et al. 2010).

In experimental laboratory conditions, monoecious hydrilla produced tubers more rapidly and in greater abundance than dioecious hydrilla. In the study, monoecious hydrilla produced twice the amount of tubers than dioecious hydrilla did (Sutton et al. 1992). Tubers were also produced under the longer days of summer on monoecious plants, while no tubers were produced under the same lighting conditions on dioecious plants (Van 1989). It was found that tuber formation in dioecious hydrilla is directly tied to short days, where monoecious hydrilla is able to produce tubers regardless of photoperiod though production is increased in response to short days (McFarland and Barko 1999). In laboratory studies, tuber sprouting was greater for monoecious hydrilla and also occurred at lower temperatures than dioecious hydrilla (Sutton et al. 1992, Van 1989).

The growth habit between the two biotypes also differ, with monoecious hydrilla more prone to grow laterally on the sediment, after sprouting while dioecious hydrilla tends to grow vertically first (Grodowitz et al. 2010, Van 1989). Therefore, dioecious hydrilla reaches the surface of the water quicker, so when monoecious hydrilla is seen, the majority of the sediment is already covered by a considerable amount of biomass (Sutton et al. 1992). This decreases the likelihood of effective control, as the complexity and difficulty of control increases as the biomass increases.

These differences enable the specific biotypes to flourish in different environments. Monoecious hydrilla, with its ability to continuously produce a large amount of tubers in a small period of time is better suited for temperate climates, while dioecious hydrilla is more suited for



tropical climates (Netherland 1997). In addition, the annual lifecycle of monoecious biotype makes the plant more suited to northern climates.

### **Issues Associated With Nuisance Aquatic Growth**

Invasive aquatic macrophytes have a significant effect on aquatic ecosystems, as aquatic systems are particularly vulnerable to invasive species (Arias et al. 2005). Eutrophication, or the nutrient enrichment of bodies of water has led to increased invasions, and has been acknowledged as one of the main origins of invasive species in developed countries (Andres and Bennett 1975). Habitat destruction and alteration, human population growth, and increased world travel by humans have also increased the range of several plants (Floerl and Inglis 2005, Pimentel et al. 2005). Examples of human alterations of waterways include the building of irrigation canals or reservoirs, or dredging existing lakes. In these instances, the exotic weed may have an adaptive advantage over native species and become dominant very rapidly (Andres and Bennett 1975). When introduced into a new habitat, noxious weeds can become environmental engineers by altering and disrupting water quality, native plant communities, and other ecosystem processes (True-Meadows et al. 2016). Following habitat loss, invasive species are considered the second greatest threat to aquatic organisms, and are found to affect over half of all species on the threatened and endangered species list (Wilcove et al. 1998).

These negative effects can be brought about by hydrilla, affecting both humans and the natural ecosystem. Large infestations of hydrilla interfere with irrigation, recreational activities, drinking water, hydropower generation, industrial uses, transportation, native plant populations, and can harbor mosquitoes that can transmit diseases to humans (True-Meadows et al. 2016). Large hydrilla mats can increase sedimentation of the water body, which slows the flow of water, and increases the chance and intensity of flooding (Sculthorpe 1967).

Thick hydrilla mats can provide suitable habitats for vectors of diseases that affect humans such as malaria, encephalomyelitis, filariasis, and schistosomiasis (Zettler and Freeman 1972). Increased disease risk is not the only effect these species have on humans, as property values are also impacted. Excessive growth of invasive aquatic macrophytes have been estimated to reduce property values from 20-40% (Halstead et al. 2003), and reduce the likelihood of housing development around the body of water by 37% after an invasion (Goodenberger and Klaiber 2016).

Swimming, boating, and recreational fishing are also impeded by thick mats of hydrilla, leading to a loss of revenue. In Florida, there was a loss of \$10 million dollars when two lakes were closed due to hydrilla treatment in one year (Pimentel et al. 2005). Swimming in a thick mat of hydrilla is considered a drowning hazard, and also impedes boat traffic reducing the recreational use of the water body (Goodenberger and Klaiber 2016).

In addition to directly altering and impacting the aquatic environment, monoecious hydrilla is able to affect terrestrial food webs indirectly. Avian Vacuolar Myelinopathy (AVM) is a fatal nervous system disease first discovered in bald eagles (*Haliaeetus leucocephalus*) in 1994, though it affects American coots (*Fulica americana*), Canadian geese (*Branta canadensis*), great horned owls (*Bubo virginianus*), and mallards (*Anas platyrhynchos*) (Wilde et al. 2005). This disease is caused by epiphytic cyanobacteria (*Aetokthonos hydrillicola*) in the order Stigonematales that has been discovered on up to 95% of hydrilla biomass in affected areas. AVM is transmitted to birds through ingestion, and appears to bioaccumulate, affecting the top predators more than the initial consumers. Hydrilla, *E. densa*, and *M. spicatum* were the most common aquatic vegetation in areas where there were cases reported of AVM.

## **Control and Management Strategies**

Due to the numerous problems associated with hydrilla, control is necessary if the body of water is to be used to its fullest potential (Sutton et al. 1992). Controlling hydrilla is difficult, expensive, and a continuous process, due to the characteristics of both the target plant and the aquatic environment (Sousa 2011). For example, the distribution and density of submersed aquatic vegetation is controlled by several different factors that interact with each other, and only some of which that can be controlled by humans (Carter et al. 1994). In addition, there are often several unrelated plant species, many of which are valuable, therefore requiring selective control methods. The flow of the system also complicates management strategies, with flowing water systems being more difficult to control. This increase of difficulty in flowing systems is due to increased regulations, downstream concerns, and decreased water contact to plants (Van et al. 1987). There are few management tools available to treat nuisance aquatic macrophytes being restricted by environmental concerns, costs, effectiveness, and public perception (Richardson 2008).

Over one billion dollars per year is spent in the US alone on aquatic vegetation control, which includes the management of hydrilla (Pimentel et al. 2005). In addition to the cost of control, there are ten million dollars per year in losses and damages in the US that are directly related to submersed aquatic vegetation (Pimentel et al. 2005). Eradication programs are not as common as control programs, as turions and tubers allow hydrilla to be extremely persistent. There are four methods of control, which include cultural, mechanical, chemical, and biological methods (Richardson 2008). Each of these methods of control have positives and negatives that need to be considered before an effective management strategy can be developed. All control methods are temporary, except for biological control, though this method takes time for the

organism to build up populations (Cuda et al. 2008). An integrated management plan can be used to increase the probability of control. After hydrilla is controlled, it is necessary to restore native plant communities, and reduce external environmental stressors (Chadwell and Engelhardt 2008). Like other invasive species, the best management strategy for hydrilla is prevention, but that is difficult (Langeland 1996.)

Another complication in control is the variety of individuals that have access to the body of water. These individuals can be homeowners, recreational users, power plant companies, and governmental agencies, all having different levels of acceptability of weed presence, knowledge of the problem, and budgets (Richardson 2008). Homeowners and swimmers generally want a completely vegetation-free body of water, which is in direct opposition to hunters and fishers, who would prefer to have aquatic vegetation. Public utility providers will only treat when plant growth interferes with energy production (Richardson 2008). Acceptable hydrilla populations are determined by economic thresholds, or where the company would lose money if the population of hydrilla exceeded the level, not based on ecological harm. As bodies of water can cross jurisdictions and even state lines, this increases the number of governmental agencies involved, and complicates management strategies (Richardson 2008).

Cultural control involves managing the weed problem by manipulating environmental conditions, and includes management actions such as water drawdowns, benthic barriers, and dyes (Poovey and Kay 1996). These control methods are temporary, expensive, difficult to implement, and are not practical on a large scale. Drawdowns involve water levels being artificially altered to expose hydrilla biomass and the hydrosol to the atmosphere to dry out. There are limitations to this management plan, and it is not always a feasible option. Some ponds and lakes are unable to have their water depth controlled, which eliminates the possibility of

using drawdowns. Hydrosol composition is an important factor in the success of a drawdown (Doyle and Smart 2001). In North Carolina, it had been reported that drawdown was completely ineffective, due to the high percent of clay in the hydrosol, meaning the tubers in the soil will not dry out enough to cause mortality (Harlan et al. 1985).

In a study conducted on monoecious hydrilla in North Carolina, a winter drawdown had no impact on the tubers in the hydrosol (Poovey and Kay 1996). Summer drawdowns may be more effective by reducing both the biomass growth and turion production (Barrat-Segretain and Cellot 2007), but hydrilla turions in sediment showed no decline in their viability throughout a 12-month continuous drawdown (Doyle and Smart 2001). Several drawdowns may be used, as the first drawdown would induce tuber sprouting, and then the second drawdown would kill the biomass before new tubers would be formed. However, summer drawdowns are not practical for most water bodies due to recreational, irrigation, drinking water, and other demands.

Benthic barriers are expensive, costing over \$4,000/ha, and are a temporary solution. Materials that are utilized include sand, clay, plastic, rubber sheets, and burlap (Nichols and Shaw 1983). Small areas, such as by boat ramps and public swimming areas would be suitable habitats to use this control method, though sediment accumulation on top of the material is a concern, as hydrilla will grow on top of the barrier (Nichols and Shaw 1983).

Liquid dyes and shade are effective in control of hydrilla, as sunlight availability is decreased. At least one pond dye has been registered with the EPA, limiting the potential of using this method of control (Richardson 2008). In addition, native plant populations and other aquatic organisms must be considered, as these organisms will also be affected by the dye. Therefore, the exclusive use of dyes and shade is limited in controlling hydrilla (Nichols and Shaw 1983).

Mechanical control is achieved by hand pulling or using machinery to remove the weed from the aquatic system. This form of control is not common because it is expensive, time consuming, encourages new growth, and has negative impacts to non-target organisms. Mechanical control can cost up to \$2,400/ha, and can require up to six treatments in the same growing season (Langeland 1996). In the Potomac River, two 450 m<sup>2</sup> locations of monoecious hydrilla were located and studied. At one location, hydrilla was mechanically harvested, and in the other area, there was no mechanical control. After three weeks, there was an increase of 34% in vegetation in the mechanically harvested site as compared to the control site (Serafy et al. 1994). In the treatment site, along with the eventual increase in hydrilla biomass, there was a 4-23% loss of fish biodiversity, as fish were caught in vegetation that was removed from the lake (Serafy et al. 1994). Another disadvantage to mechanical control is the ability of hydrilla to propagate from stem fragments, which are increased by removal. For mechanical control to be effective, all plant biomass must be removed from the body of water, which is difficult. For all these reasons, mechanical control is only a temporary solution to the problem (Serafy et al. 1994). The use of mechanical control is limited to drinking water reservoirs, fast moving water, or areas where immediate removal of the plant is necessary (Langeland 1996).

Biological control is defined as the intentional release and use of non-native organisms to help diminish the growth, ability to reproduce, or thickness of an unwanted organism. Research into using biological control for aquatic systems began in 1964 on alligatorweed (*Alternanthera philoxeroides* (Mart.) Griseb.) (Andres and Bennett 1975). Due to the success of control with insects, other forms of organisms were researched for several different aquatic weed species. The three main groups of organisms used in aquatic weed biocontrol programs are arthropods, fish, and pathogens (Cuda et al. 2008).

Both monoecious and dioecious hydrilla may be controlled by sterile triploid grass carp, *Ctenopharyngodon idella* (Cuvier and Valenciennes). These fish are sterilized by shocking the eggs with water of extreme high or low temperature or with pressure. This shock results in the eggs retaining an extra set of chromosomes, making the fish triploid and sterile (Cuda et al. 2008). Even though these fish are not selective feeders, it has been documented that hydrilla is a preferred food source. Once released, capture is difficult, and the carp will remain in the system, eating many submersed plant species.

Another biological control species has been shown to feed on hydrilla is a species of fly, *Hydrellia spp.* (Grodowitz et al. 2010). This fly has been demonstrated to control dioecious hydrilla, but has not shown any impact on monoecious hydrilla (Grodowitz et al. 2010). One reason is that monoecious hydrilla mats tend to stay directly under the water surface, where dioecious biomass is exposed to the air. This decreases the herbivory damage that can be inflicted, and also eliminates habitat area. In addition, monoecious hydrilla biomass doesn't typically overwinter, which decreases the likelihood of the insect overwintering as well (Grodowitz et al. 2010). Ongoing research is being conducted on the feasibility and practicality of using insect biological control on monoecious hydrilla.

There are currently fourteen active ingredients and one pond dye that is approved by the EPA for aquatic weed control (Richardson 2008). Chemical control costs are very expensive, with wide ranges of costs, averaging \$1,235/ha (Arias et al. 2005). Along with having steep direct costs, there is a possibility of having large indirect effects, such as impacts to non-target organisms. After a chemical treatment, there may be restrictions to drinking water, fishing, irrigation of crops, and swimming (Richardson and Getsinger 2014).

Treatment of hydrilla is complicated, and requires consistent treatment to be able to control the plant. The best way to control hydrilla is to prevent the spread of the plant, which is done by educating the public and individuals that use the bodies of water (Langeland 1996). However, after hydrilla is present, the possible treatment tools include cultural, mechanical, biological, and chemical control. Integrating more than one of these treatment tools in the management plan increases the potential effectiveness of the control (Chadwell and Engelhardt, 2008).

Despite the fact that hydrilla is one of the most commonly studied aquatic plant, there is still a large amount of knowledge not in the literature. This is due to the fact that most of the research thus far has been completed on the dioecious biotype in warm climates (True-Meadows et al. 2016). Currently, there is a large gap of knowledge of the growth and phenology of monoecious hydrilla in cool climates where the range of hydrilla is actively spreading. This knowledge is important to determine effective management plans (Netherland and Greer 2014). Additional research is needed to determine how different climate conditions, such as photoperiod and temperature affect the growth, reproduction, and competitiveness of monoecious hydrilla.



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**Taxon: *Hydrilla verticillata* L.**

Kingdom: *Plantae*- plants

Subkingdom: *Tracheobionta*- Vascular plants

Superdivision: *Spermatophyta*- Seed plants

Division: *Magnoliophyta*- Flowering plants

Class: *Liliopsida*- Monocotyledons

Subclass: *Alismatidae*

Order: *Hydrocharitales*

Family: **Hydrocharitaceae**

Genus: *Hydrilla* Rich. - hydrilla

Species: *Hydrilla verticillata* (L.f.) Royle

**Other common names:** waterthyme, Florida elodea

Figure 1-1. Taxonomic identification of *Hydrilla verticillata* (L.f.) Royle.



Figure 1-2. Axillary turions of monoecious *Hydrilla verticillata*.



Figure 1-3. Subterranean turions of monoecious *Hydrilla verticillata*.

