BIOLOGY AND CONTROL OF THE INVASIVE AQUATIC PLANT CRESTED FLOATING HEART (NYMPHOIDES CRISTATA)

By

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To my Dad and Mom who have believed in and supported me throughout the pursuit of my goals, I am forever grateful for all the opportunities you have given me

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TABLE OF CONTENTS

page

ACKNOWLEDGMENTS 4

LIST OF TABLES 8

LIST OF FIGURES 9

LIST OF ABBREVIATIONS 11

ABSTRACT 12

CHAPTER

1 INTRODUCTION AND LITERATURE REVIEW 14

Biology of Two Invasive Species of Nymphoides 14
History and Origin of Crested Floating Heart (*Nymphoides cristata*) 14
Identification and Biology 14
Habitat 16
Current Distribution 16
Invasive Potential 17
History and Origin of Yellow Floating Heart (*Nymphoides peltata*) 18
Identification and Biology 18
Habitat 19
Current Distribution 19
Invasive Potential 19
Preventative management of Nymphoides 20
Cultural Control 20
Mechanical Control 21
Biological Control 21
Chemical Control 22
Objectives 23

2 EFFECTS OF AQUATIC HERBICIDES ON CRESTED FLOATING HEART 27

Introduction 27
Materials and Methods 30
Subsurface herbicide applications 30
Foliar herbicide application 33
Results and Discussion 33
Subsurface herbicide application 33
Foliar herbicide application 40

3 THE INFLUENCE OF SEDIMENT TYPE AND FERTILITY ON THE GROWTH OF CRESTED FLOATING HEART 57

Introduction....57
Materials and Methods 58
Study 1....58
Study 2....59
Study 3....60
Results and Discussion 60
Study 1....60
Study 2....60
Study 3....62

4 THE INFLUENCE OF LIGHT AVAILABILITY ON GROWTH OF CRESTED FLOATING HEART ESTABLISHED FROM RAMETS....69

Introduction....69
Materials and Methods 69
Number of Floating Leaves 69
Dry Weight 71
Results and Discussion 71
Number of Floating Leaves 71
Dry Weight 72

5 COMPETITION BETWEEN CRESTED FLOATING HEART AND HYDRILLA IN A REPLACEMENT SERIES TRIAL 79

Introduction....79
Materials and Methods 80
Results and Discussion 81

6 A COMPARATIVE ANATOMY OF CRESTED FLOATING HEART AND YELLOW WATER LILY LEAVES AND STEMS 87

Introduction....87
Materials and Methods 88
Leaf Sections 88
Stem Sections 89
Results and Discussion 90
Leaf Sections 90
Stem Sections 92

7 CONCLUSIONS AND RECOMMENDATIONS 98

APPENDIX: ANOVA TABLES FROM CHAPTER 3 STUDY 101

LIST OF REFERENCES....102

BIOGRAPHICAL SKETCH 109

LIST OF TABLES

Table page

2-1 Subsurface concentrations, exposure times and foliar rates of herbicides used singly 45

2-2 Subsurface concentrations, exposure times and foliar rates of herbicides used in combinations. 46

3-1 Physical characterization of sediments used in sediment type/growth study. 64

3-2 Background nutrient and sediment chemistry analysis from sediments used in sediment type/growth study. 64

3-3 Hydrated lime application rates and resulting sediment pH 65

4-1 Target ambient light levels and the actual light levels measured in light intensity study. 75

4-2 *N. cristata* dry weight at various light intensities at 2 and 4 weeks after planting. 75

5-1 Comparison of *N. cristata* and hydrilla dry weight after 7 weeks of competition. 84

6-1 Features measured on *N. cristata* and *Nymphaea mexicana* for anatomical and physical comparisons.. 95

A-1 2-way ANOVA table for 6 week floating leaf data from trial 1 of Chapter 3. 101

A-2 2-way ANOVA table for 6 week dry weight data from trial 1 of Chapter 3. 101

B-1 2-way ANOVA table for 6 week floating leaf data from trial 2 of Chapter 3. 101

B-2 2-way ANOVA table for 6 week dry weight data from trial 2 of Chapter 3. 101

LIST OF FIGURES

Figure page

1-1 Image of Nymphoides cristata growing in the Santee Cooper Reservoir. 24

1-2 Ramet of N. cristata. 24

1-3 Line drawing of a crested floating heart (Nymphoides cristata) flower. 25

1-4 Image comparing leaves of the native Nymphoides aquatica and the exotic Nymphoides cristata...25

1-5 Root system of an established Nymphoides cristata.. 26

2-1 Dry weight response of N. cristata to triclopyr 4 WAT 47

2-2 Dry weight response of N. cristata to 2,4-D 4 WAT. 48

2-3 Dry weight response of N. cristata to a combination of 2,4-D (amine) + triclopyr (amine) 4 WAT.. 49

2-4 Dry weight response of N. cristata to endothall (dipotassium salt) 4 WAT 50

2-5 Dry weight response of N. cristata to 4 week static treatments with endothall (dipotassium salt). 51

2-6 Dry weight response of N. cristata to bispyribac sodium 4 WAT. 51

2-7 Dry weight response of N. cristata to flumioxazin and carfentrazone-ethyl 4 WAT. 52

2-8 Dry weight response of N. cristata to diquat 4 WAT 53

2-9 Dry weight response of N. cristata to endothall (amine salt) 4 WAT...............................54

2-10 Dry weight response of N. cristata to a combination of diquat and dipotassium salt endothall 4 WAT...55

2-11 Dry weight response of N. cristata to foliar aplpied ALS herbicides 4 WAT. 56

2-12 Dry weight response of N. cristata tofoliar applications of select herbicide combinations 4 WAT.. 56

3-1 Study 1, response of N. cristata grown in sand, muck and potting soil sediments at two fertilizer rates 6 WAT. 66

3-2 Study 2, response of N. cristata grown in sand, muck, potting soil and clay sediments and two fertilizer rates 6 WAT. 67

3-3 Linear regression of N. cristata dry weight versus sediment pH 68

4-1 Study 1, number of floating leaves of N. cristata in response to light availability 2 and 4 weeks after planting. 76

4-2 Study 2, number of floating leaves of N. cristata in response to light availability in the second study 2 and 4 weeks after planting..77

4-3 Dry weight of N. cristata in response to light availability at 2 and 4 weeks after planting 78

5-1 The dry weight of N. cristata, hydrilla, and total plant dry weight after 7 weeks of growth in a replacement series competition study...85

5-2 N. cristata and hydrilla growing in replacement series competition for 7 weeks at the 3:3 planting ratio 86

6-1 Leaf tissue samples of N. cristata and Nymphaea mexicana showing thickness, vascular bundles and supporting structures under different magnifications......................96

6-2 Stem cross section of N. cristata and Nymphaea mexicana showing diameter, vascular bundles and supporting structures under different magnification 97

LIST OF ABBREVIATIONS

A.E.	Acid equivalent
A.I.	Active ingredient
ALS	Acetolactate synthase
CET	Concentration exposure time
DAT	Days after treatment
HAT	Hours after treatment
PPO	Protoporphyrinogen oxidase
WAT	Weeks after treatment

Abstract of Thesis Presented to the Graduate School
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BIOLOGY AND CONTROL OF THE INVASIVE AQUATIC PLANT CRESTED FLOATING HEART (NYMPHOIDES CRISTATA)

By

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The invasive, floating-leaved plant crested floating heart (*Nymphoides cristata*) first documented in Florida in 1996, has been extending its range throughout Florida and the Southeastern US. To address broad information gaps on management and biology of *N. cristata*, research was conducted to evaluate herbicide efficacy and how selected environmental factors influence growth. Herbicide screening suggests *N. cristata* has broad tolerance to most registered aquatic herbicides; however, submersed applications of liquid endothall (amine salt) and diquat and foliar applied imazamox and imazapyr reduced biomass below a pre-treatment reference. Evaluation of the influence of sediment type and fertility on growth found a significant interaction between these factors. These data suggest that *N. cristata* can grow in a wide range of sediments but prefers acidic, fertile sediments. Plants exhibited a negative response to increasing pH. Growth from small submersed ramets showed a positive linear relationship between light availability and biomass, and a non-linear relationship to leaf production. Ramets grew to the water surface at 1% incident light; however, growth was greatest above 25% light. The growth of *N. cristata* from ramets versus hydrilla (*Hydrilla verticillata*) from apical shoots in a competition study showed *N. cristata* was able to produce more biomass at planting ratios greater than 1: 5 (*N. cristata*:hydrilla). Anatomical comparison of *N. cristata* to yellow waterlily (*Nymphaea*

mexicana) found that *N. cristata* leaves and stems were thinner and contained fewer supporting structures and vascular tissue. Collectively these data contribute greatly to current knowledge of the biology and management of *N. cristata*.

CHAPTER 1
INTRODUCTION AND LITERATURE REVIEW

Biology of Two Invasive Species of Nymphoides

History and Origin of Crested Floating Heart (*Nymphoides cristata*)

Crested floating heart (*Nymphoides cristata* (Roxb.)) Kuntze, hereafter referred to as *N. cristata*, is native to Asia where it is found as far south as the island of Sri Lanka and as far north as the Jiangsu Province in China along the East China Sea (Burks, 2002a). The plant is marketed in the water garden and aquarium trade as 'snowflake' or 'variegated snowflake', and it is readily available throughout North America. Within the United States, *N. cristata* has escaped from cultivation and become established in several bodies of water in Florida. *N. cristata* was first reported in 1996, in Horseshoe Lake in Collier County, Florida (Burks, 2002a) and subsequently, has been confirmed in numerous bodies of water, including, water management canals in south Florida, Lake Okeechobee, and the Big Cypress National Preserve. One of the largest *N. cristata* infestations has occurred in the 64,750 ha Santee Cooper reservoir system in South Carolina. This infestation covers over 1,416 ha (Chip Davis, personal communication, 2012) (Figure 1-1).

Identification and Biology

N. cristata, is a dicotyledonous aquatic plant of the Menyanthaceae or buckbean family. *N. cristata* can be identified by the slender bundles of tuberous roots on the underside of its floating leaves (Figure 1-2) (Burks, 2002a). The flowers are unique to the species since they bear an erect fold of tissue that runs down the length of the upper side of the petal (Figure 1-3) (Burks, 2002a).

N. cristata shares the same range as two native species of Nymphoides, *N. aquatica* and *N. cordata*. They are similar in appearance, but can be differentiated by the flowers and the undersides of the leaves. *N. cristata* has smooth ventral sides unlike the rough, pebble-like underside of *N. aquatica* leaves (Figure 1-4) (Burks, 2002a). *N. cordata* has a smooth underside of the leaf, but the flowers lack the erect 'crest' in the center of the petal.

N. cristata displays nymphaeid morphology, with the plant rooted in the submerged sediment and produces floating leaves with short petioles arising from long petiole-like stems (Burks, 2002a; Sculthorpe, 1967). Rooted plants produce many leaves, and while most float on the surface of the water some leaves can remain submersed. The ability of *N. cristata to* grow in water 3 m deep or deeper is noteworthy because many other nymphaeid plants (rooted in the sediments with floating leaves) cannot grow at this depth (Sculthorpe, 1967).

N. cristata is considered to be subdioecious in its native range since it has the ability to produce monoecious plants within a dioecious population (Burks, 2002a). Monoecious plants produce separate female and male flowers on the same plant. Recent evaluations of the floral biology of *N. cristata* suggest that populations in Florida are gynodioecious since the flowers of the plant are either pistillate, only containing female parts or hermaphroditic (perfect), containing both male and female parts (Tippery and Les, 2011). Despite the ability of *N. cristata* to produce numerous flowers, it has not been observed to produce viable seed in the US (Burks, 2002a). *N. cristata* primarily reproduces vegetatively by production of ramets. Ramets easily separate from the parent plant and can drift away in the water current to a new location to form a new colony, or sink to the bottom and remain dormant until conditions are favorable for sprouting (Burks, 2002a). Little is known about the biology or longevity of these ramets; under what environmental conditions (light, sediment type or characteristics) these propagules are stimulated to sprout. Furthermore, it is not known what conditions are necessary for these propagules to remain quiescent after separation from the parent plant. Once established, *N. cristata* forms large root systems that act as carbohydrate storage organs and anchor the plant into the sediments (Figure 1-5).

Habitat

N. cristata is typically rooted in sediments, but is also capable of survival in a free-floating form for a period of time with tuberous propagules (ramets) attached to the underside of the leaf (Burks, 2002a). The plant grows best in tropical to subtropical climate zones where it typically inhabits shallow areas of lakes, ponds, canals and areas of rivers with low current flow (Burks, 2002a). However, *N. cristata*, similar to other species in this genus, has been observed growing in water up to 3 m deep (Chip Davis, personal communication, 2011; Sculthorpe, 1967).

Current Distribution

In its native range of India, Vietnam, Taiwan and southern provinces of China, N. cristata is found from 6 – 34 degrees North latitude. In the western hemisphere this is equivalent to the area between northern South America (Columbia, Venezuela) to Chattanooga, Tennessee (Burks, 2002b). In Florida the plant ranges from the southern part of the State north towards Lake George (CAIP, 2010). In the U.S. a rapidly expanding population is found as far north as Lake Marion in South Carolina (SC DNR, 2010). Recently, *N. cristata* has also been confirmed in Texas (Invasive, 2012), North Carolina (Robert Richardson, personal communication, 2012) and Louisiana (Alexander Perret, personal communication, 2012). This has implications in Florida, since this suggests that in terms of climate suitability there are not likely to be any regions in Florida where *N. cristata* could not thrive. A Weed risk assessment performed by the USDA-APHIS (2012) suggests that Plant Hardiness Zones 8 to 13 could provide conditions in which *N. cristata* could thrive. In addition to climate, *N. cristata* has been found growing in water 3 meters deep, which is of particular concern for Florida, since the average depth of many water bodies in the state is less than 3 meters.

Invasive Potential

Within its native range, *N. cristata* is considered a common weed of rice (*Oryza sativa* L.) fields (Burks, 2002a). Reproduction and spread via vegetative reproduction is very evident, since floating leaves with the attached cluster of tubers easily detach from the branches and establish new stands. In Florida, *N. cristata* has been observed to spread rapidly in water up to 2 m deep. In Collier County, FL, a stand was observed to invade and cover 3.6 ha in four weeks (Burks, 2002a). The plant forms dense mats of overlapping leaves and this canopy greatly reduces light penetration into the water column and surface mixing of water and air, reducing native submersed plant and algal photosynthesis and therefore reducing dissolved oxygen concentration in the water under the mat (Burks, 2002a). *N. cristata* was first discovered growing in Lake Marion, South Carolina in 2006 covering 8.1 ha and now covers 1,416 ha of the water surface (Chip Davis, personal communication, June, 2012).

N. cristata was recently moved from the Florida Exotic Pest Plant Council (FLEPPC) Category II list, a plant increasing in abundance or frequency but have not altered native plant communities, to Category I, an invasive exotic plant that is altering native plant communities by displacing native species, changing community structures or ecological functions, or hybridizing natives (FLEPPC, 2009). Despite its reclassification to Category I, there is still a lack of peer-reviewed literature regarding the invasion ecology, biology and management of the plant. In 2012, United States Department of Agriculture-Animal and Plant Health Inspection Service (USDA-APHIS) performed a weed risk assessment for *N. cristata*, and determined that the plant is high risk, ranking especially high for both impact and establishment/spread potential (USDA-APHIS, 2012).