Map Source: U.S. Geological Survey, Nonindigenous Aquatic Species Database, Gainesville, FL. October 2016

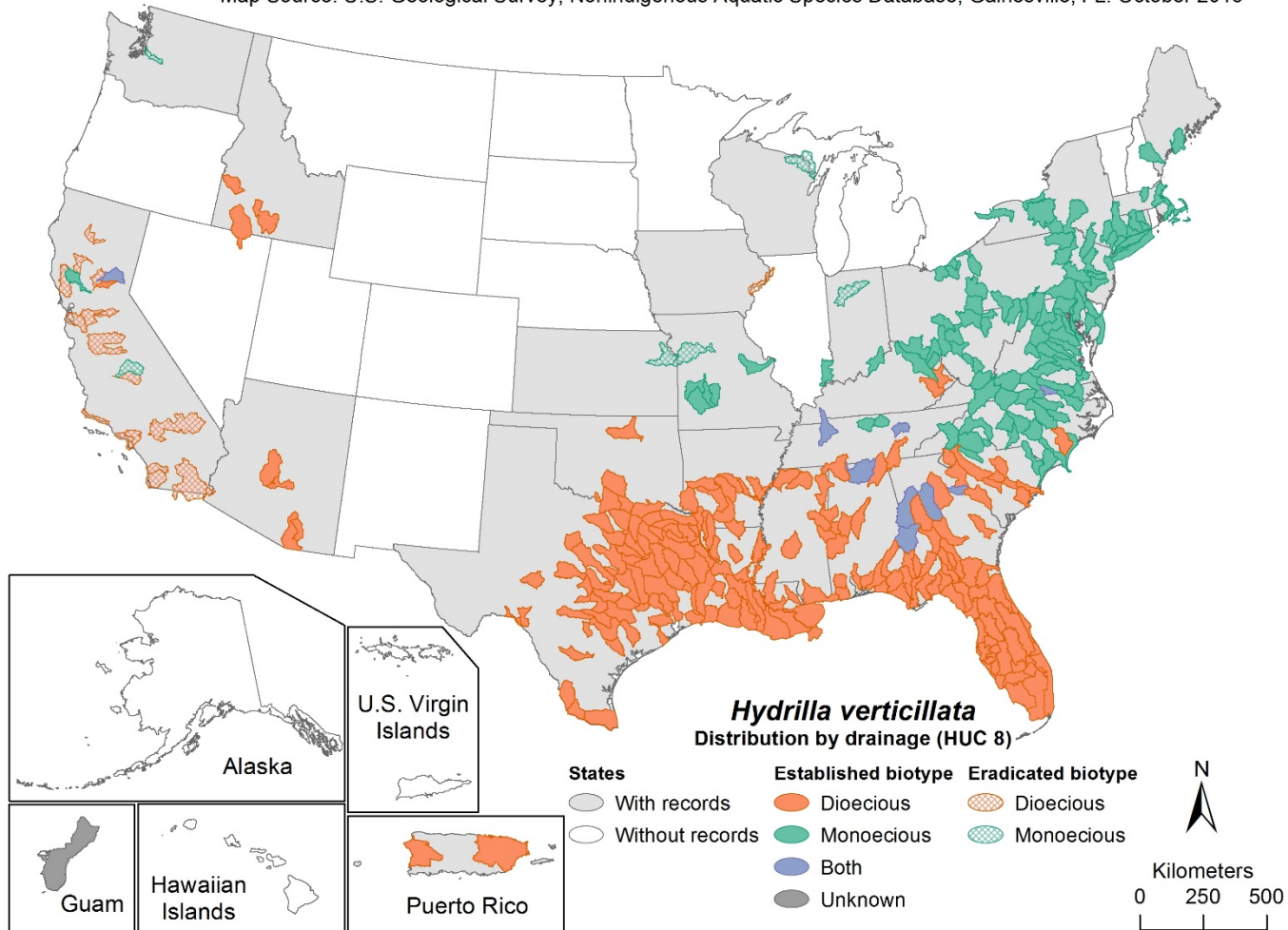


Figure 1-4. Distribution of dioecious and monoecious *Hydrilla verticillata* in the United States. U.S. Geological Survey, 2016, *Hydrilla verticillata*. Nonindigenous Aquatic Species Program, Gainesville, FL. <https://nas.er.usgs.gov/XIMAGESESERVERX/2016/20161027161554.jpg>. Accessed 12/20/2016.

## **Chapter 2 Monoecious *Hydrilla verticillata* Phenology and Growth in a Simulated Cool Climate Compared to a Warm Climate**

### **Abstract**

*Hydrilla* (*Hydrilla verticillata* (L.f) Royle), a federal noxious submersed aquatic plant, has been referred to as the perfect aquatic weed. There are two biotypes of hydrilla in the United States; a pistillate dioecious biotype and a monoecious biotype. To investigate the growth behavior of monoecious hydrilla in different climates, outdoor observational mesocosm trials were conducted at two separate research locations in Raleigh and Laurel Springs, North Carolina. Throughout the study, water and ambient temperatures were lower in Laurel Springs. All life stages occurred earlier in Raleigh than in Laurel Springs. In Raleigh, mean water temperature of turion and tuber sprouting, female and male floral initiation, turion formation, and plant senescence occurred at 12.4, 15.0, 27.7, 23.9, 24.2, and 8.5° C, respectively. In Laurel Springs, turion and tuber sprouting, female and male floral initiation, turion formation, and plant senescence occurred at 7.0, 10.1, 25.9, 23.1, 20.1, and 7.5° C, respectively.

### **Introduction**

A federal noxious weed, hydrilla has been called “the perfect aquatic weed” (Langeland 1996), and is considered one of the worst aquatic weeds worldwide (Shearer and Jackson 2006). This plant is ubiquitous, being found on every continent except Antarctica, and has the ability to survive in drastically different water systems, from small, natural ponds, man-made canals and reservoirs, to flowing water (Dayan and Netherland 2005). Hydrilla is able to grow in a wide range of pH values, temperature, dissolved oxygen (DO) and nutrient concentrations (Madsen and Owens 2000).

Two biotypes are found in the United States (US) - a triploid monoecious form and a triploid dioecious pistillate form, both of which are expanding their range in North America (Cook and Lüönd 1982). Dioecious hydrilla is typically found in the warmer climate of southern states, while monoecious hydrilla is typically found in North Carolina and north in more temperate climates. From 2000-2011, dioecious hydrilla was found in three new states, while monoecious hydrilla was found in fifteen new states. Despite this range expansion, there is little knowledge as to how monoecious hydrilla will behave in new climates (Netherland and Greer 2014). The majority of research on hydrilla has been conducted in southern climates on the dioecious biotype. Therefore, further research on monoecious hydrilla in northern climates is needed to understand plant growth and phenology (Netherland and Greer 2014).

Madeira et al (2000) stated that even though the biotypes may appear visually similar, there are important differences in the biotypes that must be understood and applied to develop effective management systems. These life strategies differences between the biotypes include physiology, reproduction, and growth behavior. Experiments have been completed to quantify the differences between monoecious and dioecious hydrilla, looking at asexual propagule formation in Florida (Steward and Van 1987; Sutton et al. 1992), and plant biomass accumulation in Texas (Grodowitz et al. 2010).

Hydrilla has two asexual propagules that are vital to the survival of the species over both time and space. These propagules are thought to be the most important source of hydrilla biomass, as monoecious hydrilla has no overwintering biomass in cooler climates (Sutton and Portier 1985). Green axillary turions, produced on stems, are leaf scales surrounding meristematic tissue, and show a longevity of less than a year (Van and Steward 1990). Subterranean turions, referred to as tubers, are formed at the end of underground rhizomes and

increase the chance of survival of the species over time, as they remain viable for up to ten years (Hodson et al. 1984). When in the soil, mature tubers are resistant to freezing, desiccation, and chemicals, complicating management strategies (Hodson et al. 1984). Due to the importance, the production potential, and the longevity of these propagules, it is vital to understand the production and maturation of these propagules to have an effective management strategy.

Monoecious hydrilla produces both female and male flowers on the same plant (Langeland 1996). Female flowers have three, white sepals and three clear petals, which arise from a single spathe (Cook and Lüönd 1982). The sepals are 10-50 mm long, and 4-8 mm wide. The flowers, attached at leaf axils close to the tip of the stems, float on the surface of the water when mature (Langeland 1996). Male flowers have three sepals that are white or brown, are smaller than female flowers, and have been observed to develop before female flowers (Cook and Lüönd 1982). Male flowers float freely on the water surface when mature, and there can be thousands floating in the water at one time, reaching densities of 310 male flowers per m<sup>2</sup> in North Carolina (Hodson et al. 1984). Monoecious hydrilla is able to produce viable seeds, though seed production is more common in tropical regions, and no seed production has been observed in North Carolina (Harlan et al. 1985).

Dissimilar climates have been previously reported to affect growth and development of monoecious hydrilla. A study by Sutton et al. (1992) showed that monoecious hydrilla biomass was able to overwinter when grown in South Florida. However, when grown in Texas, no monoecious hydrilla biomass overwintered (Owens et al 2012). In both natural systems and mesocosms in North Carolina (NC), no monoecious hydrilla biomass is present over winter (Grodowitz et al 2010; Hodson et al 1984; True-Meadows 2013). Therefore, in a more temperate

climate, there is no overwintered biomass, while in a more tropical climate, biomass is able to overwinter.

Hydrilla has a phenotypic plasticity, as its morphology is influenced by different environmental factors (Richards et al. 2006). The ability of the plant to adapt its morphology to the environment optimizes the utilization of light. In fact, species that are able to alter their morphology to survive a wide range of environmental conditions are usually more competitive than plants that are unable to adapt (Barko et al. 1981). Other environmental factors that affect plant plasticity include water velocity, temperature, photoperiod, and water chemistry, all of which are affected by the location of the body of water (Madsen 1991).

Interactions of different climatic conditions, such as increased early season photoperiod and decreased temperature have not been defined for monoecious hydrilla. It is important to understand how different environmental conditions will alter the growth, development, and phenology of monoecious hydrilla, as the range of monoecious hydrilla expands in the US (Netherland 1997). The objective of these mesocosm studies was to gain a better understanding of the growth and development of monoecious hydrilla when grown in different climates.

### **Methods and Materials**

Two different research locations were selected to investigate the effect of different climatic conditions on the growth and development of monoecious hydrilla from May 2015 to December 2016. Cool climate research was conducted at the North Carolina Department of Agriculture and Consumer Science (NCDA&CS) Upper Mountain Research Station (UMRS) in Laurel Springs, NC (36.396656, -81.306981). Warm climate research occurred in Raleigh, NC on North Carolina State University (NCSU) Research Farm #2 (35.815484, -78.732425). Laurel Springs was selected to simulate a northern climate, with yearly average temperatures similar to



Albany, New York, and Ann Arbor, Michigan (Table 2-1). Raleigh was selected as the southern research location because it is climatically different from Laurel Springs, yet still close enough that the same research team could complete the same experiments at the different locations.

Water in Laurel Springs was supplied from a well located on UMRS, while water at Raleigh was supplied by an irrigation pond. Samples of water from both locations were collected and analyzed by the NCDA&CS, Solution Analysis Section in Raleigh, NC, where water quality tests were determined in 2016 (Table 2-2). At both locations, the experiments were covered with 30% shade cloth (DeWitt Company, Sikeston, MO) to moderate the water temperatures. Daily water temperature and lumens available were measured with a HOBO Pendant® data logger (Onset Computer Corporation, Bourne, MA). Ambient temperature and photosynthetically active radiation (PAR) were measured by NCDA&CS weather stations near both research locations from May 2015 to December 2016. Raleigh weather data was collected at Reedy Creek Field Laboratory, while Laurel Springs weather data were collected at UMRS.

Sixty unsprouted monoecious hydrilla tubers were collected from Shearon Harris Reservoir (35.612378, -78.942986) in Wake County, NC on May 25, 2015. This reservoir has been infested with hydrilla since the early 1980s, has no current hydrilla management plan, and has been described in detail by Nawrocki et al (2016).

Tubers were sprouted in a greenhouse, and single sprouted tubers were planted in plastic Classic 300s six-inch nursery pots (2.9 L; 17.8x16.5 cm). Pots were filled with approximately 15 cm of Scott's Premium topsoil (Scotts, Marysville OH) and topped with 3 cm of play sand (Quikrete®, Atlanta, GA). After planting, thirty pots were evenly divided into six plastic outdoor 1040 L mesocosms (Benchmark Earth Works, Sanford, NC) at both locations. Plants were

allowed to grow and develop naturally until senescence. Visual observations were recorded on a biweekly basis noting events including as sprouting, flowering, and turion development.

On November 10, 2015, five pots were destructively harvested from Laurel Springs, while a destructive harvest was conducted in Raleigh on November 20, 2015. From these harvests, the number of turions and tubers produced throughout the growing season were counted, measured, and weighed. A greenhouse viability test was conducted in 88.7 mL microcosms with deionized water. A propagule was called viable if it sprouted, and all viability studies were run until the propagules either sprouted or rotted.

From 2015-2016, twenty-five six-inch black plastic pots were overwintered, undisturbed in outdoor mesocosms. In spring of 2016, biweekly observations were taken of the pots, noting sprouting dates of both turions and tubers. After tubers ceased sprouting, another destructive harvest was conducted at both locations, and all propagules were counted, measured, weighed, and a separate greenhouse viability test was conducted. This harvest occurred on June 5, 2016 in Raleigh and on June 18, 2016 in Laurel Springs.

The number of propagules formed, their weight, and sprouting times of both turions and tubers were recorded for both locations. Two-sample t-tests were utilized to compare ambient and water temperature between locations throughout the entire study. Number of propagules produced were compared across locations using a two-sample t-test. This study was focused on evaluating the performance of hydrilla growth, biomass quantity and propagules formed when grown in a cooler climate.

## **Results and Discussion**

During the study period, Raleigh had higher mean, minimum, and maximum ambient temperatures, with a p-value  $< 0.0001$  (Table 2-3). However, mean precipitation and PAR were

similar for each location during the duration of the study with p-values of 0.3268 and 0.7548, respectively. Raleigh temperature range was -4.5 to 35.4° C, while Laurel Springs temperatures ranged from -9.5 to 29.1° C. Mean PAR ranged from 98.0 – 580.4  $\mu\text{moles}/\text{m}^2 \text{ s}^{-1}$  in Raleigh and from 95.7 – 616.1  $\mu\text{moles}/\text{m}^2 \text{ s}^{-1}$  in Laurel Springs.

The overall mean water temperature over the entire study was higher at Raleigh, with a p-value of < 0.0001. Mean water temperature averaged 18.6° C in Raleigh, with water in Laurel Springs averaging 13.5° C (Table 2-4). There was not a difference in the amount of light available in the mesocosms between locations, with a p-value of 0.588.

In Raleigh, turions began to sprout in late March when the mean water temperature was 12.4° C with tubers sprouting in early April at mean water temperatures of 15.0° C (Table 2-5). Turion sprouting in Laurel Springs began in early April, with mean water temperature of 7.0° C and tubers sprouted in mid-April at a mean water temperature of 10.1° C. There was a two-week window of time between turions and tubers sprouting in Raleigh, while there was a one-week window between these two events in Laurel Springs (Figure 2-1).

Tubers continued to sprout until early June in Raleigh and mid-June in Laurel Springs. The length of new propagule sprouting was the same between the locations, simply offset two weeks later in Laurel Springs. There was not a difference between locations in the number of sprouted tubers after being overwintered at both locations. On average, there were 5 tubers per pot that sprouted in spring 2016 at both locations (Table 2-6). In Raleigh, approximately 30% of tubers produced sprouted, while in Laurel Springs, close to 65% of tubers sprouted in the spring. As the accumulation of tubers in the hydrosol makes treatment extremely difficult, the tuber bank is important to management. If cooler climates have a higher tuber sprouting rate than southern climates, the tuber bank might not build up as quickly. Nevertheless, a study completed

by Nawrocki et al. (2016) showed that tuber densities as low as 11 tubers per  $m^{-2}$  were adequate for a significant recovery in biomass. All mesocosms at both locations had tuber densities that were much higher than 11 tubers  $m^{-2}$ , meaning that even though tuber densities might be lower in northern climates, they still would pose serious problems to managers.

Understanding and predicting sprouting of asexual propagules is vital to an efficient management plan, as they are very important to the survival of hydrilla (McFarland and Barko 1999). Hodson et al. (1984) recorded that tubers were the primary method of monoecious hydrilla overwintering in NC lakes. In a field study, it was found that monoecious hydrilla turions in Raleigh, NC sprouted about two weeks before tubers, with sprouted tubers being found in early April (True-Meadows, 2013). The propagules observed in the Raleigh mesocosms sprouted around the same time as the field studies completed in Raleigh. However, turions and tubers sprouted later in Laurel Springs mesocosms, indicating that different temperatures found in these locations influenced the time that sprouting began. Tubers grown in the mesocosms in Raleigh had a shorter sprouting window, two months, when compared to four months found by True-Meadows in that field study. This difference might be explained by the fact that this study utilized pots that might have reached carrying capacity quicker than plants growing in a lake, resulting in a smaller sprouting window. Plants growing in a large body of water are not going to reach this carrying capacity as quickly, and therefore propagules might continue sprouting for a longer time.

Turion formation began after flowering at both locations. Turions were first observed forming in Raleigh in early September at a mean water temperature of 24.2° C while turions were formed in Laurel Springs in late September at a mean water temperature of 20.1° C (Table 2-5).

Hydrilla plants at Laurel Springs produced female flowers in late July, with an average water temperature of 25.9° C and male flowers in late August, with a mean water temperature of 23.1° C. Hydrilla plants in Raleigh produced female flowers in early August, with a mean temperature of 27.7° C and male flowers in late August, with a mean water temperature of 23.9° C. Female flowers were present until late October in both locations, while male flower observations ceased in late September.