History and Origin of Yellow Floating Heart (*Nymphoides peltata*)

The invasive yellow floating heart (*Nymphoides peltata* (S.G. Gmel.) Kuntze) hereafter referred to as *N. peltata* is a native of Europe and temperate Asia (Smits et al. 1992). *N. peltata* has been present in the United States since at least 1882, when it was collected from a pond in Massachusetts (Les and Mehrhoff, 1999). *N. peltata* has been offered for sale in the United States since 1891 (Countryman, 1970). Similar to *N. cristata*, *N. peltata* was also introduced as an escape from cultivation (Les and Mehrhoff, 1999; Stuckey, 1973).

Identification and Biology

N. peltata is a perennial aquatic plant with dark green, heart-shaped floating leaves that rise from creeping, underwater rhizomes (Washington Noxious Weed Control Board, 2007). Flowers of the plant are bright yellow with 5 petals. *N. peltata* exhibits optimal growth, and is present at highest frequencies in well buffered (high pH) water systems (Smits et al, 1992). Studies conducted on plant growth in various sediments showed that when grown in both basic and acidic soils but in buffered water, plants could successfully grow and produce numerous leaves. However, when grown in the same sediment conditions but acidic water, plants were unable to produce leaves. These results suggest that the presence or absence of the plant in different bodies of water is dependent on the water alkalinity not sediment alkalinity (Smits, 1992). It is not known how these conditions of water, sediment pH and quality effect the growth of *N. cristata*.

Floral structure of *N. peltata* is perfect (containing both male and female parts). However, unlike *N. cristata*, N. *peltata* is capable of self pollination and producing viable seed (Ornduff, 1970; Van Der Velde and Van Der Heijden, 1981). It is noteworthy that both of these species have very long flowering seasons. A long flowering season is advantageous to *N. peltata* because of the ephemeral nature of the flower (once a flower opens it withers within one day). In order to maximize the possibility of pollination, flowers have to open individually over a long period of time rather than all at once (Van Der Velde and Van Der Heijden, 1981). Even though *N. cristata* does not self pollinate it produces flowers in a similar manner and *N. peltata* is also capable of clonal propagation via fragmentation (Larson, 2006).

Habitat

N. peltata grows in shallow areas of lakes, ponds and rivers where currents are slow (Nature Conservancy of Vermont, 2003). It grows best in water 1 m deep, but has occasionally been observed growing well on the muddy shores of lakes where changing water levels have left plants above the water surface (Countryman, 1970). Climatically, this plant was not thought to persist in warmer subtropical or tropical climates (USGS, 2001).

Current Distribution

N. peltata was initially considered to have a low potential as an invasive in the southeast United States due to its preference of temperate zones (USGS, 2001). However, a weed risk assessment conducted by USDA-APHIS in 2012 suggests that *N. peltata* could persist in Plant Hardiness Zones 4 through 11, which includes Florida.

Invasive Potential

N. peltata has been shown to be competitive and aggressive when grown in competition with other species. *N. peltata* allocated most of its resources to producing above ground tissue and floating leaves when grown in competition with a species of water chestnut (*Trapa bispinosa*) and Eurasian watermilfoil (*Myriophyllum spicatum*) (Wu et al, 2006). In this study it formed a canopy over the Eurasian watermilfoil and crowded out the water chestnut. Similarly, three native species, coontail (*Ceratophyllum demersum*), elodea (*Elodea canadensis*) and fan leaved crowfoot (*Ranunculus circinatus*) were grown under varying densities of *N. peltata* (Larson, 2006). At all coverage levels of *N. peltata* present (33-100%), native species experienced significant reductions in growth rate (Larson, 2006). These studies suggest that if *N.*

peltata is introduced into a location where submersed vegetation grows without other floating vegetation, alterations to the community structure and biodiversity are likely (Larson, 2006). Studies have not been conducted to assess the competitiveness of *N. cristata* or to evaluate its direct impact on native species.

Preventative management of Nymphoides

Prevention typically include measures such as quarantines, bans on sale and importation, and creating public awareness in stopping the spread of a species or new infestations. Invasive species of Nymphoides became established after escape from cultivation, yet there are several preventive measures that can still be implemented to slow the spread. Another method used to prevent spread of invasive plant species is by creating boat and trailer cleaning stations at boat ramps. Since vegetative fragments often adhere to boat hulls and trailers, aquatic plants are easily transported and introduced to new locations in this manner. The best way to prevent further infestations is to limit Nymphoides use as a cultivated water garden plant (Les and Mehrhoff, 1999). This would require significant changes to current aquarium trade practices since *N. cristata* is readily available from numerous on-line sources.

Cultural Control

Cultural control is manipulating the habitat to achieve control of nuisance weeds. Some cultural methods include winter time water level drawdowns and altering light levels or other environmental factors (Bellaud, 2009). Cultural methods have not been successful on the Santee Cooper population of *N. cristata*. When lake levels were lowered in the winter to expose plants to drying and brief periods of freezing temperatures in 2007-2008, rapid recovery was noted in the spring and no observable impact on the *N. cristata* populations was noted (Chip Davis, personal communication, June 2011). Other options could include benthic barriers on the sediment surface early in the growing season to control new regrowth of invasive species by blocking light and creating an anoxic zone beneath the mat (Bellaud, 2009). Nonetheless, benthic barriers are very costly are difficult to deploy in large areas.

Mechanical Control

Mechanical techniques are used to physically remove plant biomass from the impacted areas. Some examples of mechanical control are dredges, harvesters and cutter/shredder boats (Haller, 2009). Mechanical control is typically expensive, generally nonselective and access to equipment is limited (Burks, 2002a). One attempted method of mechanical control for Nymphoides control is cutting the plant below the water surface; however this method proved ineffective (Middleton, 1990). Also, *N. cristata* can easily survive and recover from underwater fragments (Burks, 2002a). For *N. peltata* control mechanical harvesting has also failed with many locations becoming worse as a result of mechanical harvesting (Larson, 2006). While time and labor intensive, hand removal of the entire plant for newly established infestations has been proposed (Nature Conservancy of Vermont, 2003). This method of control is currently being attempted on Lake Okeechobee as the infestation is fairly localized and various chemical control strategies have been ineffective. Hand pulling may be effective in early detection and rapid response programs, or as a follow up to herbicide programs.

Biological Control

Biological weed control is implementing the use of a natural predator of the target plant to reduce populations of the weed. In North America, aquatic larvae of the native moth *Paraponyx seminealis* have been collected while feeding on the native *N. aquatica*. Impacts of this moth on *N. cristata* are not known (Burks, 2002a). This moth has been observed to cut large pieces of leaf tissue from floating leaves for both food and shelter (Habeck, 1974). The young larvae feed as miners, eating holes in the leaf tissue. Where present the larvae are found in large numbers for most of the year, and can cause severe injury to floating heart reducing its factor as a weed (Habeck, 1974). To date, there is no evidence that *Paraponyx seminealis* will feed on *N. cristata*; however, culture plants used for trials at the University of Florida- Center for Aquatic and Invasive Plants (CAIP) required consistent insect management to prevent loss of the culture due to insect damage from aquatic caterpillars (Leif Willey; personal observation).

The triploid grass carp (*Ctenopharyngodon idella*) is widely used for aquatic plant control. Grass carp are general herbivores that consume almost any plant material with a few exceptions (Colle, 2009). Within its native range, grass carp will not consume *N. cristata* (Van Dyke et al. 1984). Studies performed in the early 1980s in Florida, showed after grass carp had completely eliminated bladderwort (*Utricularia spp.*), umbrella grass (*Cyperus alternifolius*), and water shield (*Brasenia schreberi*) some decline in the native *N. aquatica* coverage was apparent (Van Dyke, Leslie, & Nall, 1984). In the Santee Cooper reservoir system, over 850,000 grass carp have been stocked prior to 2012 (Chip Davis, personal communication, 2012) and *N.cristata* continues to expand aggressively. Lake Fairview, near Orlando, Florida is also stocked with grass carp, yet intense chemical and physical management of *N. cristata* is regularly required since the plant was beginning to show signs of rapid expansion. Water bodies stocked with grass carp could be highly susceptible to *N. cristata* invasion due to the grass carp facilitating an open niche through the consumption of preferred vegetation. *N. cristata* could spread rapidly due to lack of competition from other species of aquatic vegetation. The lack of host-specific insect species for invasive Nymphoides control suggests that biological control is unlikely to be a management option in the near future.

Chemical Control

To date, reported success with registered aquatic herbicides on *N. cristata* has been limited, often anecdotal, and sometimes conflicting. Vermont reported in the 1970s success using applications of 2,4-D in various formulations to control *N. peltata* in Lake Champlain

(Countryman, 1970). In other areas herbicides have proven ineffective in controlling this plant in the long term.

For *N. cristata* control, various formulations and combinations of herbicides have been used, but with no long term success (Burks, 2002b). In Florida, Collier County Storm Water Management has obtained up to 4 months control using 2% glyphosate solution with a surfactant, but numerous re-treatments were required (Burks, 2002b). Herbicide treatments in South Carolina have resulted in control of the surface mats, but recovery is generally noted (SC-DNR, 2010). A recent study on herbicide efficacy on *N. cristata* in south Florida showed endothall (dipotassium salt) was most effective, giving 98-100% control at 1.5-2.5 ppm (Puri and Haller, 2010). Field trials with endothall at concentrations of 2-3 ppm resulted in 80-90% control at 8 weeks post treatment (Puri and Haller, 2010). The longer term impacts of these treatments were not reported.

Objectives

Currently, 2 publications by Burks (2002a, b) constitute a large portion of the published literature pertaining to *N. cristata*. Furthermore, the information in these publications is mainly based on field observations and reports from managers. The placement of *N. cristata* on the FLEPPC Category I list given this lack of peer reviewed literature was a major influence for this thesis research. The objectives of this research were to determine the growth responses of *N. cristata* from propagules exposed to various environmental conditions and to evaluate chemical control options for management of this plant.

Figure 1-1. Image of *Nymphoides cristata* growing in the Santee Cooper Reservoir. Photo from Chip Davis, Santee Cooper Analytical and Biological Services 2009.

Figure 1-2. Bundle of tapered, tuberous roots of the ramet developing beneath floating leaves of crested floating heart (*Nymphoides cristata*). From University of Florida Center for Aquatic and Invasive Plants (University of Florida, 2001). Available at http://plants.ifas.ufl.edu/node/291.

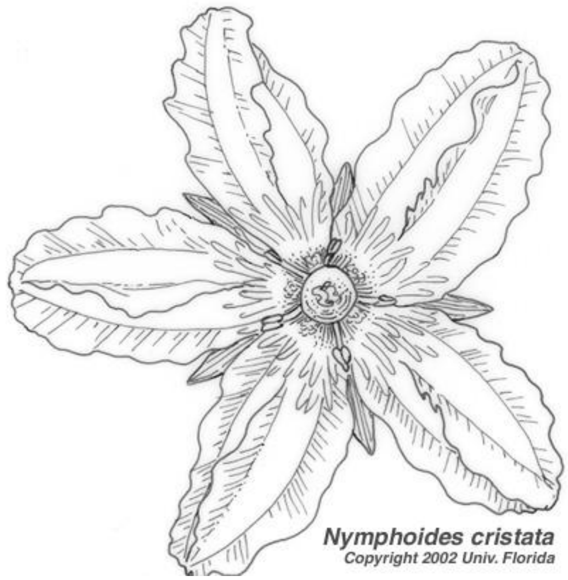


Figure 1-3. Line drawing of a crested floating heart (*Nymphoides cristata*) flower, showing the erect fold of tissue that occurs longitudinally along the center of each petal. From University of Florida Center for Aquatic and Invasive Plants (University of Florida, 2002). Available at http://plants.ifas.ufl.edu/node/291.

Figure 1-4. Image comparing the native Nymphoides aquatica leaf underside (right) showing rough texture and the exotic Nymphoides cristata leaf underside (left). From Chris Page, South Carolina Department of Natural Resources, Early Detection and Distribution Mapping System (2010). Available at http://invasiveplantatlas.org/subject.html?sub=11616.

Figure 1-5. Root system of an established *Nymphoides cristata* plant collected in January, 2011 from a South Florida Water Management District pond. Photo by Leif Willey, 2011.

CHAPTER 2
EFFECTS OF AQUATIC HERBICIDES ON CRESTED FLOATING HEART

Introduction

Crested floating heart (*Nymphoides cristata* (Roxb.) Kuntze), hereafter referred to as *N. cristata* is an invasive aquatic weed native to Southeast Asia and introduced to North America through the water garden trade. It is not known how long the plant may have been cultivated in the United States (Burks, 2002a). *N. cristata* is valued as an ornamental plant because of the expression of accessory pigments in the foliage, numerous small, white flowers, and long flowering season. It is often marketed as water snowflake because it can cover a water surface in tiny white flowers giving the appearance of snow. *N. cristata* has escaped from cultivation and exists in thriving populations in many waterways in Florida, South Carolina, Texas (Center for Invasive Species and Ecosystem Health, 2012), North Carolina (Robert Richardson, personal communication, August 2012) and Louisiana (Alexander Perret, personal communication, July 2012).

N. cristata was first reported in Florida outside of ornamental culture in 1996 in Horseshoe Lake, Collier County, FL (Burks, 2002a). One of the largest *N. cristata* infestations has occurred in the 64,750 ha Santee Cooper reservoir system in South Carolina where the infestation now covers over 2,428 ha (6,000 ac) (Westbrooks, et al. 2012). The Santee Cooper, SC infestation suggests that all water bodies in Florida are with a climate zone favorable for sustaining *N. cristata*. The magnitude of an invasion is likely to be dependent on numerous water quality variables and lake morphology. *N. cristata* has been observed growing in water from 0.6 to 3 m deep in the Santee Cooper system (Chip Davis, personal communication, July 2012). The plant can also survive extended periods in moist soil (Willey and Langeland, 2011). Lakes in Florida generally tend to be shallow. Lake Okeechobee for example, despite its size (189,000 ha), has an average depth of only 3 m (SFWMD, 2012).

In areas where it has become established, floating leaves of the plant form dense mats of overlapping leaves on the surface of the water, which interfere with boat traffic and recreational uses of the water. The mats shade the water column below, reducing light availability to submersed native vegetation, lowering dissolved oxygen levels, and reducing water flow and aeration (Burks, 2002a). Studies on similar mat-forming vegetation have shown that submersed macrophyte growth beneath these mats is significantly reduced (Janes et al. 1996). *N. cristata* reproduces and spreads by fragmentation, which can be caused by contact with boat motors, wave action and mechanical harvesting (Burks, 2002a). Spread is also facilitated through the production of clonal reproductive structures called ramets. Ramets develop beneath the floating leaves and protrude from the stems of the plant as a tuber cluster with several small leaves. These propagules easily separate from the parent plant and drift away or sink to the bottom (Burks, 2002a).

In terms of potential management options for *N. cristata*, triploid grass carp (*Ctenopharyngodon idella*) is a widely used biological control for many aquatic plants. However, previous studies have reported that grass carp do not consume *N. cristata* even when provided no other option (Van Dyke et al. 1984). A study conducted in the native range of *N. cristata* also found that grass carp would not consume the plant (Singh et al. 1966). This presents a troublesome management issue in the Santee Cooper system because which is stocked with grass carp (109,000 in 2012 in addition to 750,000 previously stocked) to control hydrilla (*Hydrilla verticillata*) (Chip Davis, personal communication, July 2012). Many lakes in Florida and other Southeastern reservoirs are stocked with grass carp for the purpose of hydrilla control; this could in turn create a niche for *N. cristata* colonization with reduced competition from hydrilla. Managers at the Santee Cooper system have also tried winter season drawdowns to expose plant parts to desiccation and potentially freezing temperatures but the plants re-grew the following spring (Page, 2010). A study conducted in the native range of *N. cristata* suggests that mechanical harvesting would be ineffective because the plant was able to recover quickly from underwater clipping (Burks, 2002a; Middleton, 1990). Effective management plans are needed to prevent further spread and reduce current populations, yet there has been very little published information on the use of herbicides as a management technique for *N. cristata*. Reports from managers tend to be anecdotal and often conflicting. Current literature from Burks (2002b), states that a maximum of 4 months of control has been achieved when using foliar applications of a 2 % glyphosate solution with a surfactant.