The floral initiation window observed in Raleigh, starting in July and lasting until October, is similar to the window described by Harlan et al (1985). However, the window is different than what was observed in the field trial completed by True-Meadows in 2013. In that study, it was discovered that floral initiation was observed in Raleigh from mid-September until late October, much later than in this study.

Altered timings of flowering times of monoecious hydrilla have been reported in different US states. Monoecious hydrilla in Umstead State Park in North Carolina (35.873154, -78.766956) began flowering in June, lasting until October (Harlan et al. 1985), while monoecious hydrilla in Ingrams Pond in southern Delaware (38.588652, -75.329175) began in September, lasting until October (Miller 1988). Flowering at these two locations were influenced by climatic conditions, and differed in both timing and duration.

As monoecious hydrilla produces both female and male flowers from one plant, it opens the possibility of seed production. However, it has been found that aquatic herbaceous perennials allocate approximately 25% of all resources to asexual reproduction, and only around 5% to sexual reproduction (Madsen 1991). Therefore, even though seed production is a possibility, it is not currently viewed as important to the annual survival of monoecious hydrilla found in the

United States. In this study, even though both male and female flowers were observed, no seed or fruit production was observed at either location.

At both locations, female flowers were observed before male flowers. This is in disagreement with Cook and Lüönd (1982), who stated that staminate flowers are produced before pistillate flowers. Observations from Delaware stated that both flowers were produced in September-October, not stating exactly what sex of flower was observed first (Miller 1988). Female flowers on hydrilla plants grown in Laurel Springs were present for a longer period of time than hydrilla grown in Raleigh. However, male flowers were present at both locations for the same amount of time. There is limited information published about the longevity of flowers in monoecious hydrilla in both artificial and natural systems.

At Laurel Springs, plant senescence occurred in early December, when mean water temperature was 7.5° C while plant senescence occurred in Raleigh late December, when mean water temperature reached 10.2° C. As expected, plants in Laurel Springs senesced earlier than Raleigh, most likely due to water cooling down earlier in the year when compared to Raleigh. The detached biomass created from plant senescence is important in the distribution and spread of turions to new areas, as some turions are still attached to the biomass (Netherland 1997). Movement of the mat of biomass is influenced by currents, animal disturbances, or wind. This floating biomass enables turions to mature and abscise in a new area to sprout the following year.

Harlan et al. (1985), reported that hydrilla senescence began in late December in North Carolina lakes, agreeing with the timing of plant senescence in this study. They also noted that across different lakes, hydrilla exhibited a range of biomass production and senescence. Therefore, it is important to look at the environmental conditions specific hydrilla populations are subject to when looking at plant senescence.

All six events measured in this study occurred when mean water temperatures were cooler in Laurel Springs than in Raleigh, demonstrating that hydrilla was able to adapt its phenology to a shorter growing season. Simply looking at water temperatures and phenology in southern climates may not be a reliable method to predict phenological events in northern climates. For example, if turion sprouting would have occurred at the same mean water temperature at both locations, the turion sprouting in Laurel Springs would have been delayed two weeks.

As this study was completed in mesocosm conditions, there was likely an effect on when life stages occurred. In a field study completed by True-Meadows (2013), timing of the events was similar, but mean temperatures for all life stages were higher in the field than what was found in this study. In another field study, it was found that monoecious hydrilla in North Carolina began sprouting in late March-early April, when water temperatures reached 11-13° C (Hodson et al. 1984). Both timing and mean water temperature of tuber sprouting in North Carolina lakes lines up with what was observed in mesocosms in Raleigh, but water temperature was much colder in Laurel Springs. As mesocosms provide an optimal environment for hydrilla growth, with reduced interspecific competition and grazing pressure (Netherland 1997), minimum temperature required to complete a physiological stage might possibly be lower. Timing of events observed at Raleigh in this study lined up with two separate field studies also completed in North Carolina. Therefore, the results from Laurel Springs can be used to give an estimate of hydrilla growth in northern climates. More *in situ* studies will need to be completed in northern climates to investigate possible differences.

From the November harvests, an average of twelve tubers were collected from Raleigh, and seven tubers from Laurel Springs (Table 2-6). An average of ten turions were collected from

Raleigh, and an average of seven turions collected from Laurel Springs. There were zero propagules that sprouted in the greenhouse viability study, as there had not been a chilling period to break dormancy before harvesting. Following the April harvest, there was an average of seven tubers collected from Raleigh and two tubers collected from Laurel Springs. An average of six turions were collected from both locations. In the greenhouse viability study, Raleigh tubers had an 85% viability rate, while turions had a 70% viability rate. Laurel Springs tubers had a viability rate of 95%, and turions had an 85% viability rate.

The destructive harvests and subsequent greenhouse viability studies conducted in this study demonstrates a strong environmentally induced dormancy on the propagules of hydrilla grown in both locations. The chilling period requirement has been well documented for hydrilla (Carter et al. 1987; Netherland 1997), and is still active in hydrilla propagules grown in cooler climates. In November, there were no propagules that sprouted, even though they were fully formed and mature. This dormancy is important to understand and incorporate into a management plan. From a natural system, monoecious hydrilla tubers consistently have a 90-95% viability rate, regardless of season (Hodson et al. 1984). Tubers from both locations had similar viability rates as seen in numerous lab viability studies.

As plants were started at the same time, and all conditions other than environmental conditions were the same, the difference in number of tubers produced was directly influenced by climate. As tuber depletion is the key aspect required to break monoecious hydrilla's life cycle (Netherland 1997), understanding tuber production is essential for effective management. This study indicated that tuber production was achieved at both locations, and these tubers were able to overwinter in their respective ambient conditions. Relying on cooler water temperatures in northern climates to reduce the viability of tubers is not a valid management strategy.



## Conclusions

Applying management practices at specific times is vital to controlling hydrilla (True-Meadows 2013), and this study demonstrates that climatic conditions can influence the timing of these events. Completing the same experiments at two separate research locations allowed an investigation into differences of hydrilla growth based on climatic conditions. Different and distinct temperatures were shown to have direct effects on the timeframe of development, along with the amount of tubers that are produced. In a warmer climate, turions and tubers sprouted earlier in the year, but at warmer water temperatures than plants in the cooler climate. Floral initiation of both sexes and plant senescence occurred later in Raleigh. In addition, plants grown in Raleigh produced a greater number of tubers, agreeing with current knowledge of hydrilla growth and development. It was also demonstrated that plants in the cooler climate reached each physiological life stage at a cooler mean water temperature than those grown in the warmer climate. However, this study was completed in artificial settings, there is a need for continued experiments that focus specifically on how hydrilla behaves in cooler climates in natural systems. Results from this research are the most conclusive to date demonstrating the plasticity of hydrilla in dissimilar climates.

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Table 2-1. Mean, minimum, and maximum temperatures, and precipitation for four cities: Raleigh and Laurel Springs, North Carolina, Ann Arbor Michigan, and Albany New York.

	Mean Yearly Temperature °C	Min Yearly Temperature °C	Max Yearly Temperature °C	Mean Annual Rainfall cm	Mean Annual Snowfall cm
Raleigh, NC	16.0	10.2	21.8	118.3	0.0
Laurel Springs, NC	9.8	3.1	16.5	139.3	58.4
Ann Arbor, MI	9.8	4.6	14.9	95.4	144.8
Albany, NY	9.0	3.7	14.3	100.1	149.9

Table 2-2. Water quality tests from Raleigh and Laurel Springs, North Carolina.

Nutrient Concentration <sup>1</sup>	Raleigh	Laurel Springs
Inorganic Nitrogen	0.82	0.94
Organic Nitrogen	0.00	0.16
Phosphorus	0.02	0.01
Potassium	1.46	4.49
Calcium	1.28	16.10
Sodium	1.94	6.35
Chlorine	4.55	5.96

Water Quality	Raleigh	Laurel Springs
pH	6.09	8.56
Electrial Conductivity <sup>2</sup>	0.03	0.15
Carbonates <sup>3</sup>	0.00	0.60
Bicarbonates <sup>4</sup>	0.10	0.43
Total Alkalinity <sup>5</sup>	5.00	51.50
Hardness <sup>5</sup>	6.41	47.40

<sup>1</sup>Parts per million.

<sup>2</sup>mS/cm.

<sup>3</sup>miliequivalent per liter (Meq/L).

<sup>4</sup>miliequivalent per liter (Meq/L).

<sup>5</sup>Parts per million of CaCO<sub>3</sub>.

Table 2-3. Ambient weather conditions in Raleigh and Laurel Springs, North Carolina from May 2015 – September 2016 as measured by NCDA&CS weather stations.

Ambient Temperatures (°C)			
Locations	Mean	Min	Max
Raleigh	19.27	-4.54	35.37
Laurel Springs	14.01	-9.53	29.13

Precipitation Per Week (cm)			
Locations	Mean	Min	Max
Raleigh	0.43	0.00	2.38
Laurel Springs	0.58	0.00	7.30

Photosynthetically Active Radiation			
Locations	Mean	Min	Max
Raleigh	382.29	98.03	580.44
Laurel Springs	383.55	95.66	616.10

Ambient temperatures were higher in Raleigh ( $p < 0.0001$ ). There was no difference in mean precipitation ( $p = 0.3268$ ) or PAR ( $p = 0.7548$ ) between locations.

Table 2-4. Water temperatures in Raleigh and Laurel Springs, North Carolina from May 2015 – September 2016, as measured by HOBO pendants.

Water Temperatures (°C)			
Locations	Mean	Min	Max
Raleigh	18.60	0.23	33.12
Laurel Springs	13.50	-3.79	27.54

Mean water temperatures were higher in Raleigh ( $p < 0.0001$ ).



Table 2-5. Mean, minimum, and maximum temperatures from March 2016 – December 2016 observed for six different physiological growth stages for monoecious *Hydrilla verticillata* at Laurel Springs and Raleigh, North Carolina.

Life Stage	Date	Laurel Springs			Raleigh			
		Temperature (°C)			Temperature (°C)			
		Mean	Min	Max	Date	Mean	Min	Max
Sprouting Turions	3-Apr	7.0	2.0	14.6	23-Mar	12.4	2.1	21.6
Sprouting Tubers	10-Apr	10.1	2.4	15.3	8-Apr	15.0	-0.1	25.4
Female Flowers	24-Jul	25.9	22.8	29.1	7-Aug	27.7	25.7	33.9
Male Flowers	21-Aug	23.1	19.3	27.5	28-Aug	23.9	18.9	31.7
Turion Formation	18-Sep	20.1	17.76	23.1	6-Sep	24.2	18.8	32.2
Plant Senescence	4-Dec	7.5	3.5	14.9	30-Dec	8.5	1.11	7.98

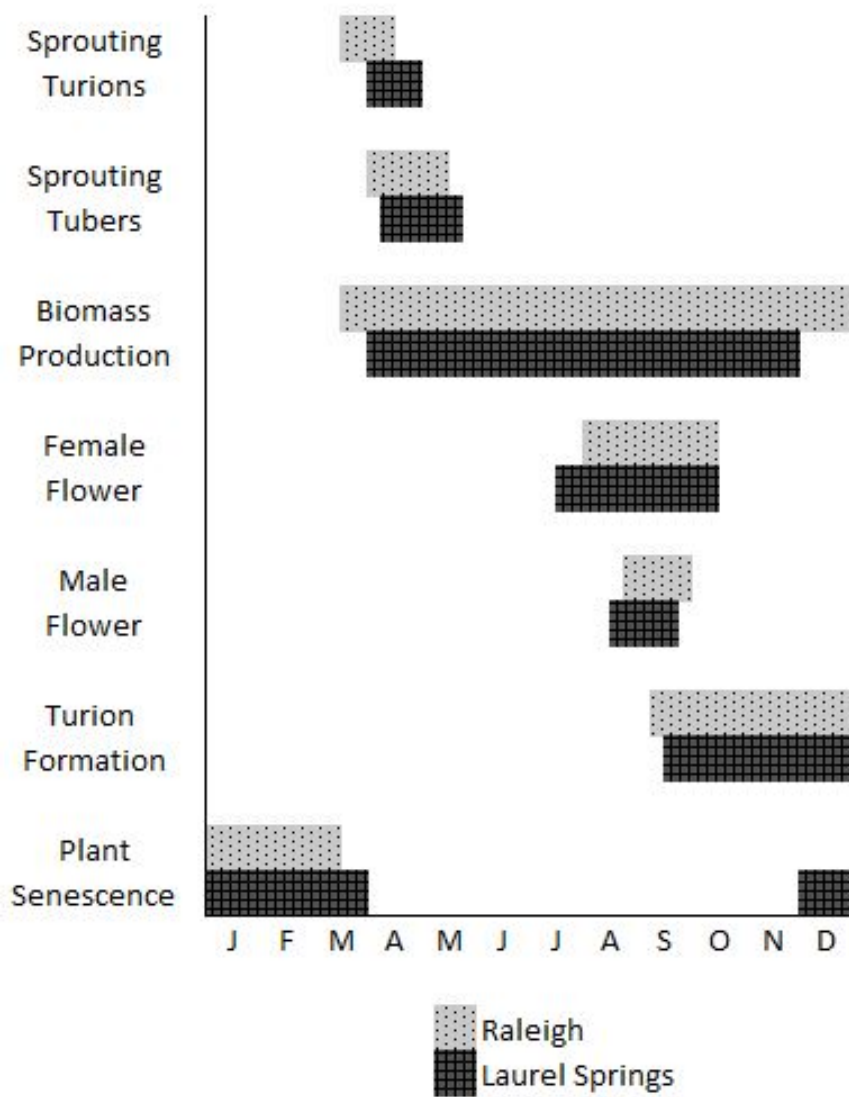


Figure 2-1. Monoecious *Hydrilla verticillata* phenology in Raleigh and Laurel Springs, North Carolina.

Table 2-6. Monoecious *Hydrilla verticillata* propagule production and viability in November 2015 and April 2016.

November Harvest						
	Average Number Tubers Collected		Average Number Turions Collected		Average % Turion Viability	
Raleigh	12	a	10	a	0	a
Laurel Springs	7	b	7	a	0	a

April Harvest						
	Average Number Tubers Collected		Average Number Turions Collected		Average % Turion Viability	
Raleigh	7	a	6	a	85	a
Laurel Springs	2	b	6	a	95	a

Values in the same column with same letters are not different, according to Tukey HSD with  $p < 0.05$ .

## Chapter 3 Competition Study Between Monoecious *Hydrilla verticillata* and Three Submersed Aquatic Plants

### Abstract

The perfect aquatic weed, hydrilla (*Hydrilla verticillata* (L.f) Royle) is a federal noxious submersed aquatic weed. A two-year competition study was completed in two mesocosm types at two research locations to compare the competitive ability of monoecious hydrilla in a warm and a cool climate. Two planting densities of hydrilla were grown with Eurasian watermilfoil (*Myriophyllum spicatum* L.), elodea (*Elodea canadensis* Michx), and vallisneria (*Vallisneria americana* Michx.). Mesocosm type affected the growth and development of hydrilla, especially in the cool climate. When grown alone, average hydrilla biomass in the warmer climate was 57.13 and 40.80 g in above and belowground mesocosms, respectively. In the cool climate, when grown alone, average hydrilla biomass was 72.17 g and 1.94 g in above and belowground mesocosms, respectively. When grown alone, average hydrilla tuber density in the warm climate was 971 and 629 tubers m<sup>-2</sup> in above and belowground mesocosms, respectively. In the cool climate when grown alone, average hydrilla tuber density was 794 and 3 tubers m<sup>-2</sup> in above and belowground mesocosms, respectively. *M. spicatum* and *E. canadensis* with hydrilla suppressed hydrilla growth and development in both mesocosm types at the cool climate compared to hydrilla alone. Hydrilla dry weight of aboveground mesocosms in the cool climate was 24.60 to 35.73 g with *M. spicatum* and 0.78 to 7.04 g with *E. canadensis*, and was 67.87 to 72.17 g when grown alone. Planting combination did not suppress hydrilla growth in either mesocosm type at the warm climate location. Hydrilla stem growth was more highly correlated in the warm climate, with  $r^2$  ranging from 0.83 to 0.89 than in the cool climate, ranging from 0.16 to 0.84. The results from the cool climate location show that in a more suitable environment for the

competitor plants, hydrilla growth and competitiveness was affected. Ensuring that bodies of water have a healthy, diverse plant community might influence the impact hydrilla can have on the aquatic ecosystems in Northern United States.

### **Introduction**

Hydrilla is a federal noxious aquatic weed that has negative effects on aquatic ecosystems, recreation, and human health (Dayan and Netherland 2005). Hydrilla has several adaptations which make it competitive, and it is referred to as “the perfect aquatic weed” (Langeland 1996). These adaptations include having several ploidy levels, numerous reproductive strategies, rapid vegetative growth, and the ability to form thick monospecific mats (Langeland 1996; Dayan and Netherland 2005). When these mats reach the water surface, it is referred to as being “topped out”.

There are two biotypes of hydrilla in the United States (US), a triploid monoecious form and a triploid female dioecious form, with both biotypes expanding their range of growth in North America (Cook and Lüönd 1982). Dioecious hydrilla is typically found in tropical climates, while monoecious hydrilla is typically found in more temperate climates. The majority of hydrilla research has been conducted in southern climates on the dioecious biotype, and results might not be able to predict monoecious hydrilla growth and development in cooler climates (Netherland and Greer 2014).

Aquatic plant community composition is an important component to consider when predicting the invasion potential of hydrilla (Smart et al. 1994). Invasive plants that fill an empty niche are much more likely to establish and thrive in an environment than when that niche is occupied by another species. It is believed that a healthy population of native plants could be a means of slowing down the invasion of hydrilla through competition (Owens et al. 2008).

Northern water bodies are known for a diverse mix of submersed aquatic vegetation (Serafy et al. 1994), adding another variable into the uncertainty as how hydrilla will perform and compete in these climates.

Hydrilla has two asexual propagules that are vital to the survival of the species over both space and time. Green axillary turions are produced on the above ground biomass, and are distributed with currents on plant fragments (Madiera et al. 2000). Subterranean turions, referred to as tubers in literature, are formed at the end of underground rhizomes and can remain viable for five to ten years (Nawrocki et al. 2016). These propagules have been identified as the most important sources for growth the following year, especially since monoecious hydrilla maintains no overwintering biomass in cooler climates (Hodson et al. 1984). Production of these propagules increases the competitive abilities of hydrilla (Chadwell and Engelhardt 2008).

Previous hydrilla competition studies have been conducted, but no study has observed the influence of climate on the competitive advantages of hydrilla and other species. In previous studies, hydrilla has been shown to be a superior competitor with many submersed aquatic plants, both native and invasive, such as *Vallisneria americana* Michx., *Egeria densa* Planch., *Myriophyllum spicatum* L., and *Ceratophyllum demersum* L. (Hofstra et al. 1999; Mony et al. 2007; True-Meadows 2013; Wang et al. 2008).

Hofstra et al. (1999) determined that when some plant species were able to establish before staminate dioecious hydrilla introduction, hydrilla growth was more likely to be suppressed in New Zealand. Therefore, the more established and diverse a plant community is, the greater resistance to hydrilla infestation an ecosystem would be. A study by Owens et al. (2008) reported that established *V. americana* plants could suppress hydrilla growth. This led the

authors to suggest planting native plants to remove empty space in an ecosystem as a practical hydrilla management method.

*M. spicatum* is an introduced, invasive submersed aquatic weed in the family Haloragaceae that is especially a problem in the northern US, though it found throughout the continental US (Trudeau 1982). This plant was originally introduced to the US from Eurasia for the aquarium plant trade before the 1880s, though it was not considered a weed problem until the 1920s (Nichols and Shaw 1986). The only submersed plant that has demonstrated the ability to compete with *M. spicatum* in North America is hydrilla (Aiken et al. 1979). A complete plant description can be found completed by Aiken et al (1979).

*E. canadensis* is a member of Hydrocharitaceae that is native to North America and shares many morphological and physiological similarities to hydrilla (Ancibor 1979). These similarities include the ability to utilize bicarbonate as an inorganic carbon source, vegetative fragmentation, growth habit and plant biomass production potential. Elodea was specifically selected for this study due to similarities in growth habit and phenology to hydrilla. *E. canadensis* does not produce asexual propagules, and relies on fragmentation for survival (Ancibor 1979). Despite being a native species to North America, it has been reported as a nuisance weed in the Chesapeake Bay, Tennessee Valley, and the Currituck Sound in recent years, due to its many adaptive features (Zettler and Freeman 1972). It is also considered a weedy species in Canada and several other countries (Spicer and Catlin 1988)

*V. americana* is a North America native plant in Hydrocharitaceae, and is an important food source for many species of waterfowl and other organisms (Catling et al. 1994). This plant is native to and widespread in eastern North America, with a range from southeastern Canada to Florida. There are several biotypes that allow the plant to grow in different climates (Lokker et

al. 1994). *V. americana* is a dioecious clonal macrophyte, and relies on winter buds to reproduce in temperate areas (Titus and Stephens 1983). The plant develops a strong root system and rhizomes, and produces winter buds to overwinter (Catling et al. 1994).

As the majority of competition studies conducted with hydrilla have been completed with the dioecious biotype in southern climates, there is uncertainty as to how competitive hydrilla will be in northern climates. Therefore, the objective of this study was to evaluate the effect of different climatic conditions on the competitive nature of monoecious hydrilla.

### **Methods and Materials**

Outdoor mesocosms were located at two separate research locations to test the effect of climate on species competitiveness. The study was repeated over two years, running from June-October and May-October in 2015 and 2016, respectively. The warm climate research site was at Reedy Creek Research Farm #2 in Raleigh, NC (35.815484, -78.732425). The cool climate research site was the North Carolina Department of Agriculture and Consumer Science (NCDA&CS) Upper Mountain Research Station (UMRS) in Laurel Springs, NC (36.396656, -81.306981). These two locations were selected because they exhibited different climates, but were close enough in proximity that one research team could complete the same experiments at both locations. Raleigh has an elevation of 96 m, while Laurel Springs has an elevation of 838 m, leading to dissimilar climates. Ambient weather temperature and photosynthetically active radiation (PAR) were measured for Laurel Springs at UMRS, and for Raleigh from the Reedy Creek Field Laboratory weather stations from May 2015-October 2016, spanning the entire study.

In 2015, plastic 120 L aboveground mesocosms (US Plastic Corp, Lima, OH) were used in Raleigh, while plastic 208 L belowground mesocosms (Benchmark Earth Works, Sanford,



NC) were used in Laurel Springs. Aboveground mesocosms had no constant exchange of water, though water was replenished biweekly to a constant height. Occasional flushing of these containers occurred to reduce algal growth as needed. Water flowed through belowground mesocosms with sixteen complete water exchanges per day. Both mesocosm types were utilized at both locations in 2016.

For both years, each research site was covered by 30% shade cloth (DeWitt Company, Sikeston, MO) to regulate water temperature and light intensity reaching the plants. Water for the Raleigh location was supplied by an irrigation pond, and a well supplied water to Laurel Springs. Water samples were processed by NCDA&CS (Table 3-1). Light intensity, measured in  $\mu\text{mol m}^{-2} \text{sec}^{-1}$  and water temperature was continuously measured every six hours with a HOBO Pendant® data logger (Onset computer Corporation, Bourne, MA) that was clipped to the pot rim.

Plants used in the study included monoecious *H. verticillata*, *M. spicatum*, *E. canadensis*, and *V. americana*. The last three plants will be referred to as the competitor species hereafter, and were selected because of their likelihood of competing with new hydrilla invasions. In late May 2015 and early May 2016, unsprouted hydrilla tubers were collected from Shearon Harris Reservoir in Wake County, North Carolina. This reservoir became infested with hydrilla in the 1980s, and has no current management plan, with a complete description by Nawrocki et al. (2016). *E. canadensis* and *M. spicatum* were collected from local sources and the northern biotype of *V. americana* was collected in New York and shipped to Raleigh. All plants, including hydrilla were allowed to grow and become established in a greenhouse in Raleigh in 3.5 L buckets until they were transplanted.

Transplanting into outdoor mesocosms occurred in late May 2016 and early June 2015. In both mesocosm types, all competitor plants were planted at a density of two plants, and were grown with a density of zero, two, or four hydrilla plants. 700 Series Nursery pots (23.02 L; 36 x 30 cm) were filled with 15 cm of bagged topsoil (Scotts Premium Topsoil, Scotts, Marysville OH), amended with 15-9-12 slow release fertilizer (Osmocote Plus, Scotts, Marysville OH) and covered in a 3 cm layer of play sand (Quikrete®, Atlanta, GA). Fertilizer was incorporated to ensure that plants initially had sufficient nutrient levels. Sand was used to reduce soil and nutrient suspension in the water, further limiting algal growth. After planting, pots were placed in the belowground mesocosms. 1000S Series nursery pots (39.7 L; 45 x 30 cm) were prepared in the same manner as previously described, except they were filled with 14 cm of bagged topsoil (Scotts Premium Topsoil, Marysville OH). These pots were planted and then placed in the aboveground mesocosms.

Biweekly measurements were taken on the longest stem lengths of all species, beginning when plants were transplanted in May until harvest in October of both years. Percent coverage of all plants were also recorded, along with water pH, temperature, and dissolved oxygen. A destructive harvest occurred in October at both locations in both years. Aboveground biomass and belowground biomass were kept separate, and rinsed before drying at 70° C for 72 hours. Asexual propagules were counted and separated from treatments with hydrilla (turions and tubers) by using 3 mm sieves. Hydrilla tuber production and aboveground dry weight were recorded with aboveground dry weight of all corresponding competitor species. These parameters were compared both within and between locations in 2016 in the same mesocosm type. 2015 results were compared to 2016 results within the same location and mesocosm type.

Treatments were replicated three times and arranged in a completely randomized block design. Plant dry weight and hydrilla propagule production were analyzed using SAS statistical software (SAS Institute Inc., Cary, North Carolina). Comparisons between climatic conditions between locations were carried out using two-tailed t-tests, with  $p < 0.05$ . Plant biomass, and propagule formation comparisons within locations were analyzed using analysis of variance (ANOVA), with means separated by Tukey's HSD ( $p < 0.05$ ). Regression analysis was run on hydrilla stem length over time, with  $p < 0.05$ .

## **Results and Discussion**

### Weather data

Raleigh had higher mean ambient temperatures in both years  $p < 0.0001$  (Figure 3-1). Temperature in Raleigh averaged 23.7° C, while Laurel Springs averaged 18.3° C from May to October in 2015 and 2016. There was no difference in the amount of precipitation  $p = 0.247$  or the mean PAR  $p = 0.877$  that each location received. Between years in the same location, there were no differences in parameters measured. Within each location, mean water temperatures were not different between the mesocosm types, but were between locations, with cooler temperatures in Laurel Springs  $p < 0.0001$  (Figure 3-2). The mean water temperature in Raleigh was 25.3° C in Raleigh and 22.4° C in Laurel Springs.

At both locations, aboveground mesocosms had more light availability as measured by the HOBO Data Loggers. In Raleigh, aboveground mesocosms received an average of 1.0  $\mu\text{mol m}^{-2} \text{s}^{-1}$  while belowground mesocosms received an average of 0.4  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . Laurel Springs aboveground mesocosms received 3.2  $\mu\text{mol m}^{-2} \text{s}^{-1}$  on average, while belowground mesocosms averaged 0.7  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . Between locations, greater lumens were measured at Laurel Springs.

As reduction in irradiance has the ability to alter submersed species composition (Barko et al. 1986), light availability has a large impact on community structure and subsequent competitiveness. Light availability decreased at both locations in all containers throughout the study due to biomass accumulation and algal accumulation on the pendants. As less overall biomass was present in Laurel Springs in both mesocosms, more available light was recorded by the pendants. Light availability and temperature impact the growth and development of aquatic submersed vegetation (Barko et al. 1986). Results from this study indicate that climatic conditions had a large impact on the growth and competitiveness of monoecious hydrilla and the three competitor plants.

The growth and development of monoecious hydrilla at Laurel Springs was not compared at different photoperiods or amounts of precipitation. Therefore, more research will need to be conducted in areas with completely different climates. In addition, *in situ* experiments will need to be conducted in areas to determine what effect the artificial environments of mesocosms had on the results.

### Mesocosm Effect

There was a mesocosm effect on both hydrilla biomass and propagule production within the same location in 2016, especially when hydrilla was grown without competition  $p < 0.0001$  (Figure 3-3). At both locations, aboveground mesocosms produced more hydrilla biomass than belowground mesocosms. This effect was more pronounced in Laurel Springs, where there was an interaction of mesocosm type on all hydrilla parameters measured.

In Raleigh aboveground mesocosms, the dry weight of hydrilla grown without competition averaged 57.13 g, while in belowground mesocosms averaged 40.80 g (Figure 3-3). In Laurel Springs aboveground mesocosms, the dry weight of hydrilla without competition

averaged 72.17 g, while belowground mesocosms averaged 1.94 g. Tuber production in Raleigh averaged 971 tubers m<sup>-2</sup> and 629 tubers m<sup>-2</sup> for above and belowground mesocosms, respectively. Laurel Springs tuber production averaged 794 tubers m<sup>-2</sup> and 3 tubers m<sup>-2</sup> in above and belowground mesocosms, respectively.

As mesocosms received the same environmental conditions and water quality was similar within locations, there was an interaction between mesocosm type and location, especially in Laurel Springs. Cooler temperatures experienced by Laurel Springs and the lower light levels experienced by belowground mesocosms most likely created a less suitable environment for hydrilla growth. Reduced light availability might have led to the greatly reduced hydrilla biomass and propagule production in Laurel Springs. However, it has been found that hydrilla can grow at extremely low levels of light (Van et al. 1976), so it is more likely that interactions between these and other climatic conditions that were experienced in Laurel Springs explains this difference.

Another aspect that might have been influenced by mesocosm size is total hydrilla biomass accumulation. Hydrilla has been shown to be able to occupy as much space as possible (Steve Hoyle, pers. comm.). In this study, hydrilla occupied all the available growing space and quickly reached the carrying capacity of the mesocosms, except for belowground mesocosms in Laurel Springs. The lower dry weights of hydrilla seen in this study compared to natural systems can be explained by limited expansion potential, as hydrilla reached growing capacity quickly in the mesocosms, especially in Raleigh.

Hydrilla plasticity is demonstrated by the changes in biomass accumulation and tuber production based on both location and mesocosm type. This study indicates that the volume, position, and set up conditions are able to affect the results of mesocosm trials, and are an

important aspect of any study. Greater understanding of how mesocosms influence research results will be important to be able to predict what might happen in natural systems, especially in temperate climates.

### Within Location Results

#### **Raleigh**

In aboveground mesocosms, hydrilla tuber production and biomass accumulation were similar, ranging from 524 to 998 tubers m<sup>-2</sup> and 34.47 to 67.00 g regardless of planting combinations in 2016 (Table 3-2). Hydrilla grown in Raleigh belowground mesocosms had no differences in tuber production or biomass accumulation. Tuber production ranged from 246 to 629 tubers m<sup>-2</sup> and dry weight of hydrilla ranged from 29.8 to 41.93 g.

In aboveground mesocosms in Raleigh, all competitor species' dry weights were suppressed when grown with any density of hydrilla (Table 3-3). *M. spicatum* dry weight was 15.57 g when grown alone, but only 0.62 and 0.43 g when grown with two and four hydrilla, respectively. *E. canadensis* dry weight was 36.67 g when grown alone, and was 11.62 and 4.76 g when grown with two and four hydrilla, respectively. *V. americana* was 56.61 g when grown alone and only 7.52 and 1.89 g when grown with two and four hydrilla, respectively. The same pattern was seen in belowground mesocosms in Raleigh. *M. spicatum* dry weight was 2.48 g when grown alone, and 0.07 and 1.01 g when grown with two and four hydrilla, respectively. *E. canadensis* dry weight was 31.83 g when grown alone, and 0.00 and 1.41 g when grown with two and four hydrilla, respectively. *V. americana* was 23.94 g when grown alone and 4.64 and 0.03 g when grown with two and four hydrilla, respectively.

In this study, hydrilla grown in both mesocosms at Raleigh was not negatively impacted by competition. This was demonstrated by the fact that there were no differences between the

number of tubers or biomass produced between hydrilla grown without competition or at any density with all competitor species. In practical terms, this means that hydrilla is a more robust competitor in warmer climates, which is what has been observed in the Potomac River (Carter et al. 1994).