Herbicide activity on *N. cristata* is not well understood. It is often hypothesized that different formulations or methods of application may also influence efficacy of aquatic herbicides (Wersal and Madsen, 2010). Foliar applications are typically easier to make than subsurface applications and may also be more economical (Wersal and Madsen, 2010). In an open system, when a herbicide is applied via a subsurface application, it immediately begins to diffuse away from the treatment zone to areas with lower herbicide concentration (Sprecher et al. 2002; Fox et al. 2002). Due to diffusion and other forces such as water flow and herbicide degradation, it is not likely to remain in the treatment zone for long periods of time, which could require higher concentrations to be applied and repeat applications to maintain specific concentrations. These issues illustrate the importance of assessing efficacy as a function of application method and potential exposure time for submersed applications. The lack of a standard management option suggests that research is needed to evaluate registered aquatic herbicides in order to improve management recommendations for control of *N. cristata*. To address this deficiency 12 registered aquatic herbicides were screened to evaluate and compare activity on *N. cristata*.

Materials and Methods

Subsurface herbicide applications

Experiments were conducted at the University of Florida, Center for Aquatic and Invasive Plants (CAIP) in Gainesville, FL in 2011 to 2012. *N. cristata* plants were collected in January 2011 from the Storm Water Treatment Areas (STA) in South Florida. Ramets were established in 1 L plastic containers that were filled with Margo Professional Topsoil[1] (92% sand, 4% silt, 4% clay) amended with fertilizer (Osmocote® 15-9-12)[2] at 1g kg^{-1} of soil. Plants were cultured in 95 L tanks in a greenhouse from January until early March then moved into outdoor 1,000 L mesocosm tanks until ramets were produced. Ramets were collected from this culture and planted in 1 L pots to conduct herbicide studies. Ramets were allowed to grow until leaves emerged at the surface and flower production was observed. When all plants had produced at least one flower treatments began. All registered aquatic herbicides were screened using both submersed and foliar applications as well as liquid and granular formulations (Table 2-1, 2-2). For the submersed applications, exposure times of 24 and 96 hours were evaluated. Current literature suggests that, depending on the rate of water exchange, size of treatment area in relation to water body size and other characteristics in a natural system the half-life of the herbicide concentration may range from as low as a few hours (Poovey et al. 2004) to as long as a few weeks (Green et al. 1989; Simsiman and Chesters, 1975; Langeland and Warner, 1986).

[1] Margo Garden Products. Folkston, GA 31537

[2] The Scotts Company. Marysville, OH 43041

Since the objective of this screening was to evaluate comparative efficacy, it was decided to focus on one short exposure time (24 h) and one long exposure (96 h) for contact herbicides. In contrast, fluridone and ALS inhibiting herbicides were tested for 3, 6 and 12 weeks under static exposure conditions in a separate greenhouse trial. All trials were conducted in 95 L tanks.

Eight herbicides and 3 combinations of herbicides were evaluated using submersed application techniques at 24 and 96 hour exposures (Table 2-1, 2-2). Herbicides were tested at maximum and half maximum label concentrations with the exception of carfentrazone which was tested at a single concentration. Plants were treated at the time of flowering. Submersed herbicide applications were made using an adjustable pipette to inject the liquid herbicide into the water column. Granular herbicides were weighed to within ± 0.02 gram of the calculated amount using a digital scale (Denver Instrument APX-203)[3] then placed into the water, avoiding the floating leaves. Water samples were collected 1 day after treatment (DAT) from all treatments of 2,4-D, triclopyr, and endothall and analyzed using an ELISA test (SDIX RaPID Assay)[4] to confirm the herbicide concentration matched the target treatment rate. A small electric pump was used 24 and 96 hours after treatment (HAT) to remove the treated water from each tank and then the tanks were refilled with untreated well water as described by Wersal and Madsen (2010). Visual observations of phytotoxicity were recorded weekly. Entire plants, including all live roots and foliage, were harvested 4 WAT and rinsed in untreated water to remove algae, sediment and dead tissue from the roots and foliage. Harvested plants were placed in labeled paper bags and dried in a drying oven (76 C) for 1 week.

[3] Denver Instruments. Arvada, CO 80004

[4] Strategic Diagnostics Inc. Newark, DE 19713

The first trial for 2,4-D, triclopyr, and a combination of the 2 herbicides was initiated June 17, 2011 and repeated July 12, 2011. The first trial for endothall, diquat and combinations of the herbicides as well as bispyribac was initiated on July 7, 2011 and repeated August 5, 2011. An additional evaluation of endothall was conducted on March 3, 2012 to evaluate the influence of cooler water temperatures (less than 20 C) on possible control.

Treatment dry weight was compared to a pre-treatment dry weight. Pre-treatment weights represent plant biomass the time of application and served as a reference to evaluate control for the herbicides. The pre-treatment stage of growth was selected as the base for herbicide activity because the untreated control is continually growing. A herbicide could result in a reduction from the untreated control, but still have higher dry weight than the time of treatment. If at 4 WAT, in two separate trials, a herbicide resulted in a significantly lower dry weight than the pre-treatment sample, it was identified as having activity on the plant (net decrease in dry weight from time of treatment) and was recommended for additional evaluation. If a herbicide did not reduce dry weight below the pre-treatment weight (no change or net increase in dry weight from time of treatment), that herbicide was determined to have limited activity on *N. cristata*.

All studies were arranged as a complete randomized design with 3 replications of each treatment. Treatment effects on dry weight were analyzed using ANOVA ($p \leq 0.05$) and graphed with 95% confidence intervals to determine differences among the treatments. Statistical analysis and graphical presentations of data were performed using SigmaPlot 11.0[5]. Data were pooled when no differences were detected between trials; however, data are presented separately for each trial in cases where data were different between trials.

[5] Systat software, Inc. San Jose, CA 95110

Foliar herbicide application

Fourteen herbicides and combinations of herbicides were screened for efficacy following foliar applications (Table 2-1, 2-2). Foliar applications were made using a CO_2 pressurized, single nozzle spray system at the time of flowering. A spray volume equivalent to 934 L ha^{-1} (100 gal ac^{-1}) was used for all foliar treatments over an area of 0.185 m^2. Output pressure was regulated at 83 to 103 kPa, which allowed for a consistent spray with minimal drift. Foliar application use rates are listed in Table 2-1. Tanks were drained following the foliar treatments and re-filled at 24 HAT to remove any confounding issues associated with herbicide concentrations in the water column. Foliage was not rinsed. Triclopyr, 2,4-D, diquat and endothall applications were performed at the same time as the submersed applications. The first trials for the ALS herbicides were initiated on June 21, 2011 and repeated on July 19, 2011. Combinations of selected herbicides were initiated on July 3, 2011 and repeated August 5, 2011.

Visual observations were recorded weekly after the herbicide treatments were applied. Whole plants, including all live roots and foliage, were harvested 4 WAT and rinsed in untreated water to remove any dead tissue and debris. Live tissue was placed in paper bags and dried at 76 C for 1 week. Statistical analyses were conducted as previously described for submersed treatments.

Results and Discussion

Subsurface herbicide application

Triclopyr, 2, 4-D, 2, 4-D + triclopyr, endothall (dipotassium salt), bispyribac, carfentrazone, and flumioxazin did not reduce dry weight below the pre-treatment dry values at 4 WAT regardless of exposure time, rate and formulation. Dry weight either remained constant or increased above the pre-treatment reference levels at 4 WAT following treatment (Figure 2-1 to 2-4, 2-6, 2-7). For these 7 herbicide treatments, visual observations of injury symptoms were not indicative of herbicide efficacy because symptoms were transient and while noticeable within days of treatment, injury symptoms were generally short-lived.

ELISA tests performed 24 HAT of the auxin-mimic herbicides showed that actual mean concentrations were within the target concentration. 2,4-D ranged from 2.25 to 2.60 mg ae L^{-1} for 2.5 mg ae L^{-1} target concentrations and 1.10 to 1.40 mg ae L^{-1} for 1.25 mg ae L^{-1} target concentrations. Triclopyr concentrations tested from 2.15 to 2.53 mg ae L^{-1} for target concentrations of 2.5 mg ae L^{-1} and 1.09 to 1.42 mg ae L^{-1} for target concentrations of 1.25 mg ae L^{-1}. An ANOVA test of the triclopyr treatments found differences among the treatments ($p<0.001$) in both trials. No treatment reduced dry weight below the pre-treatment reference (Figure 2-1 A, B). Furthermore, there were no differences found between subsurface and foliar applications or between liquid and granular formulations (Figures 2-1 A, B). No differences were found among the treatments of 2,4-D in the first trial ($p=0.329$) (Figure 2-2 A). Differences among treatments were found in the second trial ($p<0.001$) in which concentrations of 2.5 mg ae L^{-1} as both liquid and granular formulations outgrew the pre-treatment reference (Figure 2-2 B), however, no treatments reduced dry weight below the pre-treatment reference.

Similarly, a combination of 2, 4-D and triclopyr did not reduce dry weight below the pretreatment reference (Figure 2-3 A, B). In the first trial no differences were found among the treatments ($p=0.429$). Differences among treatments were found in the second trial ($p<0.001$), in which a concentration of 1.25 mg ae L^{-1} outgrew the pre-treatment reference (Figure 2-3 B). There were also no differences found between the 24 and 96 hour exposure times for these compounds. Auxin-mimic type symptoms began to develop by 1 WAT, with noticeable epinasty of the stems, elongated flower stalks and leaf curling. These symptoms were transient and did not persist, by 2 WAT and 3 WAT the plants had completely recovered (no visible symptoms).

Cessation of growth by these compounds was not observed during either of the trials. Typically broadleaved aquatic plants are highly susceptible to herbicides with auxin-mimic mode of action. Previous studies have found that subsurface applications of 2,4-D ester at 1.5 and 2.5 mg a.e. L^{-1} to fragrant waterlily (*Nymphaea odorata*) resulted in significantly less dry weight than the untreated controls (Glomski and Nelson, 2008). Spatterdock (*Nuphar advena*) dry weight was also significantly reduced by the same rates of 2,4-D ester as well as 2.0 mg a.e. L^{-1} triclopyr amine by 6 WAT (Glomski and Nelson, 2008). Studies evaluating 2,4-D and triclopyr at rates of 0.5, 1, and 2 mg ai L^{-1} using 24 and 48 hour exposure times reported that all rates controlled water chestnut (*Trapa natans*) (Poovey and Getsinger, 2007). Furthermore these compounds control submersed dicots such as Eurasian watermilfoil (*Myriophyllum spicatum*) (Netherland and Getsinger, 1992). It should be noted however, there are other dicotyledons such as cabomba (*Cabomba caroliniana*) that are more tolerant to auxin-mimic herbicides (Bultemeier et al. 2009).

Endothall is a contact herbicide that causes defoliation and tissue necrosis leading to plant death (Sprecher et al. 2002). Actual treatment concentrations were within the target concentration according to water samples analyzed at 24 HAT with an ELISA. 3.0 mg ae L^{-1} target concentrations ranged from 2.82 to 3.11 mg ae L^{-1}. 1.5 mg ae L^{-1} target concentrations ranged from 1.15 to 1.77 mg ae L^{-1}. Differences were found among the treatments in both trials ($p<0.001$), however, neither of these formulations caused a reduction in dry weight equal to or below the pre-treatment reference (Figure 2-4 A, B). In trial 1 (Figure 2-4 A) all 24 hour exposures outgrew the pre-treatment reference, while all treatments outgrew the pre-treatment reference in the second trial (Figure 2-4 B). There was a difference between exposure times in which the 96 hour exposure time caused an average of 55% and 50% greater reduction in dry weight than the 24 hour exposure time of the liquid submersed or granular treatments respectively (Figure 2-4 A, B). This suggests that increasing exposure times beyond 96 hours would improve endothall efficacy. Symptoms developed slowly after treatment. One WAT isolated spots of foliar desiccation were observed. Two WAT foliage and stems began to show signs of necrosis and dropped off the water surface, however regrowth began between 2 and 3 WAT. Endothall has been used successfully in managing several submersed invasive aquatic plants such as hydrilla and Eurasian watermilfoil (Skogerboe and Getsinger, 2001). Previous studies have shown that plants with a similar growth form to *N. cristata*, such as spatterdock and fragrant waterlily were susceptible to endothall following 5 day exposure times at concentrations of 2 and 5 mg ai L^{-1} (Skogerboe & Getsinger, 2001). Recent work on *N. cristata* has shown that endothall (dipotassium salt) was most effective in static tests resulting in 98 to 100% control using rates of 1.5 and 2.5 mg ai L^{-1} at 6 WAT (Puri and Haller, 2010). In the field, endothall (dipotassium salt) at rates of 2.0 to 3.0 mg ai L^{-1} provided 80 to 90 % control at 8WAT (Puri and Haller, 2010). To address the discrepancies between these findings and the findings of the current endothall (dipotassium salt) trials an additional trial was initiated August 5, 2011 to assess the efficacy of endothall under a 4 wk static exposure. Dry weight of the plant was reduced below that of a pre-treatment reference; however, healthy regrowth was observed by 3WAT (Figure 2-5). These results suggest that endothall (dipotassium salt) can be effective in systems where extended exposures are possible.

Bispyribac-sodium is an acetolactate synthesis (ALS) inhibiting herbicide that was recently registered for aquatic use. While differences were notes among the treatments ($p<0.001$), bispyribac-sodium did not reduce dry weight below pre-treatment levels 4 WAT regardless of exposure time (Figure 2-6). There was an exposure response observed with the 96 hour exposure time resulting in a 30% greater reduction in dry weight than the 24 hour exposure time ($p<0.001$) (Figure 2-6). Flower production ceased at 1 WAT, but by 2 WAT foliage began to turn red in color and become necrotic, however plants quickly recovered and new growth was visible by 3 WAT. Bispyribac-sodium has been found to have activity on several emergent aquatic plants (Koschnick et al. 2007). The ALS herbicides typically have very slow activity and will likely require much longer exposure to evaluate efficacy; however, I wanted to evaluate at least one ALS herbicide using a protocol similar to the auxin mimic and contact herbicides.

Carfentrazone and flumioxazin are protoporphyrinogen oxidase (PPO) inhibitors that impact chlorophyll synthesis and cause cell membrane leakage. Both are fast acting, contact herbicides (Senseman, 2007). The half-life of flumioxazin ranges from several hours to a few days depending on pH (Mudge and Haller, 2010). The pH of the water at the time of application was 6.8 and this should have provided the range of a 24 hour product half-life based on estimation of pH-dependent hydrolysis. Differences were found among the treatments ($p<0.001$) although no treatment reduced dry weight below the pre-treatment reference. Carfentrazone treated plants outgrew the pre-treatment reference as well as the 0.2 mg ai L^{-1} concentration of flumioxazin (Figure 2-7) There was an observed rate response noted between the two concentrations of flumioxazin in which the 0.4 mg ai L^{-1} concentration resulted in a 66 % reduction in dry weight from the 0.2 mg ai L^{-1} concentration (Figure 2-7). One WAT the foliage of the flumioxazin treated plants had turned necrotic and had begun to drop away from the surface, however rapid regrowth was observed 2 WAT. Similar results were observed with carfentrazone. Both herbicides have provided good activity on several floating aquatic plants (Mudge and Haller, 2010; Koschnick et al. 2004), yet activity on *N. cristata* was limited to initial injury followed by rapid regrowth.