All competitor dry weights were negatively affected when grown with any density of hydrilla. This demonstrates that both climate and competition with hydrilla impact the growth and development of the three competitor plants used in this study. A more suitable climate for hydrilla and less suitable climate for the competitor species explains the reduced growth of all competitor plants. The decreased competitive nature of the other species might be that *M. spicatum* and *E. canadensis* are cool-season plants, and more suited for growth at Laurel Springs as compared to Raleigh. Therefore, it would be expected that these species would grow less robustly in a warmer climate.

### **Laurel Springs**

In Laurel Springs aboveground mesocosms, both hydrilla tuber production and dry weight were influenced by planting combinations (Table 3-2). Hydrilla tuber production and dry weight was suppressed at both hydrilla densities when grown with either *M. spicatum* or *E. canadensis*. With *M. spicatum*, tuber production ranged between 90 to 120 tubers m<sup>-2</sup> and with *E. canadensis*, tuber production ranged from 15 to 65 tubers m<sup>-2</sup>. All other planting combinations ranged from 711 to 1,000 tubers m<sup>-2</sup>. Hydrilla dry weight was 24.60 to 35.73 g when grown with *M. spicatum* and 0.78 to 7.04 g when grown with *E. canadensis*. When grown at any density with *V. americana* or grown alone, hydrilla dry weight ranged from 67.87 to 72.17 g.

In Laurel Springs belowground mesocosms, hydrilla tuber production was not affected by planting combination (Table 3-2). Tuber production ranged from 0 to 49 tubers m<sup>-2</sup>. However,

hydrilla biomass was influenced by planting combinations. When two hydrilla plants were grown with *E. canadensis* or *V. americana*, a reduction of hydrilla biomass was observed compared to hydrilla grown alone. When grown with *E. canadensis* and *V. americana*, hydrilla dry weight was 1.09 and 0.34 g, respectively. In addition, four hydrilla grown with *M. spicatum* resulted in a suppression of hydrilla biomass. This planting combination resulted in 0.34 g of hydrilla dry weight. With all other planting combinations, hydrilla dry weight ranged from 1.40 to 7.88 g.

In Laurel Springs, the suppression of the competitor plant was not observed with *M. spicatum* or *E. canadensis* when grown in both mesocosm types (Table 3-3). In aboveground mesocosms, *M. spicatum* dry weight was 42.37 g when grown alone, and 26.91 and 38.81 g when grown with two and four hydrilla, respectively. *E. canadensis* dry weight was 83.31 g when grown alone, and 91.12 and 97.53 g when grown with two and four hydrilla, respectively (Table 3-3). *V. americana* exhibited suppressed stem dry weight accumulation when grown with both densities of hydrilla in aboveground mesocosms. *V. americana* weight was 34.41 g when grown alone and 8.88 and 5.64 g when grown with two and four hydrilla, respectively.

No competitor dry weights were suppressed when grown with any density of hydrilla in belowground mesocosms in Laurel Springs (Table 3-3). *M. spicatum* grown alone had a dry weight of 8.10 g, with 4.13 and 7.73 g when grown with two and four hydrilla, respectively. *E. canadensis* dry weight was 2.73 g when grown alone and 3.69 and 3.42 g when grown with two and four hydrilla, respectively. *V. americana* dry weight was 34.23 g when grown alone, and 34.53 and 29.23 g when grown with two and four hydrilla, respectively.

Hydrilla biomass results in Laurel Springs contradict the results found by True-Meadows et al. (2013), where hydrilla decreased the biomass of the other species of plants. At Laurel Springs, *M. spicatum* and *E. canadensis* biomass was not affected by any density of hydrilla. In



fact, these two species of plants actually suppressed the biomass accumulation of hydrilla. Differences in temperatures between locations appeared to have created a more suitable environment for the competitor species and a less suitable environment for hydrilla in Laurel Springs.

Tuber production has been found to be affected by the type of hydrosoil, season, nutrient availability, and health of plants (Basiouny et al. 1978). Differences in tuber densities found in other mesocosm trials can be explained with different environmental conditions, soils, and vitality of plants used in other studies. This study utilized amended top soil, which was different than other sediments used for mesocosm trials, and different than natural sediment.

Nawrocki et al. (2016) reported that in a reservoir in North Carolina, a tuber density of 11 tubers  $m^{-2}$  was enough to support hydrilla regrowth. With no hydrilla treatment, at the end of the growing season, densities were found to have increased over 100 fold. This exhibits the tremendous potential of hydrilla to produce tubers in one growing season. The average number of tubers produced in this study in all planting combinations in both mesocosms at both locations were greater than this threshold. This demonstrates that if hydrilla is able to produce tubers, the plant will be able to establish in that area.

In general, aboveground tuber densities in both mesocosms at Raleigh and Laurel Springs were more comparable to natural systems (Harlan et al. 1985) while Laurel Springs belowground tuber densities more closely aligned with a previous competition study (True-Meadows 2013). Discrepancies between tuber production in artificial mesocosms and natural systems have been documented (Netherland 1997). Traditionally, hydrilla grown in mesocosm trials produces a greater number of propagules. This is thought to be due to more favorable environment, with reduced grazing and competition, enhancing hydrilla growth (Netherland 1997). Added stress to

hydrilla plants from competition could help explain the lower tuber densities found in this study compared to other monoecious hydrilla mesocosm trials.

### 2016 Between Location Results

In aboveground mesocosms, hydrilla tuber production differed between locations when grown with *M. spicatum* and *E. canadensis* (Table 3-4). In Raleigh, more tubers were produced at both hydrilla densities when grown with these two plants. All other planting densities did not differ in regards to tuber production between locations in 2016. Dry weight of hydrilla differed between locations when grown at both densities with *E. canadensis*, with biomass being greater in Raleigh at both hydrilla densities. When grown alone, hydrilla produced more biomass in Laurel Springs in 2016.

In belowground mesocosms, there were differences between hydrilla tuber production, and biomass between locations based on planting combinations (Table 3-4). Tuber production was greater in Raleigh in all planting combinations except for two hydrilla with *E. canadensis*, where tuber production was similar between locations. Hydrilla dry weight was greater in Raleigh for all planting combinations.

Complete topping out of hydrilla in aboveground mesocosms occurred first at Laurel Springs, being observed in late June of both years, while topping out was not observed in Raleigh until mid-July of both years. In belowground mesocosms, hydrilla did not top out in Laurel Springs, while Raleigh plants topped out in mid-July. Even though hydrilla topping out occurred first at Laurel Springs, the competitor species' biomass was not suppressed as it was in Raleigh (Table 3-3).

In aboveground mesocosms, there was no difference between *M. spicatum* or *V. americana* regardless of planting combination biomass between locations (Table 3-3). In all

planting combinations with *E. canadensis*, more biomass was accumulated in Laurel Springs compared to Raleigh. In belowground mesocosms, *M. spicatum* biomass was greater when grown at a density of two hydrilla at Laurel Springs. No other planting combination affected *M. spicatum* biomass between locations. *E. canadensis* grown alone had more biomass in Raleigh, but did not vary when grown with either two or four hydrilla between locations. *V. americana* grown alone did not have a different biomass between locations. However, when grown in any competition with hydrilla, higher biomass was recorded in Laurel Springs.

*M. spicatum* and *E. canadensis*, while both being able to grow in several different environmental conditions, are more commonly found in northern climates. This helps explain why *E. canadensis* was more productive in Laurel Springs. The northern biotype of *V. americana* was used in this study, and biomass was not different between locations in aboveground mesocosms, but in belowground mesocosms, was greater in Laurel Springs.

Trends within location with competitor plants were similar, while trends across locations were different. Practically speaking, this means that competitor plant growth was more similar within locations than between locations. This demonstrates that ambient and water temperatures affected the competitive nature of both hydrilla and the competitor plants. The fact that when grown with any density of hydrilla in either mesocosm, the competitor plants produced more biomass in Laurel Springs supports the empty niche theory (Owens et al. 2008).

From this study, *V. americana* does not appear to be an effective competitor of hydrilla at both locations. When grown with *V. americana*, hydrilla growth and development did not differ from hydrilla grown by itself. Therefore, planting communities of this species to discourage the infestation of hydrilla may not be the most effective management strategy, especially if the

plants are not given enough time to establish. This study suggests *E. canadensis* would be a better selection of a native plant to fill any empty niches in the environment.

Hydrilla growth in all planting combinations were more highly correlated in Raleigh than in Laurel Springs, as indicated by regression analysis (Table 3-6). Correlation of stem length ranged from 0.83 to 0.89 in Raleigh, and from 0.16 to 0.84 in Laurel Springs. Maximum growth, or the point where stem lengths did not increase, were achieved earlier in Raleigh than in Laurel Springs in all planting combinations except for two hydrilla with *V. americana*. However, hydrilla alone at Raleigh and both hydrilla densities grown with *E. canadensis* in Laurel Springs did not reach maximum growth within the confines of this study.

Higher correlations in Raleigh indicate that hydrilla grew in a more predictable way than in Laurel Springs. This was not unexpected, as there are large knowledge gaps in the literature as to how monoecious hydrilla will behave in cooler climates (Netherland and Greer 2014). Results from this study supports this concept, by illustrating the greater unknowns of hydrilla growth and development in cooler climates. Understanding the invasive potential and management implications of monoecious hydrilla in northern states is important, as monoecious hydrilla behaves differently in dissimilar climates (Owens et al. 2012; Sutton et al. 1992).

In Raleigh, hydrilla without competition did not reach stem growth maximum, while in Laurel Springs, both hydrilla densities grown with *E. canadensis* did not reach stem growth maximum. This indicates that in Raleigh, hydrilla would have been increasing in stem length if the study was continued. In Laurel Springs, it indicates that *E. canadensis* interfered with hydrilla growth, and suppressed stem elongation in aboveground mesocosms.

## Conclusions

Based on results of this study, hydrilla is not as competitive in a cool climate, producing less biomass and fewer tubers when planted with any competitor species than hydrilla in a warm climate. However, hydrilla was able to establish both years in all planting combinations in both containers in Laurel Springs. Therefore, even though hydrilla growth was suppressed when grown with a competitor plant, if hydrilla was introduced into a new water body, it has the potential to become established.

The existing plant community structure is very important to excluding hydrilla or slowing down the invasion. If these communities are established and diverse, hydrilla infestations may cause problems at a slower pace. However, there are already several reports of thick, monospecific areas of hydrilla in northern states, demonstrating that hydrilla is able to compete in the Potomac River (Carter et al. 1994). More information is needed to understand how monoecious hydrilla will behave in new climates, especially with different light availability and temperatures.

Along with establishing biomass, both propagules were produced in all aboveground mesocosms, and in all planting combinations except *E. canadensis* with two hydrilla in belowground mesocosms. This increases the potential for long term infestations, which will need to be investigated further. This research project looked at one season of growth with a similar introduction time. The effect of overwintering structures already being present in the hydrosol were not addressed. More research will need to look into these aspects in different climates to understand and predict the growth and development of monoecious hydrilla.

As the northern US has greater biodiversity of aquatic species, it would be likely that hydrilla invasions would be slower in overtaking areas. This suppression of hydrilla would be

more pronounced in areas with well-established plant communities. However, there are already reports of serious infestations of hydrilla in many states, so it has demonstrated that it is able to survive in different climates. Hydrilla has been called the first aquatic weed that could become an issue for the entire continental US (MD Netherland, pers. comm.), and management plans will need to incorporate climatic conditions to be effective.

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Table 3-1. Water quality tests from Raleigh and Laurel Springs, North Carolina.

Nutrient Concentration <sup>1</sup>	Raleigh	Laurel Springs
Inorganic Nitrogen	0.82	0.94
Organic Nitrogen	0.00	0.16
Phosphorus	0.02	0.01
Potassium	1.46	4.49
Calcium	1.28	16.10
Sodium	1.94	6.35
Chlorine	4.55	5.96

Water Quality	Raleigh	Laurel Springs
pH	6.09	8.56
Electrial Conductivity <sup>2</sup>	0.03	0.15
Carbonates <sup>3</sup>	0.00	0.60
Bicarbonates <sup>4</sup>	0.10	0.43
Total Alkalinity <sup>5</sup>	5.00	51.50
Hardness <sup>5</sup>	6.41	47.40

<sup>1</sup>Parts per million.

<sup>2</sup>mS/cm.

<sup>3</sup>miliequivalent per liter (Meq/L).

<sup>4</sup>miliequivalent per liter (Meq/L).

<sup>5</sup>Parts per million of CaCO<sub>3</sub>.

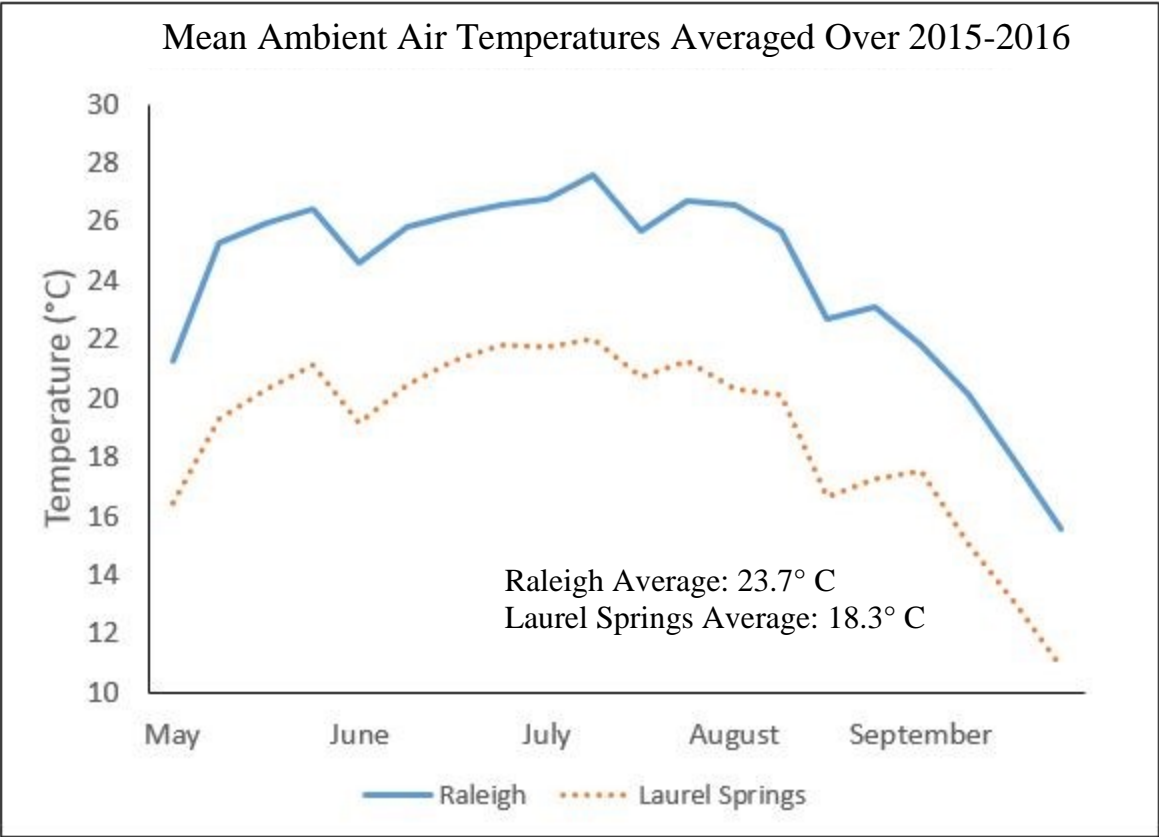


Figure 3-1. Mean Ambient Air Temperatures Recorded by NCDA&CS Weather Stations in 2015 and 2016. Temperatures were higher in Raleigh ( $p < 0.0001$ ).

Mean Water Temperatures in Raleigh and Laurel Springs,  
North Carolina.

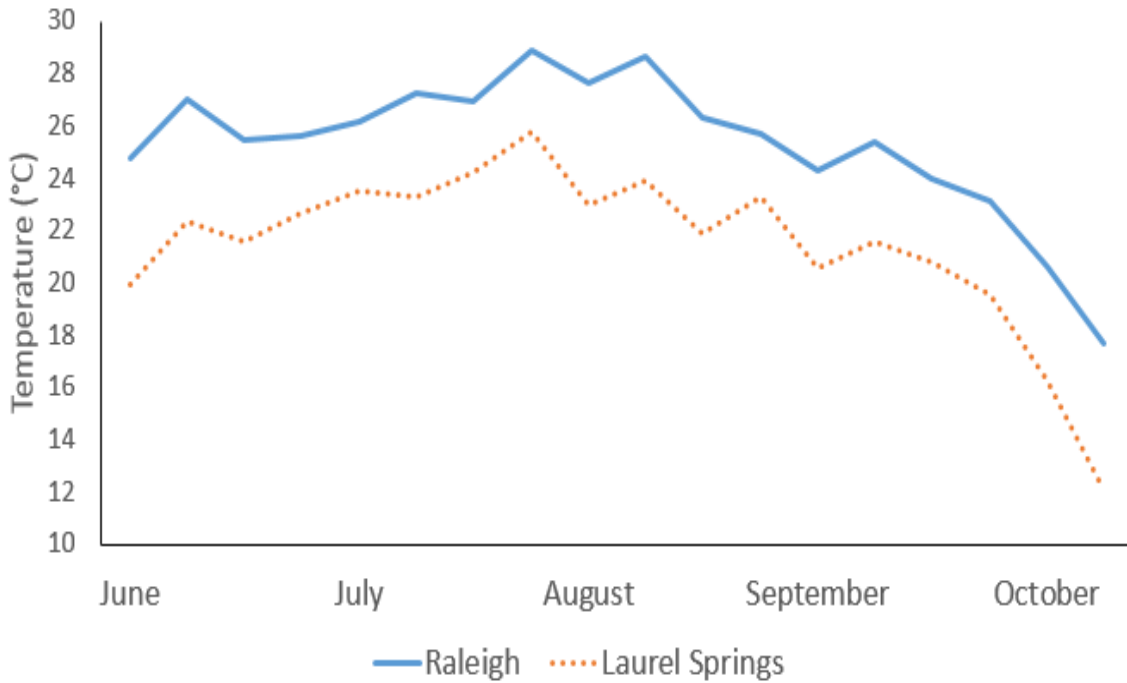


Figure 3-2. Mean water temperatures at Raleigh and Laurel springs, North Carolina. Temperatures were higher in Raleigh ( $p < 0.0001$ ).

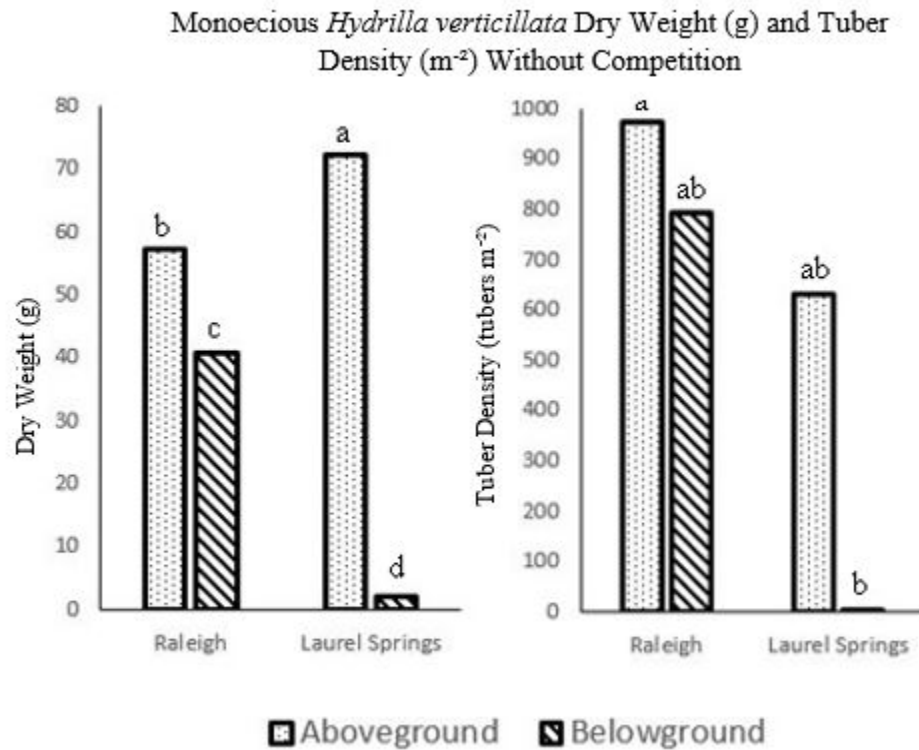


Figure 3-3. Mesocosm effect on monoecious *Hydrilla verticillata* growth in Raleigh and Laurel Springs, North Carolina when two *H. verticillata* were grown alone. Values separated by different letters are similar, according to Tukey HSD ( $p < 0.05$ ).

Table 3-2. Mean tuber density (tubers m<sup>2</sup>) and dry weight (g) for monoecious *Hydrilla verticillata* grown alone and with three competitor plants in Raleigh and Laurel Springs, North Carolina in above and belowground mesocosms.

Competitor	Tuber Production <sup>1</sup>															
	Aboveground Mesocosms								Belowground Mesocosms							
	Raleigh				Laurel Springs				Raleigh				Laurel Springs			
	<i>H. verticillata</i> Density		<i>H. verticillata</i> Density		<i>H. verticillata</i> Density		<i>H. verticillata</i> Density		<i>H. verticillata</i> Density		<i>H. verticillata</i> Density		<i>H. verticillata</i> Density			
	Two	Four	Two	Four	Two	Four	Two	Four	Two	Four	Two	Four	Two	Four		
	tubers m <sup>2</sup>								tubers m <sup>2</sup>							
<i>M. spicatum</i>	778	ab	769	abc	90	cde	120	bcde	403	ab	573	a	49	b	3	b
<i>E. canadensis</i>	524	abcde	740	abcd	15	e	65	de	246	ab	344	ab	0	b	0	b
<i>V. americana</i>	998	a	876	a	711	abcd	1000	a	403	ab	570	a	3	b	33	b
<i>None</i>	971	a			794	ab			629	a			3	b		

Competitor	Biomass Production <sup>2</sup>															
	Aboveground Mesocosms								Belowground Mesocosms							
	Raleigh				Laurel Springs				Raleigh				Laurel Springs			
	<i>H. verticillata</i> Density		<i>H. verticillata</i> Density		<i>H. verticillata</i> Density		<i>H. verticillata</i> Density		<i>H. verticillata</i> Density		<i>H. verticillata</i> Density		<i>H. verticillata</i> Density			
	Two	Four	Two	Four	Two	Four	Two	Four	Two	Four	Two	Four	Two	Four		
	(g)								(g)							
<i>M. spicatum</i>	53.87	ab	62.47	ab	24.60	bc	35.73	abc	32.03	a	29.8	a	7.03	b	0.34	b
<i>E. canadensis</i>	34.47	abc	64.67	a	0.78	c	7.04	c	38.8	a	31.23	a	1.09	b	1.4	b
<i>V. americana</i>	61.80	ab	67.00	a	67.87	a	69.93	a	36.97	a	41.93	a	0.34	b	7.88	b
<i>None</i>	57.13	ab			72.17	a			40.8	a			1.94	b		

<sup>1</sup>Mean monoecious *H. verticillata* tuber production in one growing season at a planting density of two or four hydrilla with three competitor plants. Values followed by the same letter are not different according to Tukey HSD  $p < 0.05$  within the same mesocosm type.

<sup>2</sup>Mean hydrilla biomass, measured as dry weight produced in one growing season at a planting density of two or four hydrilla with three competitor species. Values followed by the same letter are not different according to Tukey HSD  $p < 0.05$  within the same mesocosm type.

Table 3-3. Competitor species shoot biomass dry weight when grown with zero, two or four monoecious *Hydrilla verticillata* at Raleigh and Laurel Springs, North Carolina in above and belowground mesocosms.

Raleigh Aboveground						
<i>H. verticillata</i>	Dry Weight (g)					
Density	<i>M. spicatum</i>		<i>E. canadensis</i>		<i>V. americana</i>	
Zero	15.57	a	36.67	a	56.61	a
Two	0.62	b	11.62	b	7.52	b
Four	0.43	b	4.76	b	1.89	b

Laurel Springs Aboveground						
<i>H. verticillata</i>	Dry Weight (g)					
Density	<i>M. spicatum</i>		<i>E. canadensis</i>		<i>V. americana</i>	
Zero	42.37	a	83.31	a	34.41	a
Two	26.91	a	91.12	a	8.88	b
Four	38.81	a	97.53	a	5.64	b

Raleigh Belowground						
<i>H. verticillata</i>	Dry Weight (g)					
Density	<i>M. spicatum</i>		<i>E. canadensis</i>		<i>V. americana</i>	
Zero	2.48	a	31.83	a	23.94	a
Two	0.07	b	0.00	b	4.64	b
Four	1.01	b	1.41	b	0.03	b

Laurel Springs Belowground						
<i>H. verticillata</i>	Dry Weight (g)					
Density	<i>M. spicatum</i>		<i>E. canadensis</i>		<i>V. americana</i>	
Zero	8.10	a	2.73	a	34.23	a
Two	4.13	a	3.69	a	34.53	a
Four	7.73	a	3.42	a	29.23	a

*Myriophyllum spicatum*, *Elodea canadensis*, and *Vallisneria americana* shoot dry weight grown at a density of zero, two, or four monoecious *Hydrilla verticillata* plants. Values in columns followed by the same letter are not different, among species according to Tukey HSD  $p < 0.05$ .

Table 3-4. Raleigh vs Laurel Springs 2016 above and belowground mesocosms monoecious *Hydrilla verticillata* tuber production and shoot biomass.

		Aboveground						Belowground											
Competitor Plant	Location	Tuber Production <sup>1</sup>				Biomass <sup>2</sup>				Competitor Plant	Location	Tuber Production <sup>1</sup>				Biomass <sup>2</sup>			
		<i>H. verticillata</i> Density		<i>H. verticillata</i> Density		<i>H. verticillata</i> Density		<i>H. verticillata</i> Density				<i>H. verticillata</i> Density		<i>H. verticillata</i> Density		<i>H. verticillata</i> Density			
		Two	Four	Two	Four	Two	Four	Two	Four			Two	Four	Two	Four	Two	Four		
		tubers m <sup>-2</sup>				dry weight (g)						tubers m <sup>-2</sup>				dry weight (g)			
<i>M. spicatum</i>	Raleigh	778	a	769	a	53.87	a	62.47	a	<i>M. spicatum</i>	Raleigh	403	ab	573	a	32.03	a	29.80	a
	Laurel Springs	90	b	120	b	24.60	a	35.73	a		Laurel Springs	49	bc	0	c	7.03	b	0.34	b
<i>E. canadensis</i>	Raleigh	524	ab	740	a	34.47	b	64.67	a	<i>E. canadensis</i>	Raleigh	246	a	344	a	38.80	a	31.23	a
	Laurel Springs	15	c	65	cb	0.78	c	7.04	c		Laurel Springs	0	a	7	a	1.40	b	1.09	b
<i>V. americana</i>	Raleigh	998	a	876	a	61.80	a	67.00	a	<i>V. americana</i>	Raleigh	391	a	570	a	36.97	a	41.93	a
	Laurel Springs	711	a	1000	a	67.87	a	69.93	a		Laurel Springs	3	b	33	b	7.86	b	0.34	b
None	Raleigh	971	a			57.13	b			None	Raleigh	629	a			40.80	a		
	Laurel Springs	794	a			72.17	a				Laurel Springs	3	b			1.94	b		

<sup>1</sup>Mean monoecious hydrilla tuber production in one growing season at a planting density of two or four hydrilla plants with three competitor species. Values followed by the same letter are not significantly different within the same planting combination according to Tukey HSD with  $p < 0.05$ .

<sup>2</sup>Mean monoecious hydrilla biomass, measured as dry weight, produced in one growing season at a planting density of two or four hydrilla with three competitor species. Values followed by the same letter are not significantly different within the same planting combination according to Tukey HSD with  $p < 0.05$ .



Table 3-5. Competitor stem weight comparison between Raleigh and Laurel Springs, North Carolina when grown with zero, two, and four monoecious *Hydrilla verticillata*.

Aboveground							Belowground								
<i>H. verticillata</i>		Dry Weight (g)					<i>H. verticillata</i>		Dry Weight (g)						
Density	Location	<i>M. spicatum</i>	<i>E. canadensis</i>	<i>V. americana</i>			Density	Location	<i>M. spicatum</i>	<i>E. canadensis</i>	<i>V. americana</i>				
Zero	Raleigh	15.57	a	36.67	b	56.6	a	Zero	Raleigh	2.48	a	31.83	a	23.94	a
	Laurel Springs	42.37	a	83.31	a	34.433	a		Laurel Springs	8.1	a	2.73	b	34.23	a
Two	Raleigh	0.62	a	11.62	b	7.516	a	Two	Raleigh	0.07	b	0	a	4.64	b
	Laurel Springs	26.91	a	91.12	a	8.88	a		Laurel Springs	4.13	a	3.69	a	34.53	a
Four	Raleigh	0.43	a	4.76	b	1.892	a	Four	Raleigh	1.01	a	1.41	a	0.03	b
	Laurel Springs	38.81	a	97.53	a	5.643	a		Laurel Springs	7.73	a	3.42	a	29.23	a

*Myriophyllum spicatum*, *Elodea canadensis*, and *Vallisneria americana* shoot dry weight grown at a density of zero, two or four hydrilla plants. Values followed by the same letter are not different among species according to Tukey HSD with  $p < 0.05$ .

Table 3-6. Regression equations for monoecious *Hydrilla verticillata* stem length from May – October 2016 when grown alone and with three competitor plants in aboveground mesocosms in Raleigh and Laurel Springs, North Carolina.

Location	Hydrilla Density	Competitor Species	Regression Equation <sup>1</sup>	p-value	Weeks Until Maximum Growth ( $X_0$ ) <sup>2</sup>	Maximum Growth (cm)	$r^2$
Raleigh	2	None	$y = 13.6 + 12.33x - 0.548x^2$	<0.0001	---	83	0.86
	2	<i>M. spicatum</i>	$y = 11.2 + 14.32x - 0.845x^2$	<0.0001	17	72	0.85
	2	<i>E. canadensis</i>	$y = 2.6 + 18.66x - 1.279x^2$	<0.0001	15	71	0.83
	2	<i>V. americana</i>	$y = 11.7 + 14.78 - 0.869x^2$	<0.0001	17	74	0.85
	4	<i>M. spicatum</i>	$y = 5.9 + 17.5x - 1.145x^2$	<0.0001	15	73	0.89
	4	<i>E. canadensis</i>	$y = 20.1 + 12.05x - 0.736x^2$	<0.0001	16	69	0.83
	4	<i>V. americana</i>	$y = 12 + 14.44x - 0.901x^2$	<0.0001	16	70	0.89
Laurel Springs	2	None	$y = 1.8 + 15.81x - 0.897x^2$	<0.0001	18	72	0.81
	2	<i>M. spicatum</i>	$y = 7.5 + 12.55x - 0.614x^2$	<0.0001	20	72	0.75
	2	<i>E. canadensis</i> <sup>3</sup>	---	---	---	---	---
	2	<i>V. americana</i>	$y = (-0.9) + 18.56x - 1.294x^2$	<0.0001	14	66	0.84
	4	<i>M. spicatum</i>	$y = 2.7 + 14.17x - 0.786x^2$	<0.0001	18	67	0.73
	4	<i>E. canadensis</i>	$y = 2.5 + 15.14x - 1.424x^2$	0.0026	---	72	0.16
	4	<i>V. americana</i>	$y = 17 + 10.65x - 0.577x^2$	<0.0001	18	66	0.43

<sup>1</sup>Regression equations and corresponding p-values for each significant model.

<sup>2</sup>Weeks until maximum growth indicate the  $X_0$  value at which maximum growth was achieved, and no significant increases in stem length were observed beyond this plateau. Treatments without  $X_0$  values did not reach a plateau within the confines of this study, and were regressed with a standard quadratic regression model, instead of a quadratic plateau model.

<sup>3</sup>No regression equation fit two hydrilla with *E. canadensis* in Laurel Springs.