Diquat is a cell membrane disruptor that interrupts photosynthetic electron transport and diverts electrons to oxygen resulting in the formation of radical oxygen which causes cell membrane damage and death (WSSA, 2007). Diquat readily binds to organic matter and sediment particles which can impact activity in turbid water and also likely explains the lack of a granular formulation. Water clarity was optimal for diquat activity in these trials. Differences were found among treatments in both trials ($p<0.001$). None of the treatments reduced dry weight below the pre-treatment reference in trial 1, but all treatments were less than the untreated control (Figure 2-8 A, B). A rate response was observed during the second trial in which the maximum labeled concentration (0.38 mg L^{-1}) yielded 47% less dry weight than the half maximum concentration (Figure 2-8 B). There was no observed response between the 24 and 96 hour exposure times at the maximum label rate. Diquat treated plants rapidly developed symptoms. One WAT, foliage was necrotic and stems had dropped away from the water surface, however new leaves began to re-grow by 2 WAT. During re-growth, new foliage would open on the water surface and would become chlorotic, and then would either die or very slowly recover. Plants began to recover between 3 and 4 WAT, but re-growth remained limited with some new foliage remaining chlorotic near the midvein of the leaf. Diquat applied as a submersed application strategy was shown to be effective in controlling other invasive aquatic species with emergent foliage such as parrotfeather even when exposed to short exposure times (Wersal and Madsen, 2010).

The amine salt formulation of endothall applied into the water column was the most effective herbicide evaluated. ANOVA determined there were differences among the treatments ($p<0.001$). Liquid subsurface treatments caused a 100% reduction in dry weight compared to the pre-treatment reference (Figure 2-9 A, B). Actual treatment concentrations for the target concentration of 0.5 mg ae L^{-1} ranged from 0.30 to 0.62 mg ae L^{-1} and target concentrations of 0.25 mg ae L^{-1} ranged from 0.11 to 0.35 mg ae L^{-1}. Treated plants showed necrosis by 24 HAT. One WAT all plant material had dropped away from the water surface and by 4 WAT there was no observed regrowth. There was no observed rate or time response since both concentrations and exposures tested resulted in 100% control of the plant. Granular applications of endothall were less effective than liquid applications and were highly variable. There was a rate response to the granular herbicide in the first trial (Figure 2-9 A) in which the maximum concentration resulted in 50% greater reduction than the half maximum concentration with a 24 hour CET.

The first round of Endothall (amine salt) treatments occurred in June and July when the water temperatures were high. It is thought that uptake of this herbicide may be increased under higher temperatures (Haller and Sutton, 1973). While endothall (amine salt) was the only herbicide to result in 100% control at both rates tested, there are some issues associated with use of this herbicide. While the amine salt is 2 to 3 times more active than the dipotassium salt on submersed plants, activity on algae, potential rapid drops in dissolved oxygen, and toxicity to fish are all significant concerns (Sprecher et al. 2002).

Combining herbicides could have a synergistic effect (two herbicides together result in greater efficacy than either of the herbicides used singly). Other advantages of herbicide combinations may be reduced herbicide quantity used and greater selectivity (Pennington et al. 2001). Analysis of a combination of diquat + endothall (dipotassium salt) found differences among treatments ($P<0.001$). Dry weight was reduced below that of the pretreatment reference (Figure 2-10) and exposure time did not affect efficacy of the treatment. The combination of 2,4-D + triclopyr, did not result in dry weight less than the pre-treatment and results were similar to 2,4-D and triclopyr used alone (Figures 2-1 A,B; 2-2 A, B; 2-3 A, B).

In summary, the screening procedure identified endothall amine salt as the most active compound followed by diquat. The lack of activity of the dipotassium salt of endothall was unexpected since previous research suggested good activity for this herbicide (Puri and Haller, 2010). We were able to achieve improved control with the dipotassium salt of endothall under static conditions; however, many sites and treatment scenarios will not provide this level of exposure time.

Foliar herbicide application

Generally, foliar herbicide applications offer a more economical treatment option when compared to subsurface applications (Wersal & Madsen, 2010), since the herbicides are placed directly on the foliage and not subject to dilution. Foliar herbicide trials were performed simultaneously with the sub surface applications to determine any differences between application strategies.

Foliar applications of triclopyr did not reduce dry weight (Figure 2-1A, B) and similar results were observed with 2,4-D (Figure 2-2 A, B). Typical auxin-mimic symptoms began to develop within 1 WAT but the plants quickly recovered by 2 WAT and continued growing until the study was harvested. There were also no differences found between foliar and subsurface applications of these herbicides. Studies performed on other broadleaf, emersed plants like parrotfeather (*Myriophyllum aquaticum*), have shown that 2, 4-D is more effective when foliar applied (Wersal and Madsen, 2010). Other studies examining effects of foliar applications have shown that rates of 2,4-D amine up to 4.48 kg ha^{-1} cause symptom development in spatterdock, but did not result in death of the plant tissue (Hanlon and Haller, 1990). Waterlily has also been reported to be sensitive to triclopyr amine via foliar application, but no details were given pertaining to rates (Glomski and Nelson, 2008; Langeland et al. 1993; Gettys et al. 2009).

Foliar applications of diquat and endothall (dipotassium salt) did not reduce dry weight. Endothall (dipotassium salt) showed similar results (Figure 2-4 A, B and 2-8 A, B). Diquat treated foliage died back from the surface quickly and regrowth began 2 WAT. Regrown foliage would reach the water surface and become chlorotic. This chlorotic regrowth suggests that diquat may be having a temporary impact on the new foliage. While the growth was initially slow, normal growth was noted by 3 WAT. When applied to foliage at rates of 2.3 L ha^{-1}, diquat reduced biomass of duckweed (*Lemna minor*), water hyacinth (*Eichhornia crassipes*) and water lettuce (*Pistia stratiotes*) greater than 90% (Langeland et al. 2002). Alligator weed (*Alternanthera philoxeroides*) has also shown similar results to *N. cristata*. When sprayed with diquat at rates of 5.6 and 22.4 kg ha^{-1}, alligator weed showed complete foliar control within the first 2 weeks, but by 4 WAT only achieved 80 and 89 % control and by 8 WAT 45 and 60 % control respectively (Blackburn, 1963). Endothall (dipotassium salt) treated plants began to show symptoms 1 WAT as bronzed spots on the foliage. These damaged areas did not expand and the foliage never became totally necrotic or fell from the water surface. Diquat applied to the foliage was less effective than sub surface applications, while endothall was more effective when foliar applied than 24 hour sub surface applications but less effective than 96 hour applications (Figures 2-4 A and B and 2-8 A and B). Diquat and endothall have also been evaluated against other species of floating vegetation. Giant salvinia (*Salvinia molesta*), when treated with endothall at 5.04 kg ha^{-1} and a surfactant resulted in 80% control 4 WAT and when treated with diquat at 2.24 and 1.12 kg ha^{-1} resulted in 100 and 98% control respectively (Nelson et al. 2001).

Four amino acid inhibiting herbicides were also screened for foliar application. An ANOVA test of the treatments determined that there were differences among the treatments ($p<0.001$). Three of the herbicides (imazamox, imazapyr and penoxsulam) are ALS inhibitors, while the fourth (glyphosate) is an EPSP synthase inhibitor. Glyphosate and penoxsulam were largely ineffective compared to imazamox and imazapyr which reduced dry weight below the pre-treatment reference by 83% and 84% respectively (Figure 2-11). All herbicides resulted in cessation of growth by 1 WAT. Two WAT foliage from imazamox and imazapyr treated plants was not present on the surface while live foliage was still present on glyphosate and penoxsulam treated plants. No regrowth had occurred 4 WAT on imazamox and imazapyr treated plants suggesting the herbicide had translocated into the root system; however, the root systems were still intact upon harvesting. Penoxsulam resulted in no reduction in dry weight compared to the pre-treatment reference. However, when plants were harvested it was noticed that even though shoot material did not die off, the root system sustained severe damage and was almost entirely dead tissue. Glyphosate treated plants began to show signs of recovery 4 WAT with appearance of new flowers. The result for glyphosate is interesting since it is considered a broad spectrum herbicide, yet seemed to have little impact on this plant. Other emergent plants such as water lily, spatterdock, and lotus (*Nelumbo lutea*) are effectively controlled with glyphosate. Spatterdock and water lilies have been reported to be controlled at rates of 2.5 kg ai ha^{-1}(Baird et al., 1983; Seddon, 1981). Lotus is a third emergent plant that is controlled with glyphosate using rates of 2.4 kg ai ha^{-1} with little to no regrowth 8 WAT (Nathan Eddy, personal communication, 2012). Numerous applicators include glyphosate in combination with imazapyr as part of their current treatment regime for *N. cristata* (Burks, 2002).

Combinations of foliar-applied herbicides (Table 2-2 and Figure 2-12) were analyzed via ANOVA and no differences were detected among treatments ($P<0.001$). All combinations tested reduced dry weight below the pre-treatment reference however results were comparable to at least one of the two herbicides used. The combination of triclopyr + penoxsulam reduced dry weight 43% below the pre-treatment reference and this effect was not observed when triclopyr or penoxsulam were tested alone (Figure 2-1, 2-11, 2-12). Foliar combinations have been used in the field on several other emergent plants. A combination of triclopyr and imazapyr 1.75 and 0.15 kg ai ha^{-1} respectively, applied to the foliage of parrot's feather resulted in 85% control 8 WAT (Nathan Eddy, personal communication, 2012). This same combination was used in a pond treatment of yellow floating heart (*Nymphoides peltata*), but only provided 3 weeks of control (Nathan Eddy, personal communication, 2012). In deep water treatments at the Santee Cooper Reservoir a combination of glyphosate (4.8 kg ai ha^{-1}) and imazamox (2.4 kg ai ha^{-1}) has resulted in good control with limited regrowth at 8 WAT (Chip Davis, personal communication, 2012).

The screening of foliar applied herbicides has identified imazamox and imazapyr as having good activity on *N. cristata*. When making foliar applications of herbicides, translocation plays a critical role in the efficacy of a treatment. In the Santee Cooper system, it has been noted that post-treatment regrowth is less at depths less than1.5 m (Chip Davis, personal communication, 2011). Furthermore, managers in south Florida have reported greater control in shallower, quiescent areas (Burks, 2002). As a result of these observations it is hypothesized that water depth may impact the ability of herbicides to translocate from foliage to roots (Chip Davis, personal communication 2011). *N. cristata* has been found to grow in up to 3 m of water, if water depth (stem length) has an impact on the ability of a herbicide to translocate, it may be very difficult to manage this plant in deep water when using foliar treatment strategies.

This screening has identified 4 herbicides; liquid endothall (amine salt), imazamox, imazapyr and diquat (high concentration and long CET) that were able to reduce dry weight of *N. cristata* below the pre-treatment reference. In addition, there were no differences between liquid and granular formulations with the exception of endothall (amine salt), in which the granular formulation was less effective than the liquid. These findings also suggest that in most cases foliar or sub surface applications have no influence on treatment efficacy and that foliar applications of a herbicide result in similar efficacy as would be observed using subsurface applications of the same herbicide. This result is similar to that reported by Wersal and Madsen, (2010). Management of *N. cristata* will continue to remain a challenge, due to the small number of herbicides that show good activity on this invasive plant.

Table 2-1. Single herbicide subsurface treatment concentrations and foliar rates, exposure times and application method used in the herbicide screening on *N. cristata*. Spray volume of foliar treatments was equivalent to 934 L ha-1. All foliar applications made with methylated seed oil as surfactant at a rate of 0.25 % vol : vol.

Herbicide treatment	*Exposure time (hr)*	*Application method*[a]
2,4-D (amine) 2.5 and 1.25 mg ae L^{-1}	24,96	L
Diquat 0.370 and 0.185 mg ai L^{-1}	24,96	L
Flumioxazin[b] 0.4 and 0.2 mg ai L^{-1}	24,96	L
Bispyribac-sodium[b] 0.03 mg ai L^{-1}	24,96	L
Triclopyr (amine) 2.5 and 1.25 mg ae L^{-1}	24,96	L,G
Endothall (dipotassium) 3.0 and 1.5 mg ae L^{-1}	24,96	L,G
Endothall (amine) 0.5 and 0.25 mg ae L^{-1}	24,96	L,G
2,4-D (ester) 2.5 and 1.25 mg ae L^{-1}	24,96	G
Imazapyr 1.2 kg ae ha^{-1}	-	F
Penoxsulam 0.1 kg ai ha^{-1}	-	F
2,4-D (amine) 2.2 kg ae ha^{-1}	-	F
Diquat 2.2 kg ai ha^{-1}	-	F
Glyphosate 2.4 kg ae ha^{-1}	-	F
Endothall (dipotassium) 2.5 kg ae ha^{-1}	-	F
Imazamox 1.2 kg ae ha^{-1}	-	F
Triclopyr (amine) 3.5 kg ae ha^{-1}	-	F

[a] Abbreviations: L, liquid subsurface; G, granular subsurface; F, foliar.
[b] Liquid stock formulations were prepared from granular and wettable powder formulations for submersed liquid applications.

Table 2-2. Herbicide combination subsurface treatment concentrations and foliar rates, exposure times and application method used in the herbicide screening on N. cristata. Spray volume of foliar treatments was equivalent to 934 L ha-1. All foliar applications made with methylated seed oil as surfactant at a rate of 0.25% vol : vol.

Herbicide treatment	*Exposure time (hr)*	*Application method*[a]
2,4-D (amine)1.94 and 0.98 mg ae L^{-1} + triclopyr (amine) 0.55 and 0.27 mg ae L^{-1}	24,96	L,G
Diquat 0.2 mg ai L^{-1} + endothall (dipotassium salt) 0.8 mg ae L^{-1}	24,96	L
Glyphosate 2.4 kg ae ha^{-1} + imazapyr 0.6 kg ae ha^{-1}	-	F
Glyphosate 2.4 kg ae ha^{-1} + imazamox 0.6 kg ae ha^{-1}	-	F
Triclopyr (amine) 1.7 kg ae ha^{-1} + imazapyr 0.6 kg ae ha^{-1}	-	F
Triclopyr (amine) 0.44 kg ae ha^{-1} + penoxsulam 0.07 kg ae ha^{-1}	-	F
Diquat 0.5 kg ai kg ha^{-1} + imazamox 0.9 kg ae ha^{-1}	-	F
Endothall 0.6 kg ae ha^{-1} + imazamox 0.9 kg ae ha^{-1}	-	F

[a] Abbreviations: L, liquid subsurface; G, granular subsurface; F, foliar.