## **Chapter 4 Effects of Temperature during Dormancy on Viability of Monoecious *Hydrilla verticillata* Propagules**

### **Abstract**

*Hydrilla* (*Hydrilla verticillata* (L.f) Royle) is a federal noxious, submersed aquatic weed that causes much economic and environmental harm. This study utilized two research locations- a cool climate and a warm climate to understand climatic effects on the production and viability of asexual hydrilla propagules after an overwintering period in cooling chambers at three different temperatures. Tubers produced in the cooler climate were heavier, averaging 0.113 g compared to 0.096 g in the warmer climate. The cooler climate had higher viability, averaging 89% when overwintered at 4° C and 20% when overwintered at 0° C, while the warmer climate hydrilla tubers had an average viability of 63% when overwintered at 4° C, and 0% when overwintered at 0° C. Tuber densities were different between locations, with the cool climate averaging 823 tubers m<sup>-2</sup>, while the warm climate averaged 2142 tubers m<sup>-2</sup>.

### **Introduction**

*Hydrilla* is an invasive, noxious submersed aquatic weed that has been called “the perfect aquatic weed” (Langeland 1996), and is considered one of the worst submersed aquatic weeds worldwide (Thullen 1990). This plant is ubiquitous, being found on every continent except Antarctica, and based on the current range of hydrilla in China, has the ability to grow anywhere in the continental United States (US) (Balciunus and Chen 1993). *Hydrilla* has the ability to survive in drastically different water systems, from small, natural ponds, man-made canals and reservoirs, to flowing water (Dayan and Netherland 2005). *Hydrilla* has a large range of suitable environmental conditions such as pH, temperature, dissolved oxygen (DO), and nutrient content

(Madsen and Owens 2000). Due to the adaptive abilities of this plant, hydrilla has been called the first aquatic weed that has a potential to be a problem nationwide (MD Netherland, pers. comm.).

There are two biotypes of hydrilla in the US, a triploid monoecious form and a triploid female dioecious form, and both are expanding their range of growth in North America (Cook and Lüönd 1982). Dioecious hydrilla is typically found in the warmer climate of southern states, while monoecious hydrilla is typically found in temperate climates. From 2000-2011, dioecious hydrilla was found in three new states, while monoecious hydrilla was found in fifteen new states. Despite the recent range expansions, there is little knowledge as to how monoecious hydrilla will grow and compete in new climates (Netherland and Greer 2014). The majority of research on hydrilla has been conducted in southern climates on the dioecious biotype. Therefore, research on monoecious hydrilla in northern climates is needed to fill in many unknown plant behaviors (Netherland and Greer 2014).

Madeira et al. (2000) stated that even though the biotypes may visually appear similar, there are important differences that must be understood and applied to effective management systems. These include differences in physiology, reproduction, and phenology. Experiments comparing asexual propagule formation have been reported between monoecious and dioecious hydrilla (Steward and Van 1987; Sutton et al. 1992). Therefore, as the range of both dioecious and monoecious hydrilla expand and start to overlap, it will be important to alter management strategies for greatest impact (Netherland 1997).

Asexual propagules have been determined to be the most important to perennation and dispersal of hydrilla (Haller et al. 1976). Hydrilla produces two kinds of these asexual propagules: an axillary turion and a subterranean turion, often called tubers for simplicity (Netherland 1997). Axillary turions are formed on aboveground biomass in axillary buds on the

stem, and is a single apical meristem surrounded by leaf scales (Netherland 1997). Turions are able to remain viable for only a year after production. Tubers are formed at the end of rhizomes in the hydrosol, and contain several buds surrounded by leaf scales (Netherland 1997). Monoecious hydrilla tubers were able to remain viable for up to seven years following intense management in North Carolina waters, supporting the survival of the species over time (Nawrocki et al. 2016).

There appears to be an environmentally induced quiescence of tubers, though the exact mechanisms are not well understood (Netherland 1997). This quiescence prevents the quick depletion of tubers in the hydrosol, allowing for accumulation in sediment (Van and Steward 1990). However, when tubers are removed from sediment, sprouting rates are consistently high in laboratory conditions. Increased light and oxygen and decreased levels of carbon dioxide (CO<sub>2</sub>) are believed to induce these high sprouting rates (Netherland 1997). Turions appear to have an environmentally induced dormancy, and show a seasonality of sprouting in laboratory conditions (Harlan et al. 1985). Periods of chilling have been shown to be the optimal way of breaking turion dormancy. Exposing turions to 2° C for thirty-three days resulted in the highest sprouting rate for monoecious hydrilla turions (Sastroutomo 1980).

It is important to understand the production and maturation of these propagules to develop an effective management strategy, due to the reproductive potential of just one single tuber or stem fragment (Sutton et al. 1992). Being able to predict how northern climates will affect the dormancy and sprouting rate of monoecious hydrilla is imperative to effective control. True-Meadows et al. (2016) suggested that the increased chilling period in northern climates might increase the sprouting rate.

This study examined the ability of propagules produced in two different climates to withstand cool temperatures for different lengths of time. Research evaluating propagule survival in freezing temperatures has been completed on dioecious hydrilla, but not on monoecious hydrilla from a temperate climate. The results from this study will enable managers to better understand and predict tuber behavior in natural systems, and to effectively control hydrilla infestations.

### **Methods and Materials**

This one year experiment was conducted at two locations to test the effect of climate on the viability of propagules from May 2015-September 2016. The warm climate research was completed at North Carolina State University's Reedy Creek Research Farm in Raleigh, North Carolina (35.816907, -78.725034). Cool climate research was conducted at the North Carolina Department of Agriculture and Consumer Science (NCDA&CS) Upper Mountain Research Station (UMRS) in Laurel Springs, NC (36.396470, -81.307561). These locations were selected because they exhibit dissimilar climates due to difference in elevation, with 125 m at Reedy Creek, and UMRS at 975.36 m. Laurel Springs has a similar annual mean temperature as Albany, New York, and Ann Arbor, Michigan. The annual mean temperature of Raleigh is 16.1° C, while Laurel Springs, Albany, and Ann Arbor, are 9.8°, 9.0°, and 9.7° C, respectively (Arguez et al. 2010).

Throughout the entire study, mean, minimum, and maximum temperature, photosynthetically active radiation (PAR), and precipitation were recorded on a daily basis at NCDA&CS weather stations. Raleigh weather data was collected at Reedy Creek Field Laboratory, while the Laurel Springs weather data was collected at the UMRS. Temperature and light regulation was achieved by utilizing 30% shade cloth that covered all mesocosms. Pond

water was used in Raleigh, while well water was used in Laurel Springs. Water quality tests were processed at each location by NCDA&CS Agronomic Services (Table 4-1).

In early May 2015, unsprouted monoecious hydrilla tubers were collected from Shearon Harris Lake (35.612378, -78.942986) in Wake County, North Carolina and sprouted in a greenhouse. In late May, sprouted tubers were planted into six-inch plastic nursery pots (2.9L; 17.8 x 16.5 cm), with a single tuber per pot. Substrate used in the pots was topsoil (Scotts Premium Topsoil, Marysville OH) with three centimeters of sand (Quikrete®, Atlanta, GA) to cover the top, which was utilized to prevent soil from entering the water column, and to also discourage algal growth. The soil was unamended with fertilizer for this study. A total of 164 pots were planted for each location, for a total of 328 plants for this study. Pots were placed in plastic outdoor mesocosms (1040 L; Benchmark Earth, Sanford, NC), with a complete water exchange every 80 hours. Plants were allowed to grow and develop undisturbed throughout the growing season. Once being placed outside, the plants grew undisturbed throughout the summer until natural senescence.

After senescence, 144 pots were removed from the mesocosms in late November in Laurel Springs and early December in Raleigh. All remaining aboveground biomass was removed from the pot, and the pot was placed in a 2 mm plastic bag with two inches of water. Pots were evenly divided and placed in three different coolers set at 4, 0, and -3° C. Temperatures in the cooling chambers were regulated by a Thermostar Digital Temperature Controller (Northern Brewer LLC, Roseville, MN), and were accurate within one degree. Soil in the pots remained moist for the entirety of the study while in cooling chambers, due to the plastic bags.

A destructive harvest was also completed in Laurel Springs in late November and in Raleigh in early December on five pots from each location. These pots were sifted using a 3 mm sieve to collect all turions and tubers present in the sediment. Each propagule was individually measured and weighed, and a greenhouse viability test was completed. Fresh weight of all propagules were determined. Individual propagules were placed in 88.7 ml microcosms and put in a glass greenhouse under natural photoperiod. Greenhouse temperatures were maintained between 26-32° C. Daily observations were recorded to note sprouting in both propagules. Propagules were called viable if they sprouted, and all tests were run until every propagule either sprouted or rotted.

The remaining fifteen pots were left to overwinter in ambient weather conditions at each location. Starting early March 2016, these pots were evaluated on a biweekly basis to determine the emergence date for sprouting tubers. The pots in the coolers remained undisturbed until two weeks after the outdoor pots sprouted at each location. At this time, the first of six monthly destructive harvests began. The first harvest occurred in mid-April in Raleigh, and in early May in Laurel Springs, due to the fact that tubers in Raleigh sprouted before tubers in Laurel Springs.

The harvest process consisted of randomly selecting four pots from each of the three different temperatures to be placed in outdoor mesocosms, to observe undisturbed sprouting rates at each harvest at both locations. In addition, four random pots were selected to be destructively harvested from each of the three cooling chambers at each location. These pots were sifted and all propagules were collected. The turions and tubers collected from the destructive harvest were counted, weighed, and measured in the lab. A viability study was conducted in a greenhouse, using microcosms to hold an individual propagule that correlated to the quantitative laboratory measurements previously recorded. Daily observations noted any sprouting, which resulted in



average viability rates of each different temperature for each harvest across both locations. A total of seventy two pots were destructively harvested at each of the two locations over the course of the study.

In each of the harvest months, forty unsprouted tubers were collected from Shearon Harris Lake. These tubers were processed in the same way as the propagules collected from the harvests and used as controls for both size and viability rates. Viability studies were conducted on these tubers simultaneously with each of the monthly harvests. Each of the monthly studies were ran until all the propagules either sprouted or rotted, and viability in this study was defined as sprouting.

Two weeks after tuber sprouting ceased in outdoor mesocosms, a destructive harvest was completed on five pots that were overwintered in mesocosms from each location. This harvest was completed in the same manner as the harvest in November to understand the percentage of tubers that had sprouted at each location.

Data for propagules, weight, and viability was analyzed using SAS statistical software (SAS Institute Inc., Cary, North Carolina). Number and mass of asexual propagules were analyzed using analysis of variance (ANOVA), with means being separated by Tukey HSD tests ( $p < 0.05$ ). Differences between viability rates were calculated with a Chi-Squared test, with ( $p < 0.05$ ).

## **Results and Discussion**

### Weather

During the study, Raleigh had higher mean, minimum, and maximum ambient temperatures ( $p$  values  $< 0.0001$ ) (Table 4-2). There was not a difference in weekly precipitation, ( $p = 0.466$ ) or mean PAR, ( $p = 0.869$ ) received at each location. There were no differences in

measured parameters between 2015 and 2016 in the same location except for precipitation in Laurel Springs. In 2015, there was more weekly precipitation than in 2016 ( $p = 0.035$ ).

As expected, both ambient temperature and water temperature were cooler in Laurel Springs. Temperature has been demonstrated as an important factor of submersed macrophyte growth and development (Barko et al. 1986). Therefore, differences can be expected between locations with hydrilla development. Although though there were differences in temperatures, PAR, and precipitation did not differ between locations. Differences in precipitation levels at Laurel Springs between years did not have an impact on all other measured parameters between years.

#### Production of propagules

At both locations, monoecious hydrilla was able to produce turions and tubers (Table 4-3). When combining all harvests, hydrilla grown in Raleigh produced more tubers than plants grown in Laurel Springs. Tuber density at each harvest ranged from 1008 to 3696 tubers  $m^{-2}$  in Raleigh, with a mean tuber density of 2142 tubers  $m^{-2}$  (Table 4-4). The tuber density at Laurel Springs ranged from 224 to 2352 tubers  $m^{-2}$ , with a mean tuber density of 823 tubers  $m^{-2}$ . Turion densities ranged from 0 to 1680 turions  $m^{-2}$  in Raleigh with a mean of 529 turions  $m^{-2}$ . In Laurel Springs, turion densities ranged from 0 to 2016 turions  $m^{-2}$  with a mean of 593 turions  $m^{-2}$ .

Within locations, tuber numbers collected from each harvest did not vary over harvests (Table 4-3). Between locations, the amount of tubers collected in Raleigh was higher than in Laurel Springs except for the September harvest. The number of tubers collected in this last harvest was not different between locations. In addition, the amount of tubers collected from Raleigh during the September harvest were not different from the June harvest at Laurel Springs.

When combining tubers and turions across all harvests at Laurel Springs, there were differences in average propagule densities between harvests (Table 4-3). The July and August harvests both had lower numbers of propagules, both months averaging 560 tubers m<sup>-2</sup>, than the September harvest, which averaged 1120 tubers m<sup>-2</sup>. All other harvests at Laurel Springs did not vary in the average number of propagules collected.

Tuber production depends on many environmental factors, such as nutrient availability, health of the plant, hydrosoil composition, and climatic conditions (Basiouny et al. 1978). All of these factors interact with the production of tubers. However, since nutrient content and hydrilla health were similar at both locations, the differences in number of tubers produced in this study can be contributed to different temperatures experienced by the two locations. This has large implications on management, as being able to predict how temperatures will impact tuber production is a key aspect of hydrilla management (McFarland and Barko 1999). This study demonstrated that warmer temperatures facilitated greater tuber production with greater uniformity than cooler temperatures.

Although mesocosm tuber densities are traditionally higher than densities found in natural systems (Netherland 1997), this was not the case in this study. Tuber densities in Raleigh were comparable to monoecious tuber densities in North Carolina lakes (Harlan et al. 1985; Hodson et al. 1984), and were less than other mesocosm trials (Steward and Van 1987; Sutton et al. 1992), likely due to longer experiment times in these other studies.

Plants grown in Laurel Springs produced heavier tubers compared to plants grown in Raleigh (Table 4-5). On average, tubers from Raleigh had a fresh weight of 96 mg, while tubers had a fresh weight of 113 mg from Laurel Springs. The control tubers that were collected every month had an average fresh weight of 110 mg. Tubers had statistically different weights based on

when they were harvested. Control tubers had the lowest weight in August, as smaller, less mature tubers were collected, lowering the average weight. In September, the tubers were more mature, increasing the average tuber weight.

There were no differences in the average fresh weight of turions between locations, with 41 mg from Raleigh and 42 mg from Laurel Springs (Table 4-6). No turions were collected from natural systems to act as controls. Within the same natural system, propagules vary significantly in weight (True-Meadows et al. 2016). Therefore, weight differences between locations are expected to be great. Fresh weight of monoecious hydrilla turions average 36 to 77 mg (Spencer et al. 1987), while tubers average between 30 to 320 mg in North Carolina (Nawrocki 2011). Both propagules from each location in this study fell within these ranges, though on the lower end.

Carbohydrate reserves are important to the survival of submersed aquatic macrophyte propagules (Titus and Adams 1979). It is believed that larger propagules are able to withstand greater stress and still remain viable. Therefore, heavier tubers in Laurel Springs would increase their chance of surviving colder temperatures for a longer period of time, which was observed in the greenhouse viability studies.

#### Overwintered temp effects- Greenhouse Studies

Due to the constant darkness and above freezing temperatures experienced by propagules overwintered at 4° C, propagule disintegration occurred at both locations, but to a greater degree in Raleigh. Propagules were considered disintegrated when they were soft and falling apart at harvest. Less tuber disintegration occurred in 0° C, while no tuber disintegration was observed when kept at -3° C. Tubers were more resilient to disintegration than turions in all temperatures at both locations.

Tubers from Laurel Springs were subjected to and survived freezing temperatures for seven months. It has been found that in natural settings, mature tubers are resistant to freezing temperatures when insulated by sediment (Hodson et al. 1984). As turions are not buried in sediment are less resistant to freezing, monoecious hydrilla turions survived only a week in freezing conditions (Sastroutomo, 1982).

In natural systems the specific temperatures experienced by the pots in this study would not be seen uniformly. The effectiveness of using a winter drawdown to expose sediment to cold temperatures is highly site specific. Tubers in the upper portion of sediment might be affected by the temperatures; however, tubers would be more likely to survive deeper in the sediment. Another downside of using drawdown is that the results from this study demonstrate that even when sediment was exposed to freezing temperatures, tubers were able to remain viable for seven months in Laurel Springs. Therefore, even higher viability rates can be expected in natural systems, due to the temporal difference in sediment temperatures. Because of this, relying on colder, longer winter conditions to kill tubers is not a viable management option.

When combining viability rates for the same overwintered temperature, tubers from Laurel Springs had a higher viability rate at both 0 and 4° C (Table 4-6). Turions from Laurel Springs had a higher viability rate than those from Raleigh, with an average viability rate of 78% from Laurel Springs and 38% from Raleigh. There was no difference in viability rates at all other overwintering temperatures. When tubers were overwintered at 0° C, in Raleigh, no tubers were viable (Table 4-9). In Laurel Springs, initial viability was 57% and declined to 0 to 3% for harvests three through six.

There was not a difference in tuber viability from both locations when overwintered at 4° C (Table 4-7). The viability rates from Raleigh ranged from 29 to 93%. Viability rates of tubers from Laurel Springs ranged from 73 to 100%. Control tubers viability rates ranged from 90 to 100% (Table 4-8). In Raleigh, when overwintered at 4° C, turion viability did not differ based on harvest time, and ranged from 19 to 62% (Table 4-7). However, in Laurel Springs the number of viable turions was different for the July harvest, at 18% compared to 70 to 99% for all harvests.

When all harvests across locations were combined, tubers took longer to sprout at Laurel Springs, but there was no difference for turions between locations (Table 4-10). Tubers produced in Raleigh exhibited no difference in the amount of time required for sprouting. However, turion sprouting from Raleigh varied, with June turions requiring the longest period of time to sprout. Laurel Springs tubers did not differ in the time to sprouting, while turions did across harvests. Turions harvested in July required a longer sprouting time than all other harvests. When data collected from both locations were combined across all harvests, both tubers and turions showed differences in sprouting time. The number of days for tuber and turion sprouting at both locations was 4.1 days. Over the course of the study, the length of time it took tubers and turions to sprout did not differ.

Propagules that overwintered in constant temperatures had faster and more uniform sprouting times than those overwintered in ambient conditions at both Laurel Springs and Raleigh. Monoecious hydrilla tubers have synchronous sprouting in cooler climates, meaning that sprouting occurs in a narrow time period instead of yearlong sprouting, as observed in warmer climates (Michael Netherland, pers. comm.). Therefore, as photoperiod was different between monthly harvests, but tuber sprouting in outdoor mesocosms did not vary in length to sprout or amount, temperature is likely the most important cue for synchronous sprouting.

Greenhouse viability studies had higher sprouting rates than the mesocosm studies at each month. However, high propagule sprouting in greenhouse trials has been well documented (Netherland 1997; personal experience). It is hypothesized that when tubers are removed from the anoxic conditions found in the hydrosol, and exposed to oxygen, the environmentally imposed dormancy is broken (Netherland 1997). In natural systems, hydrilla would benefit if some viable tubers did not sprout immediately, allowing the plant to build up a tuber bank.

#### Mesocosms trials

When placed into the outdoor mesocosms, tubers in this study were able to sprout regardless of natural photoperiod or ambient temperatures. All pots overwintered at 4° C sprouted at each harvest, from April to September. At both locations after each harvest, it took an average of two weeks for the first sprouted tuber to be observed. There was not a difference in length of sprouting time between harvests or locations. On average, five to seven tubers sprouted per pot at both locations. There was not a difference in tuber sprouting between locations, though the percent of tubers that sprouted was higher in Laurel Springs.

Results from the mesocosm trial represents a more natural setting, as tubers were not removed from the hydrosol. Therefore, it can be expected that longer chilling times would increase the amount of tubers that sprouted in northern climates, which was hypothesized by True-Meadows et al. (2016). This study supports that hypothesis, as a greater proportion of tubers sprouted in Laurel Springs compared to Raleigh. If this trend continues in natural systems, it will impact monoecious hydrilla management in northern climates. If more tubers sprout in a season, current management strategies will need to change. In southern climates, the accumulation of tubers is rapid, complicating management efforts (Nawrocki et al. 2016). Though if timely management strategies are implemented after tubers sprout but before new

tubers are formed, in northern states, large tuber banks may not accumulate as quickly compared to southern states. Even if this is the case, Nawrocki (2016) found that in a North Carolina reservoir, a tuber density of just 11 tubers  $m^{-2}$  was enough to repopulate an area with hydrilla. In addition, when no management was implemented, the tuber bank increased over 1000% in just one growing season. Tuber densities found in Laurel Springs after tubers ceased sprouting in the spring was approximately 187 tubers  $m^{-2}$ . Therefore, even though the tuber bank is smaller, it is significant.

Plants grown in Raleigh had zero viable tubers at any harvest when overwintered at either  $0^{\circ}C$  or  $-3^{\circ}$ . In Laurel Springs, six pots that were overwintered at  $0^{\circ}C$  had viable tubers through the first two harvests. After the second harvest, there were no viable propagules overwintered at  $0^{\circ}C$  when placed in the outdoor mesocosms in Laurel Springs.

Pots harvested in June demonstrated that there were viable tubers at both locations that did not sprout in the undisturbed mesocosm. In Laurel Springs, pots averaged 4 unsprouted tubers, while Raleigh averaged nine unsprouted tubers. Average tuber viability rates were 98% from Raleigh and 95% from Laurel Springs when removed from the sediment and placed in the greenhouse. Even though these tubers were viable, they required longer to sprout. Tubers collected from Raleigh averaged 8.7 days to sprout, while Laurel Springs tubers averaged 4.8 days to sprout. One tuber from Raleigh required 21 days to sprout, while the longest time to sprouting at Laurel Springs was 7 days. A larger percentage of tubers sprouted in Laurel Springs in the mesocosms, creating a smaller tuber bank than at Raleigh.

#### November 2016 Year Harvest

At the harvest in November 2016, pots from Laurel Springs averaged 896 tubers  $m^{-2}$  and 336 turions  $m^{-2}$ , while Raleigh averaged 1008 tubers  $m^{-2}$  and 0 turions  $m^{-2}$ . There was no



difference in amount of propagules collected or their weight at this harvest. Laurel Springs had a 97% viability rate of tubers and a 75% survival of turions, which was not different than rate measured throughout the six monthly viability tests. Raleigh had a 90% viability rate of tubers, which was not different than rates observed previously in greenhouse trials. As there were no turions collected from Raleigh, there was no viability rate. The number of propagules collected from Raleigh was lower than harvests for the six monthly harvests, due to the disintegration of propagules. However, tuber weights at Raleigh were not different than those observed in the monthly harvests. All propagules from both locations required a longer time to sprout at the year harvest than the six monthly trials.

### **Conclusions**

Interactions between two or more climatic conditions were not captured with this study, as temperature was the major climatic difference seen between locations. Other interactions, especially with photoperiod length and light intensity may influence growth and development of hydrilla. However, temperature difference influenced tuber production, weight, and viability. These tuber dynamics are vital for effective management. From this study, tuber production was more variable in Laurel Springs when compared to Raleigh, having a wider range of production. However, Raleigh hydrilla produced a greater amount of tubers. Tuber weights were larger in Laurel Springs, theoretically allowing these propagules to withstand more intense environmental stress and still remain viable. Within locations, tubers were more resistant to environmental stresses, having higher viability rates and surviving in colder temperatures in Laurel Springs for longer. In addition, turions were more susceptible to rotting than tubers in the same overwintering temperature.

Tubers were present at a high enough density after sprouting ceased in the spring, that the tubers would act as perennation means for at least one season. Therefore, tuber bank management is still required even though a greater proportion of tubers sprouted in Laurel Springs compared to Raleigh. In addition, relying on the colder, longer winters in northern US to reduce viability of tubers will not be an effective management strategy, as tubers were able to withstand seven months of freezing temperatures and still remain viable.

More research is required to observe how interactions of climatic conditions affect hydrilla biomass and production of asexual propagules. Longevity of propagules and the accumulation of tubers were not measured in this study, and need to be understood to be effectively incorporated into management and control strategies of monoecious hydrilla in northern climates.

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Table 4-1. Water quality tests from Raleigh and Laurel Springs, North Carolina.

<b>Nutrient Concentration<sup>1</sup></b>	<b>Raleigh</b>	<b>Laurel Springs</b>
Inorganic Nitrogen	0.82	0.94
Organic Nitrogen	0.00	0.16
Phosphorus	0.02	0.01
Potassium	1.46	4.49
Calcium	1.28	16.10
Sodium	1.94	6.35
Chlorine	4.55	5.96

<b>Water Quality</b>	<b>Raleigh</b>	<b>Laurel Springs</b>
pH	6.09	8.56
Electrical Conductivity <sup>2</sup>	0.03	0.15
Carbonates <sup>3</sup>	0.00	0.60
Bicarbonates <sup>4</sup>	0.10	0.43
Total Alkalinity <sup>5</sup>	5.00	51.50
Hardness <sup>5</sup>	6.41	47.40

<sup>1</sup>Parts per million.

<sup>2</sup>mS/cm.

<sup>3</sup>miliequivalent per liter (Meq/L).

<sup>4</sup>miliequivalent per liter (Meq/L)

<sup>5</sup>Parts per million of CaCO<sub>3</sub>.

Table 4-2. Ambient temperature, photosynthetically active radiation (PAR), and precipitation per week in Raleigh and Laurel Springs, North Carolina from May 2015 – September 2016, as measured by NCDA&CS weather stations.

Average Ambient Temperatures °C			
	Mean	Minimum	Maximum
Raleigh	19.27	13.98	24.55
Laurel Springs	14.01	8.82	19.19

Range of Ambient Temperatures °C		
	Minimum	Maximum
Raleigh	-4.50	35.37
Laurel Springs	-9.50	29.13

Photosynthetically Active Radiation (PAR)		
	Mean	Maximum
Raleigh	382.29	2445.57
Laurel Springs	383.55	2726.43

Precipitation Per Week (cm)		
	Mean	Maximum
Raleigh	0.43	2.86
Laurel Springs	0.58	7.3

Raleigh had higher mean, minimum, and maximum temperatures than Laurel Springs over this time period. PAR and precipitation did not vary between locations.

Table 4-3. Average number of tubers and turions (m<sup>-2</sup>) collected at each monthly harvest at Raleigh and Laurel Springs, North Carolina.

Harvest	Month	Tubers Collected				Turions Collected			
		Raleigh		Laurel Springs		Raleigh		Laurel Springs	
1	April	2128	a <sup>1</sup>	784	c	2128	a	784	c
2	May	2016	a	784	c	2016	a	784	c
3	June	2016	a	1008	bc	2016	a	1008	bc
4	July	1904	a	784	c	1904	a	784	c
5	August	1904	a	784	c	1904	a	784	c
6	September	1792	ab	1586	abc	1792	ab	1586	abc

<sup>1</sup>Values not followed by the same letter are different among propagule type, according to Tukey HSD  $p < 0.05$ .



Table 4-4. Monoecious *Hydrilla verticillata* propagule density at Raleigh and Laurel Springs, North Carolina over all harvests.

Location	Propagule Density (per m <sup>2</sup> )					
	Tubers			Turions		
	Mean	Min.	Max.	Mean	Min.	Max.
Raleigh	2142 a <sup>1</sup>	1008	3696	529 a	0	1680
Laurel Springs	823 b	224	2352	293 a	0	2016

<sup>1</sup>Tuber densities are compared across locations. Mean values not followed by the same letter are different among propagule types, according to Tukey HSD  $p < 0.05$ .

Table 4-5. Monoecious *Hydrilla verticillata* tuber and turion fresh weight over six harvests.

Harvest	Month	Tuber Fresh Weight				Turions Fresh Weight			
		Raleigh		Laurel Springs		Raleigh		Laurel Springs	
1	April	0.104	b <sup>1</sup>	0.118	a	0.043	a	0.043	a
2	May	0.093	b	0.117	a	0.039	a	0.038	a
3	June	0.095	a	0.113	a	0.035	a	0.04	a
4	July	0.093	a	0.104	a	0.057	a	0.045	a
5	August	0.098	b	0.113	a	0.043	a	0.039	a
6	September	0.094	b	0.112	a	0.033	a	0.047	a
All		0.096	b <sup>2</sup>	0.113	a	0.042	a	0.042	a

<sup>1</sup>Mean fresh weight comparisons between locations at each harvest within propagule type. Values with different following letters are significantly different between locations within the same harvest, according to Tukey HSD  $p < 0.05$ .

<sup>2</sup>Overall mean fresh weight comparison between locations within propagule type. Values with different following letters are significantly different over all harvests, according to Tukey HSD  $p < 0.05$ .

Table 4-6. Overall mean viability rates of monoecious *Hydrilla verticillata* tubers and turions from Raleigh and Laurel Springs, North Carolina.

Temperature	Tuber Viability Rate		Turion Viability Rate			
	Location	Mean Viability Rate	Location	Mean Viability Rate		
4° C	Raleigh	63%	b <sup>1</sup>	Raleigh	41%	b
	Laurel Springs	89%	a	Laurel Springs	78%	a
0° C	Raleigh	0%	b	Raleigh	0%	a
	Laurel Springs	20%	a	Laurel Springs	0%	a
-3° C	Raleigh	0%	a	Raleigh	0%	a
	Laurel Springs	0%	a	Laurel Springs	0%	a

<sup>1</sup>Viability rates are combined across all harvests within the same location. Values not followed by the same letter are different among temperature and propagule type, according to Tukey HSD  $p < 0.05$ .

Table 4-7. Monthly viability rates of tubers and turions overwintered at 4° C from Raleigh and Laurel Springs, North Carolina.

Harvest	Month	Tubers				Turions			
		Raleigh		Laurel Springs		Raleigh		Laurel Springs	
1	April	93	a <sup>1</sup>	100	a	20	a	97	a
2	May	29	a	93	a	19	a	99	a
3	June	51	a	98	a	38	a	92	a
4	July	75	a	75	a	62	a	18	a
5	August	66	a	98	a	56	a	97	a
6	September	63	a	73	a	40	a	70	a

<sup>1</sup>Values in the same column followed the same letter are not different, according to Tukey HSD  $p < 0.05$ .

Table 4-8. Average monthly viability rates of tubers collected from Shearon Harris Reservoir between April and September 2015.

Harvest	Month	Control	
1	April	90	a <sup>1</sup>
2	May	95	a
3	June	99	a
4	July	100	a
5	August	93	a
6	September	90	a

<sup>1</sup>Values followed the same letter are not different, according to Tukey HSD  $p < 0.05$ .

Table 4-9. Monthly viability rates of tubers overwintered at 0° C from Raleigh and Laurel Springs, North Carolina.

Harvest	Month	Raleigh		Laurel Springs	
1	April	0	a <sup>1</sup>	57	a
2	May	0	a	58	a
3	June	0	a	3	b
4	July	0	a	0	b
5	August	0	a	0	b
6	September	0	a	0	b

<sup>1</sup>Values in the same column followed the same letter are not different, according to Tukey HSD  $p < 0.05$ .

Table 4-10. Mean days to sprout for monoecious *Hydrilla verticillata* tubers and turions within and between Raleigh and Laurel Springs, North Carolina in greenhouse viability studies.

Harvest	Month	Tubers		Turions	
		Raleigh	Laurel Springs	Raleigh	Laurel Springs
1	April	4.7 a <sup>1</sup>	4.6 a	3.0 cb	4.1 b
2	May	3.4 b	3.8 a	4.2 b	3.0 c
3	June	4.1 ab	4.6 a	6.4 a	4.6 b
4	July	3.9 ab	4.8 a	4.2 b	6.5 a
5	August	3.2 b	3.4 a	2.4 c	3.2 c
6	September	4 ab	4.4 a	3.1 cb	3.8 bc
All	Average	3.9 b <sup>2</sup>	4.3 a	4.0 a	4.2 a

<sup>1</sup>Single harvest mean number of days to sprout comparisons within locations. Values not followed by the same letter are significantly different within location, according to Tukey HSD  $p < 0.05$ .

<sup>2</sup>Overall six harvest mean number of days to sprout comparisons between locations. Values not followed by the same letter are significantly different within propagule type, according to Tukey HSD  $p < 0.05$ .