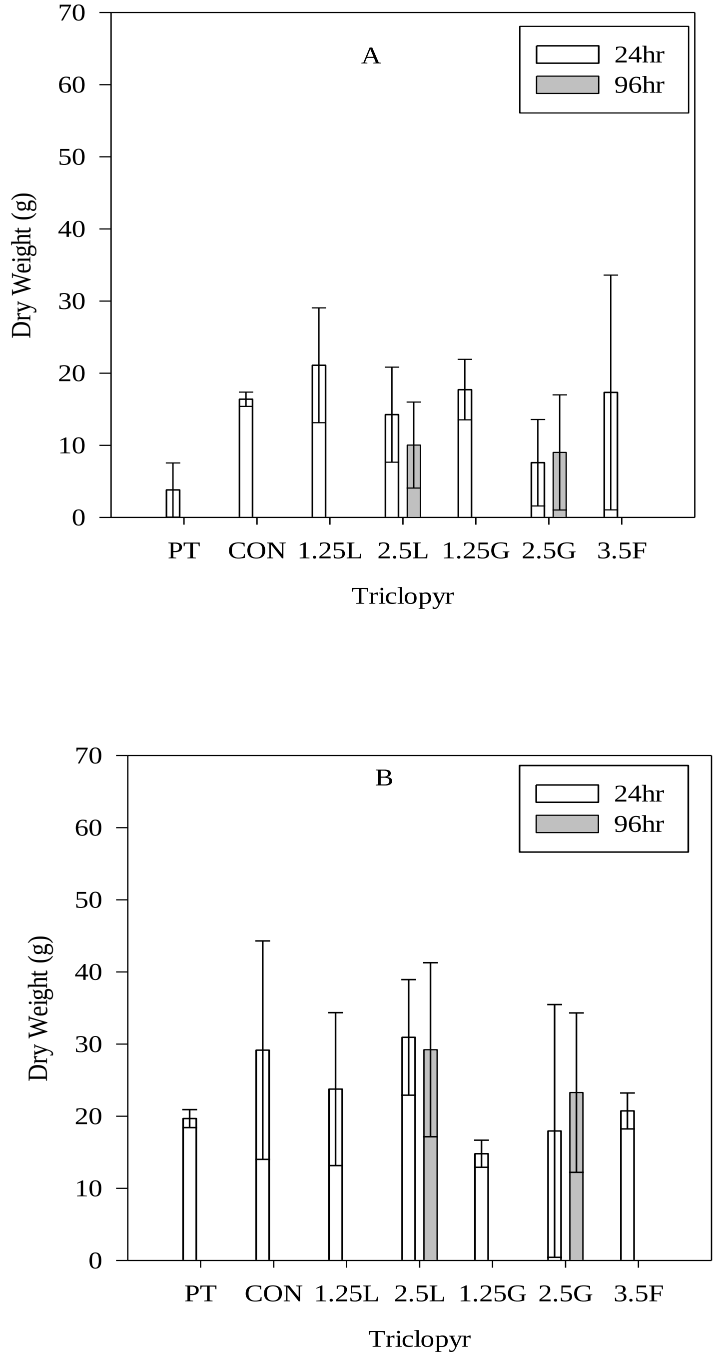


Figure 2-1. Combined dry weight of live roots and foliage of *N. cristata* at 4 WAT in response to triclopyr at 24 and 96 h exposures in trial 1 (A) and in trial 2 (B). PT = pre-treatment reference, CON = untreated control, L = liquid subsurface, G = granular subsurface and F = foliar rate in kg ae ha^{-1}. Numbers in front of L and G are concentrations in mg ae L^{-1}. Error bars represent ± 95% confidence intervals.

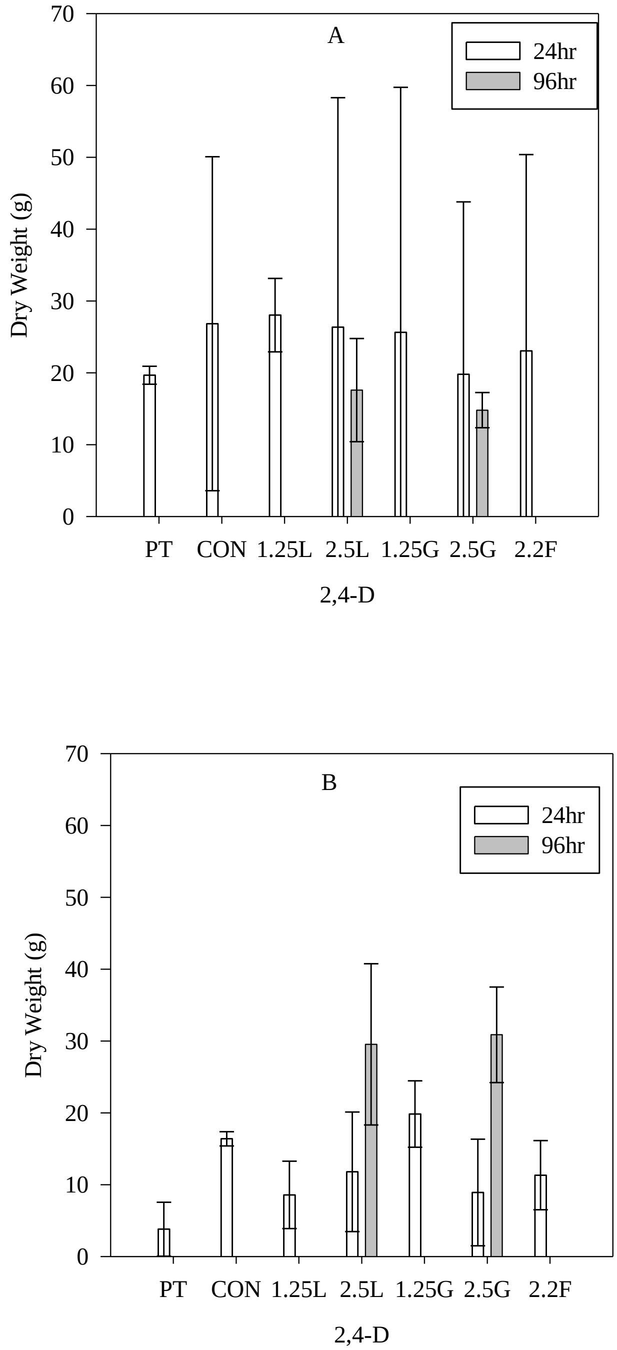


Figure 2-2. Combined dry weight of live roots and foliage of *N. cristata* at 4 WAT in response to 2,4-D for 24 and 96 h exposures in trial 1 (A) and in trial 2 (B). PT = pre-treatment reference, CON = untreated control, L = liquid subsurface, G = granular subsurface and F = foliar rate in kg ae ha^{-1}. Numbers in front of L and G are concentrations in mg ae L^{-1}. Error bars represent ± 95% confidence interval.

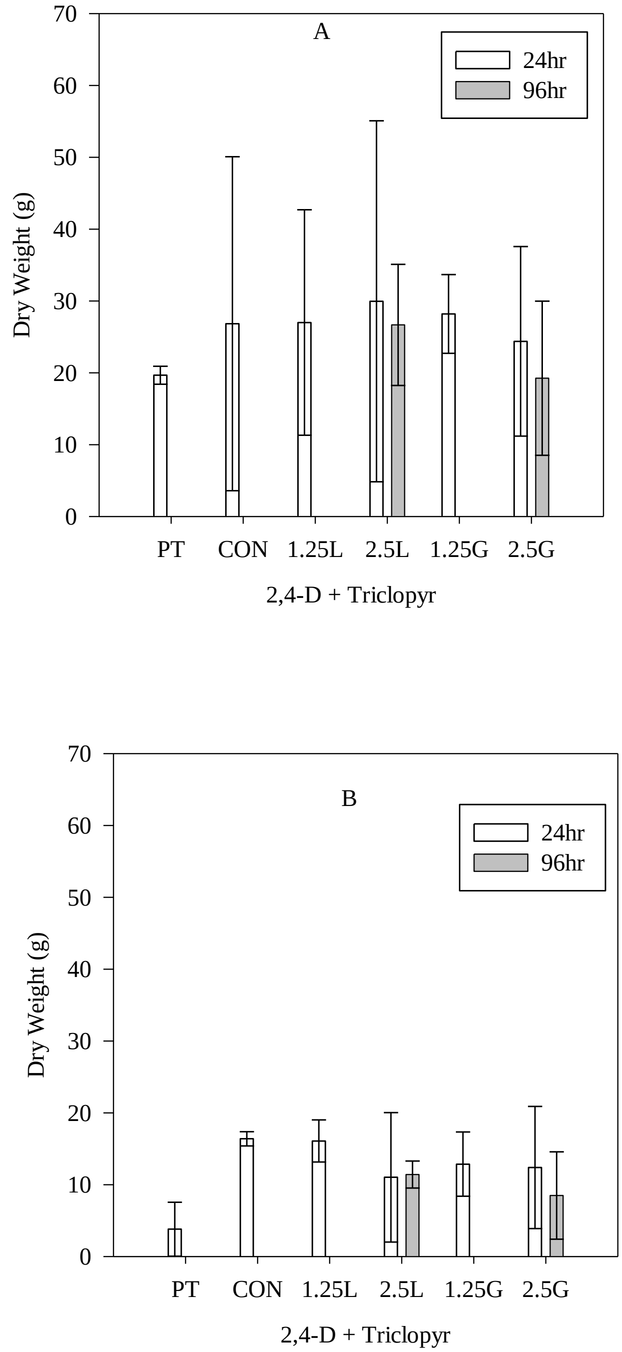


Figure 2-3. Combined dry weight of live roots and foliage of *N. cristata* 4 WAT in response to a combination of 2,4-D (amine) + triclopyr (amine) at 24 and 96 h exposures in trial 1 (A) and in trial 2 (B). PT = pre-treatment reference, CON = untreated control, L = liquid subsurface, G = granular subsurface. Numbers in front of L and G are concentrations in mg ae L^{-1}. 2.5 concentration is 1.94 mg ae L^{-1} 2,4-D + 0.55 mg ae L^{-1} triclopyr. 1.25 concentration is 0.98 mg ae L^{-1} 2,4-D + 0.27 mg ae L^{-1} triclopyr. Error bars represent ± 95% confidence interval.

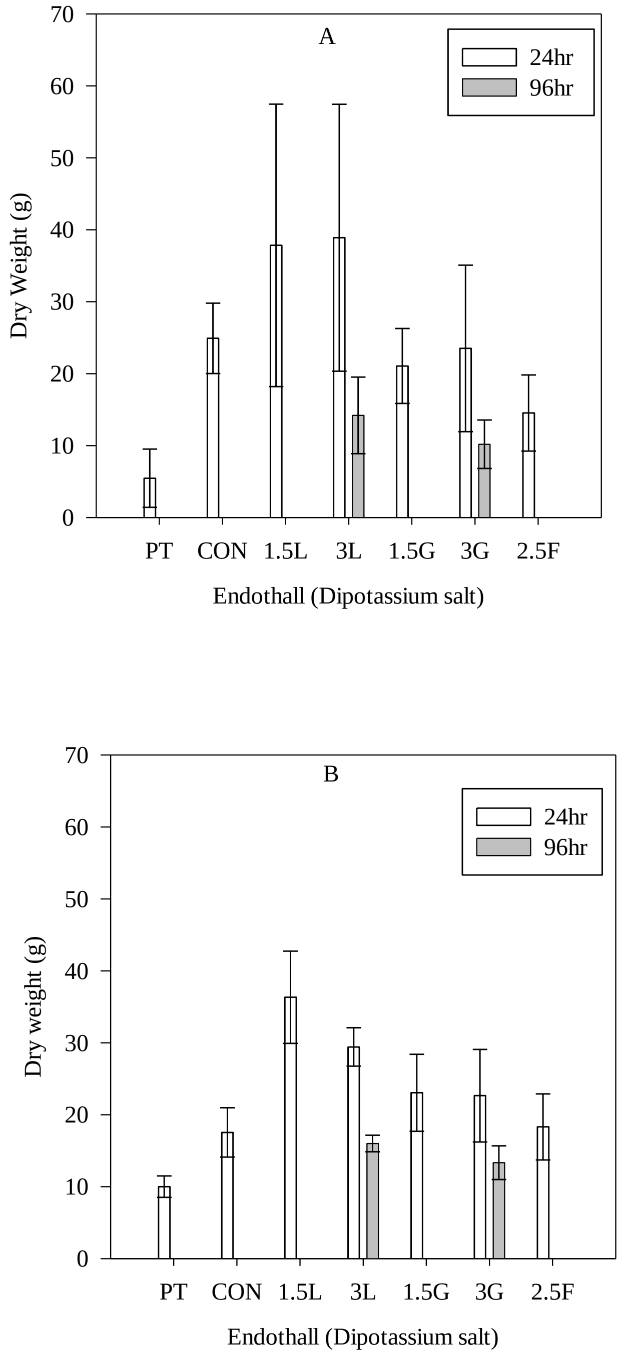


Figure 2-4. Combined dry weight of live roots and foliage of *N. cristata* 4 WAT in response to endothall (dipotassium salt) at 24 and 96 h exposures in trial 1 (A) and in trial 2 (B). PT = pre-treatment reference, CON = untreated control, L = liquid subsurface, G = granular subsurface and F = foliar rate in kg ae ha^{-1}. Numbers in front of L and G are concentrations in mg ae L^{-1}. Error bars represent ± 95% confidence interval.

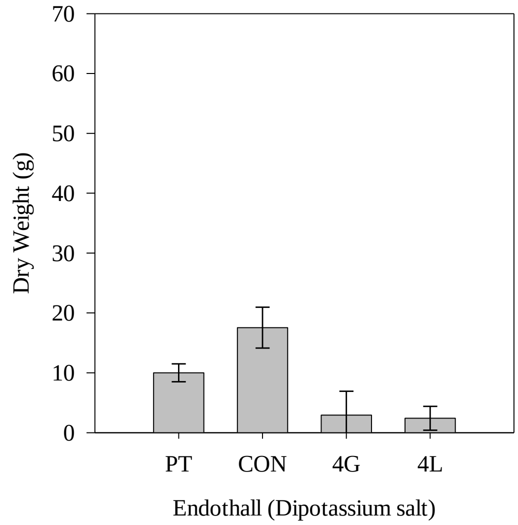


Figure 2-5. Combined dry weight of live roots and foliage of *N. cristata* 4 WAT in response to dipotassium salt endothall for 4 weeks. PT= pre-treatment reference, CON = untreated control, G = granular subsurface, L = liquid subsurface. Concentrations are 4 mg L^{-1} ae for both liquid and granular. Different letters indicate significant differences. Error bars represent ± 95% confidence interval.

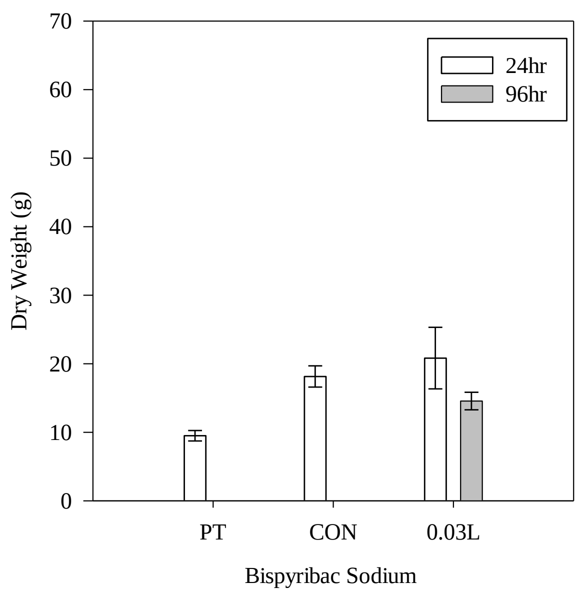


Figure 2-6. Combined dry weight of live roots and foliage of *N. cristata* 4 WAT in response to bispyribac sodium (0.03 mg ai L^{-1}_ at 24 and 96 h exposures, both trials combined. PT = pre-treatment reference, CON = untreated control, L = liquid subsurface. Different letters indicate differences between herbicide treatments. Error bars represent ± 95% confidence interval.

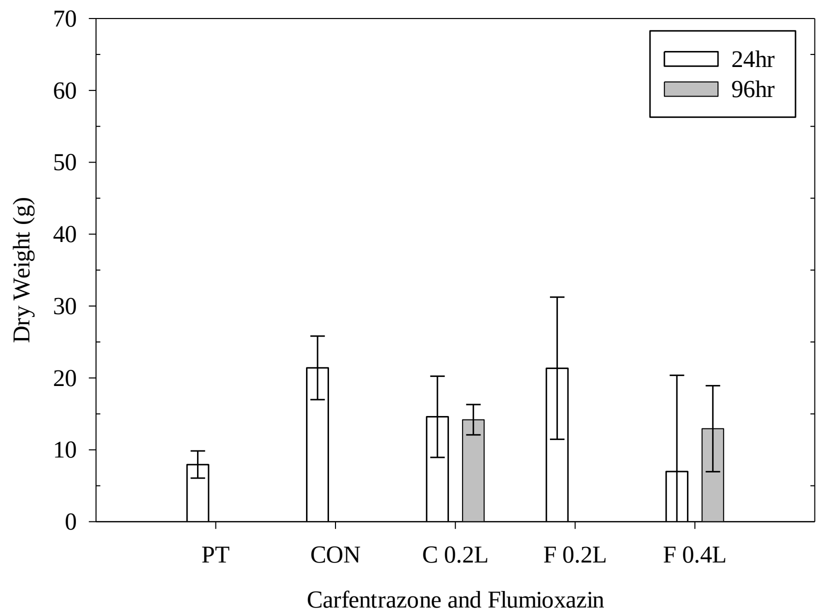


Figure 2-7. Combined dry weight of live roots and foliage of *N. cristata* 4 WAT in response to carfentrazone ethyl (C) and flumioxazin (F) at 24 and 96 h exposures, both trials combined. PT = pre-treatment reference, CON = untreated control, L = liquid subsurface. Concentrations are in mg ai L^{-1}. Different letters indicate differences between herbicide treatments. Error bars represent ± 95% confidence interval.

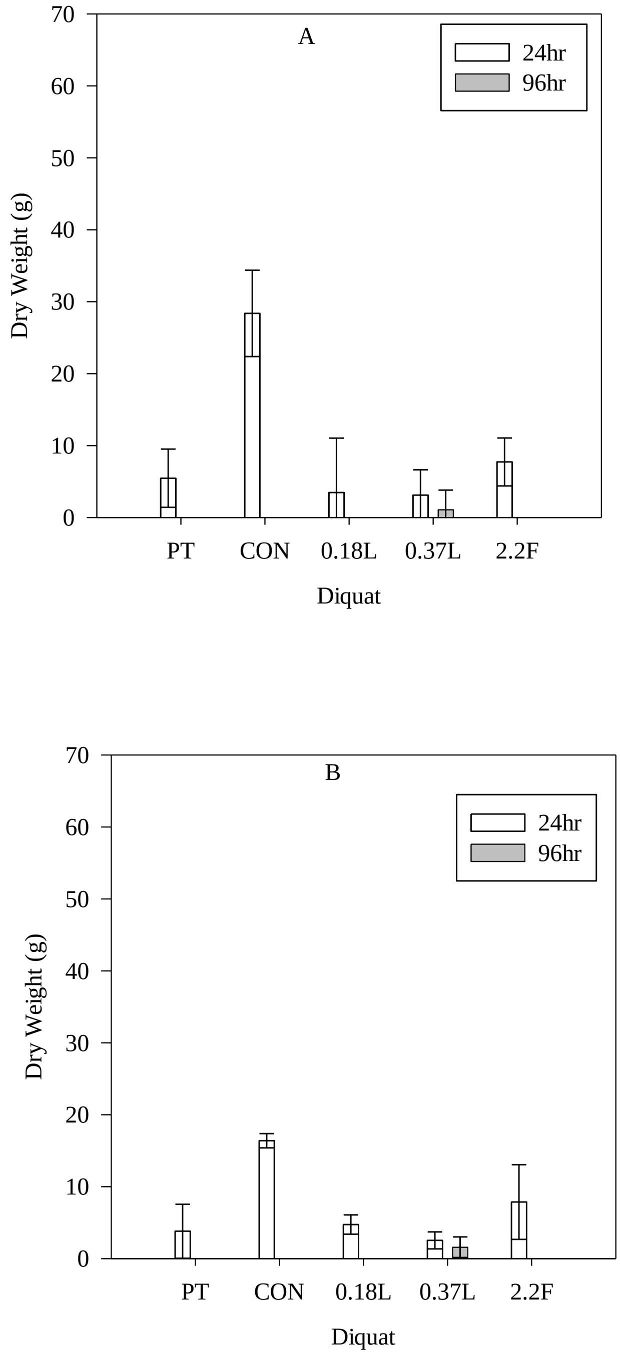


Figure 2-8. Combined dry weight of live roots and foliage of *N. cristata* 4 WAT in response to diquat at 24 and 96 h exposures in trial 1 (A) and in trial 2 (B). PT = pre-treatment reference, CON = untreated control, L = liquid subsurface and F = foliar rate in kg ae ha^{-1}. Liquid subsurface concentrations are in mg ai L^{-1}. Error bars represent ± 95% confidence interval.

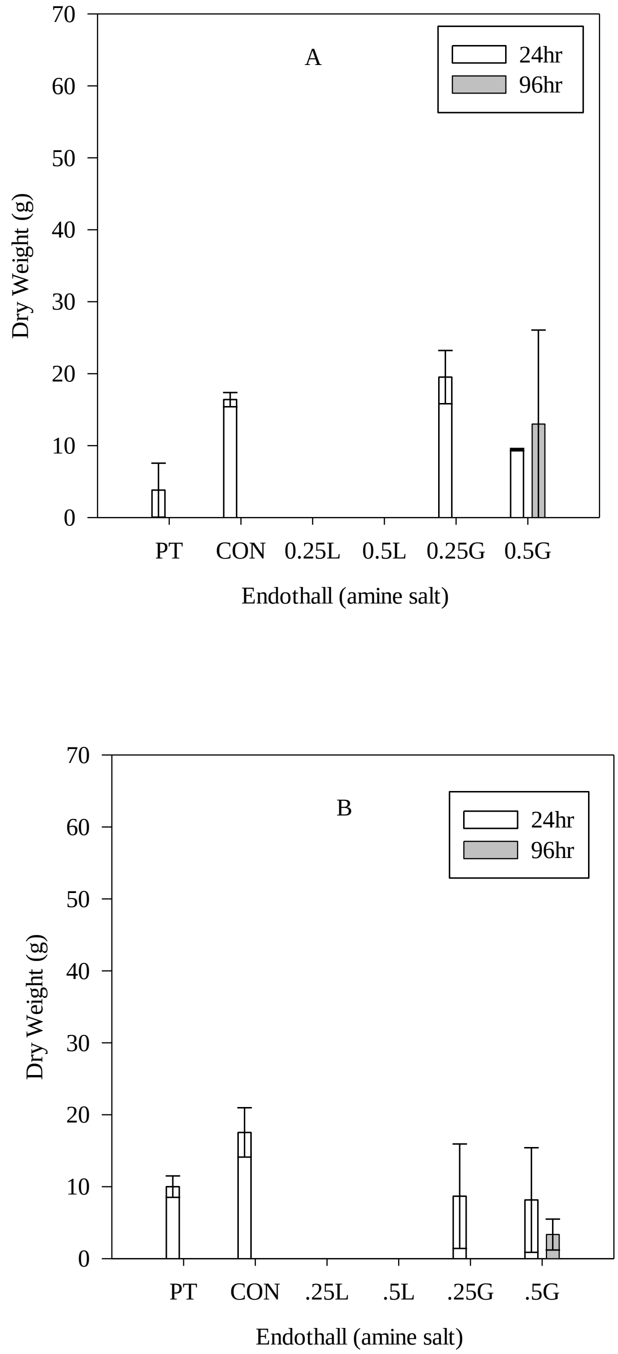


Figure 2-9. Combined dry weight of live roots and foliage of *N. cristata* 4 WAT in response to endothall (amine salt) for 24 and 96 h exposures in trial 1 (A) and in trial 2 (B). PT = pre-treatment reference, CON = untreated control, L = liquid subsurface, G = granular subsurface. L and G subsurface concentrations are in mg ae L^{-1}. Error bars represent ± 95% confidence interval.

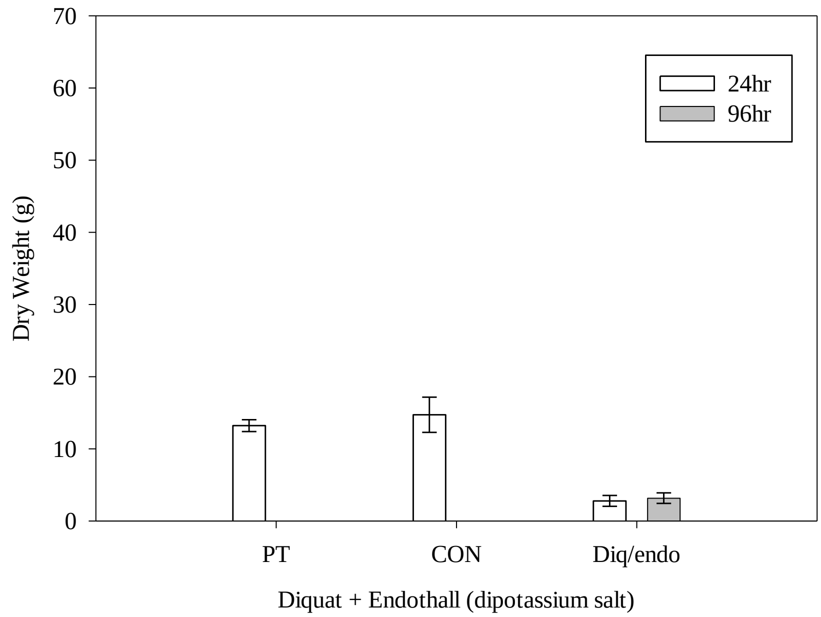


Figure 2-10. Combined dry weight of live roots and foliage of *N. cristata* 4 WAT in response to a combination of diquat (0.2 mg ai L^{-1}) + dipotassium salt endothall (0.8 mg ae L^{-1}) at 24 and 96 hours. PT = pre-treatment reference, CON = untreated control, Diq/endo = diquat + endothall. Error bars represent ± 95% confidence interval.

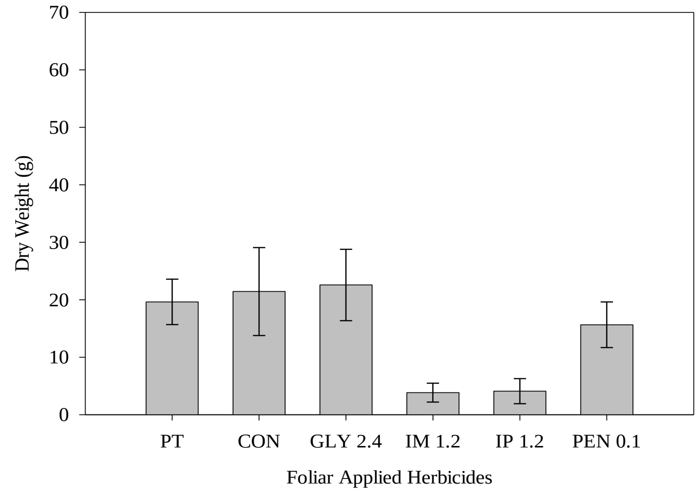


Figure 2-11. Combined dry weight of live roots and foliage of *N. cristata* 4 WAT in response to foliar applied amino acid inhibiting herbicides. GLY = glyphosate, IM = imazamox, IP = imazapyr, PEN = penoxsulam. Numbers represent treatment rate in kg ai ha^{-1}. Error bars represent ± 95% confidence intervals.

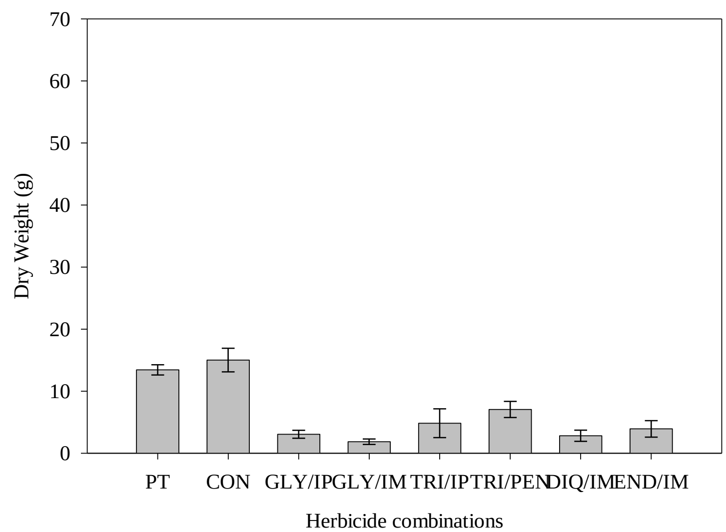


Figure 2-12. Combined dry weight of live roots and foliage of *N. cristata* 4 WAT in response to foliar applications of selected herbicide combinations. GLY = glyphosate, IP = imazapyr, IM = imazamox, TRI = triclopyr, PEN = penoxsulam, DIQ = diquat, END = endothall (dipotassium salt). Error bars represent ± 95% confidence intervals. Herbicide rates are listed in Table 2-2.

CHAPTER 3
THE INFLUENCE OF SEDIMENT TYPE AND FERTILITY ON THE GROWTH OF CRESTED FLOATING HEART

Introduction

Crested floating heart (*Nymphoides cristata* (Roxb.) Kuntze), hereafter referred to as *N. cristata* is an invasive aquatic plant native to Southeast Asia that has recently become established in the southeastern United States including Florida (Burks, 2002). *N. cristata* grows rooted in the submersed sediments in water up to 3 m deep in lakes, ponds, reservoirs and canals; however it can persist in the absence of standing water given sufficient soil moisture (Willey and Langeland, 2011). Plants of the nymphaeid growth form (rooted in sediments with floating or emergent leaves) such as *N. cristata* rely on the submersed sediments to provide many of the nutrients required for growth (Twilley et al. 1977). Nutrients are only absorbed from the water column when leaves are in the submerged stage; even then nutrient absorption is highest in the roots (Twilley et al. 1977).

It is unknown what characteristics of the sediments allow *N. cristata* to thrive or if there are certain conditions that may inhibit growth and spread in some sediments, but some aquatic plant nurseries suggest planting in enriched sediments that are mildly acidic to mildly basic (Anonymous, 2012). It is known that many plants grow better in certain substrates than others and nutrient availability can play a major role in plant invasions (Van et al. 1999). Research on the invasive plant yellow floating heart (*Nymphoides peltata*) suggests that the sediment pH was not as important to its growth compared to water pH, due to the observation that *N. peltata* grew equally well in basic and acidic sediments when the pH of the water was high (Smits et al. 1992).

The objective of this study was to determine the influences of sediment type, nutrient availability and the interaction of these two factors on the growth of *N. cristata* when established from ramets.

Materials and Methods

Study 1

Mature *N. cristata* plants were collected from the storm water treatment areas (STAs) in South Florida in January 2011. Sediment/growth studies were conducted at the University of Florida Center for Aquatic and Invasive Plants (CAIP). Ramets and root crowns were planted in 1 L pots filled with a commercial potting soil mixture with 7% organic matter and a mineral content of 92% sand, 4% silt and 4% clay. Pots were placed into 95 L tanks inside a closed greenhouse for 2 months to initiate growth in January through February 2011. In March 2011, plants were then moved into outdoor 1,000 L mesocosm tanks at the time of prolific production of ramets. Ramets were collected and used for these studies. The first study was conducted May 15 through July 12, 2011 and repeated August 23 through October 4, 2011.

In the first study the effects of 3 sediment types (sand, muck and potting soil) on the growth of *N. cristata* ramets were evaluated. A sand and muck sediment were used because they are representative of the two ends of the substrate spectrum common lake sediments in Florida lakes. The potting soil was used because it had been used to grow *N. cristata* stock for other studies and it has been widely used in other mesocosm trials. Sediments were collected from lakes with the exception of the commercial potting soil. Sand sediment was collected from West Lake Tohopekaliga (Toho) near Kissimmee, FL and muck (organic) sediment was collected from Orange Lake (Orange) located Southwest of Gainesville, FL (Table 3-1). Each sediment type was amended with 2 rates of fertilizer (Osmocote® 15-9-12); 1, or 4 g kg^{-1} dry sediment (Mony et al. 2007) and a non-amended control for each sediment type was also used. One L pots were filled with sediment and then fertilizer was mixed into the soil and a single ramet was planted 0.6 to 1.2 cm into the sediment. Nine pots per sediment type were used as unplanted sediment controls (3 per fertilizer rate) to compare nutrient content of planted and unplanted sediments.

Pots were randomly placed in 12 uncovered 1,000 L mesocosms and whole plants including roots and foliage were harvested and rinsed with untreated well water to remove sediments and dead plant tissue at 6 weeks after planting.

The number of floating leaves was counted at harvest and plants were bagged and placed in a drying oven for one week at 76 C to determine dry weights of each plant. Sediment was saved for additional nutrient analysis and after harvesting samples were dried and sent to the Analytical Research Lab (ARL) at the University of Florida in Gainesville, FL to be evaluated for organic matter content (loss on ignition), total Kjedahl nitrogen, phosphorus (Mehlich 1 test) and particle size composition. All treatments were arranged as a completely randomized design with 5 replicates. SigmaPlot 11.0 statistical analysis software was used to analyze data. Data were analyzed using a 2-way factorial ANOVA ($p \leq 0.05$) to test for effects of sediment, fertilizer and the interaction of these factors on the number of floating leaves produced and dry weight. To test for differences among fertilizer treatments within a soil type and for differences among the soil types within a fertilizer treatment ANOVA ($p \leq 0.05$) was used and a post-hoc Fishers protected LSD test was used to separate the treatment means. For the 6 week harvest, dry weight data were log transformed to meet the equal variance assumption for ANOVA. Non-transformed data are presented in the results. ANOVA tables from this study can be found in the appendix.

Study 2

In addition to the 3 sediments described above a clay based sediment collected near Lewisville, TX was included in the repeat of the study (Table 3-1). The clay sediment has been used in numerous mesocosm trials with a wide range of aquatic species. Methods of planting, harvest and data analysis remained the same as those described in study 1.

Study 3

An third study was performed to evaluate the effects of pH on growth using builders sand with a base pH ranging from 6.6 to 6.8. Hydrated lime with a calcium carbonate equivalent (CCE) of 110% was added at 6 rates in addition to a control (Table 3-3). Lime was thoroughly mixed with the sand and fertilized with Osmocote® 15-9-12 at 1g kg^{-1} dry sediment. Ramets were planted and allowed to grow for 4 weeks then whole plants were harvested and dried in an oven for 1 week at 76 C then weighed. Sediment was saved for pH analysis following the procedure described by Batjes (1995). Data were analyzed using linear regression ($Y= a +bx$); where Y is the dependent (response) variable, a= Y-intercept (value of Y when x=0), b= slope of the line, x= independent (explanatory) variable.

Results and Discussion

Study 1

The 6 week harvest data showed a significant interaction between soil type and increased fertility with respect to the number of floating leaves and increase in dry weight ($p<0.001$; Table A-1, A-2). Leaf production and dry weight increased with increasing fertilizer rates across all sediment types (Figure 3-1 A, B) at the 6 week harvest. Growth, as measured by changes in dry weight remained very limited in the non-fertilized sand and muck sediments through 6 WAT. Plants grown in sand at the highest fertilizer rate produced 2 times more dry weight than muck and potting soil (Figure 3-1 B). Furthermore, within the sand sediment biomass increased 16 fold from fertilizer rates of 0 to 1 g kg^{-1} and an additional 2.7 fold from the 1 to 4 g kg^{-1} fertilizer rates demonstrating the importance of nutrients in the sand sediment (Figure 3-1 B).

Study 2

The 6 week harvest data showed a significant interaction between soil type and increased fertility with respect to the number of floating leaves ($p<0.001$; Table B-1) and increase in dry weight ($p<0.001$; Table B-2). Plants at this harvest had prolific growth and exhibited a clear and strong response to increasing fertilizer rates in the sand and muck sediments (Figure 3-2 A, B). Plants grown in sand sediment increased dry weight by 20 fold compared to the non-amended treatment and were double the dry weight of the 1 g kg^{-1} treatment. Increased dry weight through increasing fertilizer treatments was also observed in the muck sediment. In contrast, plants grown in potting soil and clay sediments exhibited poor growth and a weak but varied response to increasing fertilizer (Figure 3-2 A, B).

The first 2 studies provides important initial information regarding the potential role of substrate type and fertility in supporting growth of *N. cristata*. The results suggest that nutrient availability plays a key role in the ability of this plant to grow in two different sediment types (sand and muck) but were not a factor in other sediment types (potting soil and clay). This finding seems intuitive because a plant is expected to grow better with additional nutrients; however, the comparatively poor growth observed in the clay sediment and potting soil suggests factors other than nutrients play a key role in *N. cristata* growth. This study has shown that sand and muck sediments were capable of supporting dense growth of *N. cristata* although both of these sediments differ greatly in organic matter content and mineral composition (Table 3-1). The ability of *N. cristata* to grow well in these two sediment types, despite their physical differences suggests that a wide range of sediment textures can support the growth of this plant. It is important to note that growth in clay potting soil sediment, while reduced produced healthy, but less robust plants in these trials.

There were no differences in macronutrient content between blank (no plants grown) and 6 week harvest sediment samples within sand, potting soil and clay sediments indicating ample macronutrients remained available in all sediments at 6 weeks to continue to support plant growth. An exception to this is phosphorus (P) in the muck sediment in which P content was 16 to 342 times lower in the blank pots than in those with plants (Table 3-2). A possible explanation for this is that the presence of *N. cristata* actually enhances P retention in the sediment. One study conducted on Lake Michigan found that total P was higher in areas where vascular aquatic plants were present and when tested *in situ* found that in the absence of vascular plants P was released in greater amounts to the water (Jaynes and Carpenter, 1986). In aquatic sediments P is generally not limiting since the sediment is the primary source for this macronutrient (Barko et al. 1991). While P detection was very low in the clay sediment, it should be noted that the clay sediments received the same fertilizer regime as the other sediments and that P detection can be very difficult in clay sediments (Gatiboni et al. 2010). Given that *N.cristata* growth was also greatly reduced in potting soil (with much higher concentrations of P), a significant role for P limitation on *N. cristata* growth is not indicated (Table 3-2). It is interesting to note that nitrogen (N) levels were much higher in clay sediments than sand sediments (Table 3-2) but this did not result in increased plant growth in the clay sediment.

Study 3

The pH of sand and muck sediments ranged from 5.8 to 6.4 while the pH of potting soil and clay sediments ranged from 7.8 to 8.3. In the follow up study using builders sand plant dry weight regressed against sediment pH showed a negative growth response (slope coefficient -0.334; $p<0.001$) of *N. cristata* to increasing pH (Figure 3-3). Some plants are very sensitive to pH and have specific requirements for optimum growth. Another plant in the Nymphoides genus, *N. peltata* shows little apparent pH preference and instead growth is more dependent on water pH (Smits et al. 1992). In Southeast Asia, the native range of *N. cristata*, a predominant wetland soil type is the gleysol, an acidic, fertile soil (FAO, 2012).

These data suggest *N. cristata* may grow best in acidic, fertile sediments which support rapid growth and establishment; however, it is shown to be capable of growth in a wide variety of sediment types and in various levels of nutrient availability. This information could be useful to predict which lakes may be more or less susceptible to rapid colonization and spread of *N. cristata* based on the sediment profiles. Additional studies on the sediment pH, sediment type and nutrient availability need to be conducted confirm the preliminary results obtained in these studies.

Table 3-1. Characterization of sediments used in study to determine effects of sediment type, nutrient addition and their interaction on growth of *N. cristata*.

Sediment type	Collection location	Mineral content			Organic matter
		Sand	Silt	Clay	
		------------------------%------------------------			
Sand	Lake Toho, FL	97	1.5	1.5	0.9
Muck	Orange Lake, FL	21.8	0.5	0.5	77
Clay	Lewisville, TX	22.5	33.7	33.7	10
Potting soil[a]	CAIP,[b] FL	85.5	3.7	3.7	7

[a]Margo Garden Products. Folkston, GA 31537
[b]University of Florida Center for Aquatic and Invasive Plants

Table 3-2. Background nutrient and sediment chemistry analysis with standard error from sediment samples collected during the sediment type by nutrient addition study to determine their impact on growth of N. cristata.

Soil	*Fertilizer*	*Sample(n)*	*TKN[a]±SE*	*P[b]±SE*	*pH±SE*
Sand	0g kg^{-1}	6	386 ± 63	0.38 ± 0.13	6.4 ± 0.02
	0 g[NP]	6	467 ± 29	1.05 ± 0.68	5.8 ± 0.06
	1 g kg^{-1}	6	363 ± 53	11.40 ± 0.32	6.1 ± 0.08
	1 g[NP]	6	443 ± 45	24.97 ± 7.37	5.6 ± 0.05
	4g kg^{-1}	6	549 ± 17	22.70 ± 2.54	5.8 ± 0.08
	4g[NP]	6	565 ± 31	45.36 ± 4.7	5.6 ± 0.10
Muck	0g kg^{-1}	6	19535 ± 1401	5482.50 ± 54.93	6.0 ± 0.07
	0 g[NP]	6	20008 ± 1217	16.71 ± 12.47	5.8 ± 0.18
	1g kg^{-1}	6	15124 ± 2783	5327.90 ± 23.15	5.8 ± 0.12
	1 g[NP]	6	8939 ± 4643	210.71 ± 21.60	5.6 ± 0.04
	4g kg^{-1}	6	18140 ± 1610	5666.10 ± 16.59	5.7 ± 0.09
	4 g[NP]	6	19070 ± 2429	354.73 ± 83.85	5.5 ± 0.1
Potting	0g kg^{-1}	6	1002 ± 109	20.79 ± 2.26	7.8 ± 0.05
	0 g[NP]	6	1110 ± 41	20.56 ± 1.29	7.8 ± 0.01
	1g kg^{-1}	6	909 ± 86	24.47 ± 1.9	8.0 ± 0.06
	1 g[NP]	6	1182 ± 64	22.78 ± 0.71	8.0 ± 0.08
	4g kg^{-1}	6	1195 ± 34	28.80 ± 1.50	7.8 ± 0.1
	4 g[NP]	6	1174 ± 33	31.80 ± 2.46	7.8 ± 0.1
Clay	0g kg^{-1}	3	1403 ± 63	0 ± 0	8.3 ± 0.01
	0 g[NP]	3	1411 ± 105	0 ± 0	8.4 ± 0.02
	1g kg^{-1}	3	1435 ± 85	0 ± 0	8.3 ± 0.10
	1 g[NP]	3	1233 ± 67	0 ± 0	8.3 ± 0.03
	4g kg^{-1}	3	1716 ± 52	0 ± 0	8.2 ± 0.05
	4 g[NP]	3	1666 ± 30	0 ± 0	8.2 ± 0.06

[a] TKN is Total Kjedahl Nitrogen in mg kg^{-1}
[b] P is phosphorus from Melich 1 test in mg kg^{-1}
[NP] No Plant grown in sediment

Table 3-3. Strudy 3. Impact of hydrated lime (CCE 110%) application rates on sediment pH and growth of *N. cristata* grown in sand sediment amended with 1 g kg^{-1} fertilizer (15-9-12). N=5

Lime rate (kg m^{-3})	*Mean pH±SE*
0	6.7 ± 0.04
0.02	7.8 ± 0.11
0.04	8.7 ± 0.15
0.12	9.6 ± 0.13
0.24	9.7 ± 0.11
0.32	9.6 ± 0.30
0.36	10.3 ± 0.04

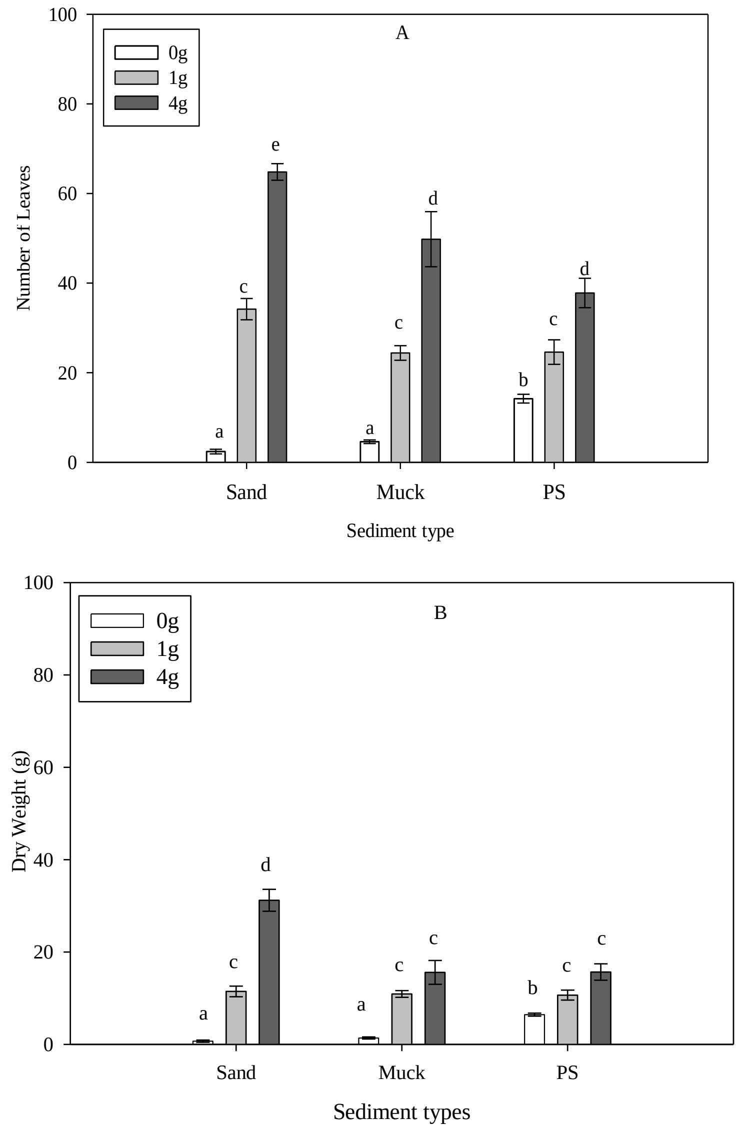


Figure 3-1. Study 1, response of *N. cristata* grown in sand, muck and potting soil sediments at two fertilizer rates. A) Six week number of floating leaves produced. B) Six week total plant dry weight. PS = potting soil. Different letters indicate significant differences among treatments, Fishers protected LSD ($p \leq 0.05$). Error bars are ± SE. N=5.

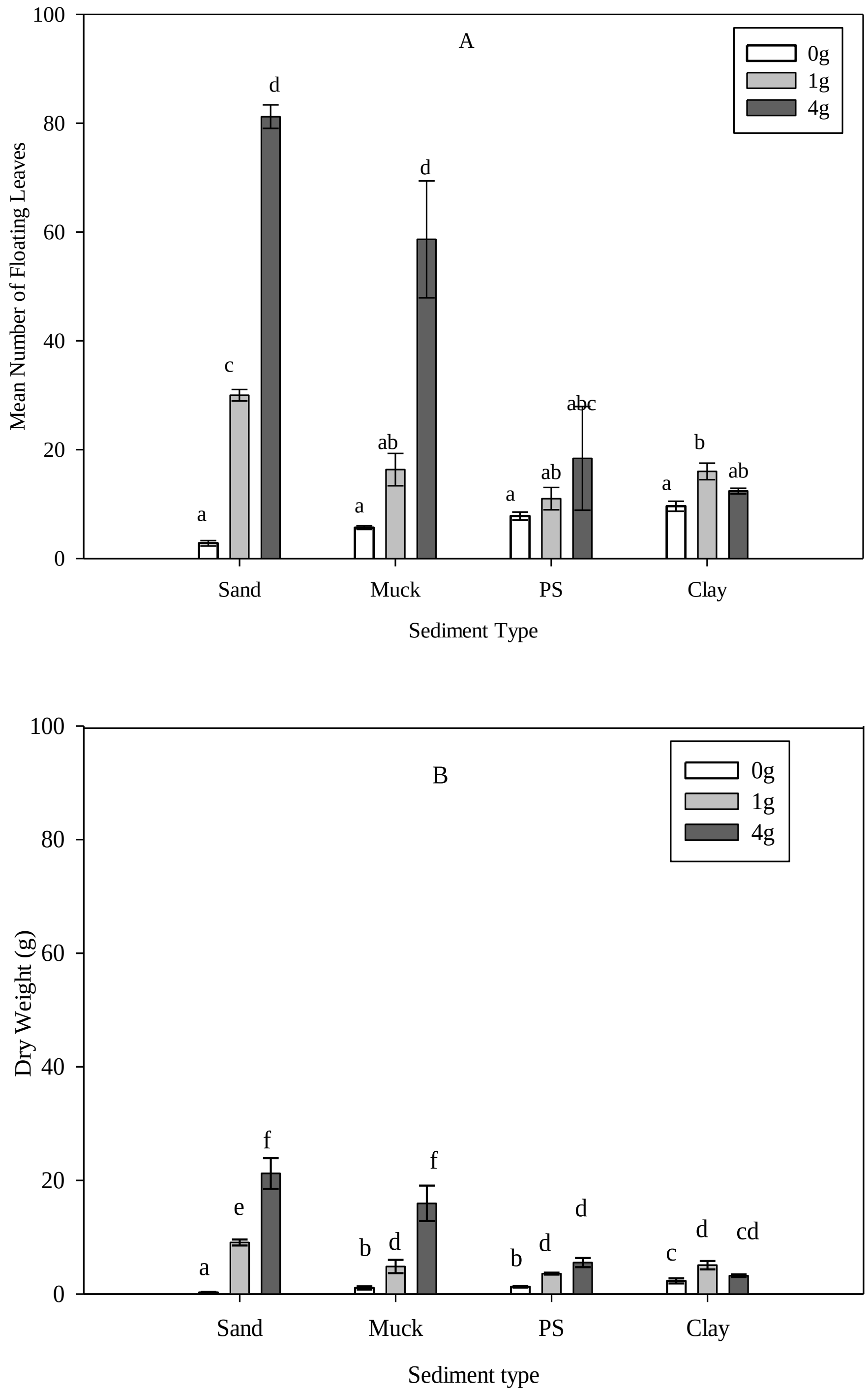


Figure 3-2. Study 2, response of *N. cristata* grown in sand, muck, potting soil and clay sediments and two fertilizer rates. A) Six week number of floating leaves produced. B) Six week total plant dry weight. PS = potting soil. Different letters indicate significant differences among treatments. Fishers protected LSD ($p \leq 0.05$). Error bars are ± SE. N=5.

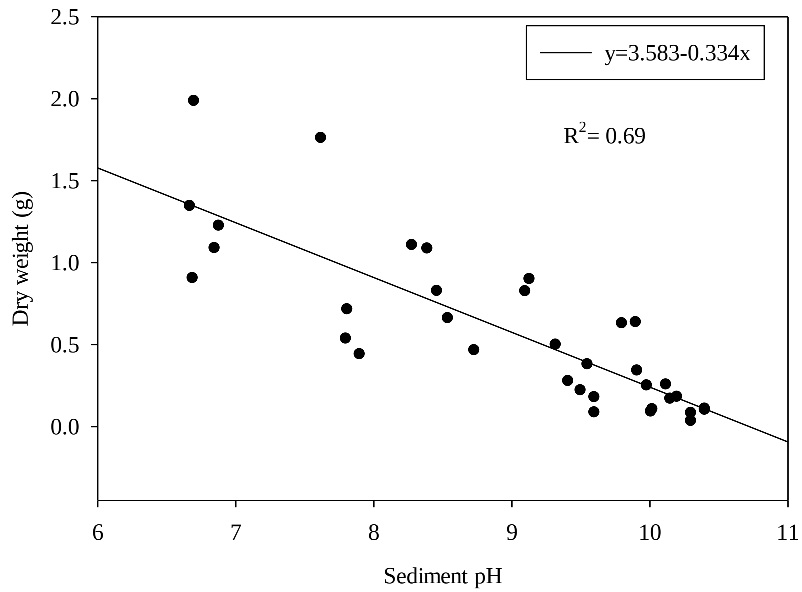


Figure 3-3. Study 3 linear regression of *N. cristata* dry weight versus sediment pH. Sand sediment amended with hydrated lime (CCE 110%).

CHAPTER 4
THE INFLUENCE OF LIGHT AVAILABILITY ON GROWTH OF CRESTED FLOATING HEART ESTABLISHED FROM RAMETS

Introduction

Many aquatic plants are adapted to life in low light environments. Some aquatic invasive plants such as hydrilla (*Hydrilla verticillata*) have a particularly low light compensation point and can thrive and dominate in deeper or more turbid systems where light is limited (Bowes et al. 1977) which provides the plant with the ability to grow to the water surface. Crested floating heart (*Nymphoides cristata*), hereafter referred to as *N. cristata* is an invasive aquatic plant that has been expanding throughout Florida and the Southeast US. While *N. cristata* exhibits nymphaeid morphology (plants rooted in submersed sediments with floating leaves at the end of long stems) clonal reproductive structures called ramets represent a fully submersed form of the plant. These ramets are also the initial propagules that must establish in order for the plant to spread. *N. cristata* has been found growing in water up to 3 m deep suggesting that the small ramets are likely tolerant to low light conditions. Impacts of light availability on the growth and establishment of *N. cristata* ramets have not been reported and this lack of information on such a basic environmental factor and the impact on growth suggests research is needed to determine how *N. cristata* ramets respond to varying degrees of reduced light availability. The objective of this study was to assess the growth response of *N. cristata* ramets to different light levels.

Materials and Methods

Number of Floating Leaves

In January 2011, mature *N. cristata* plants were collected from the storm water treatment areas (STA) in Southern Florida and planted in 1 L pots filled with Margo ® commercial potting soil with a mineral composition of 85.5% sand, 3.7% silt and 3.7% clay and organic matter content of 7%. Pots were placed in 95 L tanks filled with well water inside of a greenhouse for 2 months to initiate growth and then moved into outdoor, 1,000 L tanks until prolific production of ramets was observed. A sample of 5 ramets was collected to determine initial mean dry weight prior to the beginning of each study. The first 4 week trial was conducted from June 9, 2011 through July 7, 2011 and repeated July 5, 2011 through August 4, 2011.

To evaluate the effect of light on ramet growth, five target light levels were selected. These were 1, 5, 25, 50 and 100% ambient light availability at the water surface. A total of fifteen, 1,000 L mesocosm tanks were filled with well water to a depth of 50 cm. Frames were constructed to fit over the top of each mesocosm and shade cloth was pulled over the frames and layered until the desired amount of light was reached. Shade cloth was secured to the frame using 15.25 cm plastic cable-ties. Light measurements were taken at the water surface with a LI-COR model LI-250A light meter[1]. Table 4-1 lists the desired light levels with the range of light availability that was actually achieved. Ramets were planted 0.6 to 1.2 cm deep in 1 L pots filled with potting soil and amended with Osmocote® 15-9-12. A thin layer of sand was added over the potting soil to prevent any turbidity that may be caused by floating or suspended organic matter from the potting soil.

Light treatments were applied randomly to the mesocosms and each treatment was replicated 5 times. One set of plants was harvested at 2 weeks after planting and the second set was harvested 4 weeks after planting. Data were collected for the number of floating leaves at the time of harvest. Nonlinear regression analysis was performed on data with light as the independent variable and the number of floating leaves as the dependent variable using SigmaPlot 11.0 statistical analysis software. A 3-parameter rectangular hyperbola model was fit

[1] LI-COR Biosciences. Lincoln, NE 68504

to data ($y = y_0 + \frac{ax}{b + x}$). The treatment-by-experimental run interaction was significant ($p<0.001$) therefore data were analyzed separately. Data from both trials are presented.

Dry Weight

After the number of floating leaves had been counted at 2 and 4 WAT, entire plants including all live roots, stems and foliage were cleaned of debris and placed in a drying oven at 76 C for 1 week. Dry weight was recorded, and data were analyzed using a linear regression with light as the independent variable and dry weight as the dependent variable ($y = y_0 + ax$); where y_0 is the value of y (dependent variable) when x = 0, a is the slope of the line and x is the independent variable. Differences between 2 and 4 weeks dry weight were also determined using a t-test ($p<0.05$). An additional t-test was used to test for differences in initial mean dry weight and the mean dry weight at 1% light. The treatment-by-experimental run interaction was not significant ($p=0.279$) therefore data were pooled.

Results and Discussion

Number of Floating Leaves

The leaves of ramets grown in 25 to 100% light reached the water surface in 3 to 4 days after planting. Plants grown at 1 and 5% light had their first leaves reach the surface in 8 and 6 days after planting, respectively. In the first study, the number of leaves did not increase across light levels above 25% available light during the 2 week harvest (Figure 4-1 A). A similar trend was observed in the second study (Figures 4-2 A). The R^2 values (0.65 and 0.51 in study 1 and 2, respectively) suggest that initial light availability was not strongly predictive of the ability of ramets to produce floating leaves.

During the first study leaf production increased as light availability increased from 1 to 25% ($p=0.03$) but no increase in leaf production was noted as light levels were increased beyond 25%

during the 4 week harvest ($p=0.44$) (Figure 4-1 B). Results from the second trial showed leaf production after 4 weeks responded to light availability in a slightly different manner. The regression line showed a gradual but nonlinear increase ($p=0.0009$) across all light levels. In the second study (Figure 4-2 B) the R^2 value was much higher (0.90), suggesting that light played a more important role in the plants ability to produce floating leaves at 4 weeks compared to the first study.

Plants grown at 100% available light produced green foliage with red-violet pigment expression in the leaf margins after 4 weeks. In contrast, plants grown under lower light levels (1 to 50%) did not exhibit a similar response, suggesting the appearance of red-violet coloration in the foliage is a response to exposure to the higher light intensities. Variegation has been shown to be a heritable, nonlethal genetic mutation in some plants (Orakwue and Crowder, 1983). Since variegation is a heritable trait, the red-violet pigmentation observed in *N. cristata* grown in full sunlight is believed to be a response to high light conditions. This accessory pigment expression likely protects the chlorophyll inside the thin leaves and potentially produce biologically active compounds against herbivores (Schaefer and Rolshausen, 2005; Zhang et al., 2010). Plants grown under the lowest light levels produced the fewest number of leaves and also never produced flowers suggesting these plants were not utilizing carbohydrates to produce leaves or reproductive structures in an environment where the plants' productivity was limited. Flower production was observed at 2 weeks in the 50 and 100% light levels. Ramet production was observed 2 weeks after planting at 100% light and after 4 weeks in 50% light.

Dry Weight

The initial dry weight of ramets was 0.06 grams (± 0.009) g. Two weeks after planting, there was a positive linear response (slope coefficient 0.0049; $p<0.0001$) of dry weight to light availability (Figure 4-3 A). From 2 to 4 weeks after planting, dry weight increased as light increased from 5 to 100%. Linear increases in dry weight were also noted at the 4 week harvest (slope coefficient 0.044; $p<0.0001$). In contrast, no change in dry weight was noted at the 1% light level between the 2 harvest dates (Table 4-2). However, it was found that plants were able to grow at 1% light compared to the initial sample dry weight ($p<0.001$). The relationship based on linear regression shows that light availability plays an important role in growth of *N. cristata* (Figure 4-3 A, B). The fact that dry weight and floating leaf production showed different responses to light intensity suggests that leaf production is related to the ability of the ramet to produce leaves independent of light intensity, while subsequent growth is influenced by light intensity to a much larger degree (Figure 4-3 A, B).

In general, increases in leaf production were less pronounced as light intensities increased above 25% light; suggesting leaf production was likely a response of carbohydrate content in the ramets. Light levels at which the cessation of additional leaf production occurred was within the range of 20 to 40% incident light that has been estimated in previous studies to support active growth of many submersed aquatic plants (Abernethy et al. 1996; Middelboe and Markage, 1997; Van et al. 1976). This light response only applies to the submersed stage of growth for *N. cristata* since it is unlikely that floating leaves would be subject to a sustained low light environment upon emergence.

While the results of this study show a clear relationship between higher light availability and dry matter production by *N. cristata*, a key finding was that even at ~1% available light (99% shade) ramets were able to produce stems that rapidly elongated until small leaves emerged on the water surface in approximately 1 week. Rapid stem elongation by ramets under low light conditions suggests a mechanism that allows *N. cristata* to become established in deep or turbid water which may allow the plant to grow where other submersed aquatic plants or nymphaeid species would be unable to survive, further enhancing the invasive potential of *N. cristata*.

Rapid stem elongation by *N. cristata* under low light conditions may be somewhat analogous to rapid shoot elongation observed for the submersed invasive species hydrilla (Van et al. 1976, Bowes et al. 1979, Steward, 1991). Similarities between *N.cristata* and hydrilla include rapid elongation under low light, formation of dense canopies near the water surface, and prolific production of vegetative propagules. All of these factors contribute to the success of these invasive species within the introduced range.

While the ramets are quite small, they serve as a source of carbohydrate for the establishment of new plants. Due to their ability to tolerate and overcome low light environments, it is likely that many water bodies in Florida will provide a light environment that favors establishment and growth of *N. cristata*.

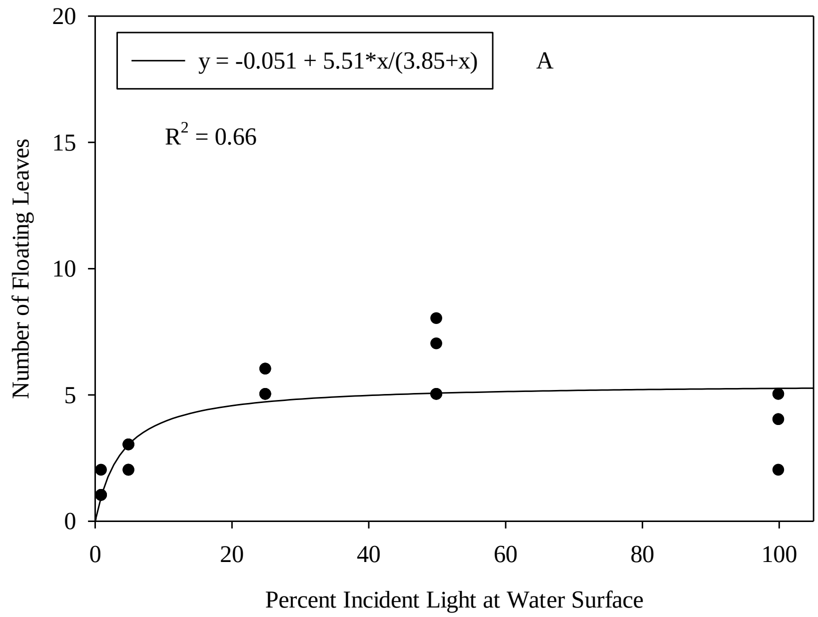
Table 4-1. Target ambient light levels and the actual light levels measured at the water surface. Light readings were taken between noon and 1pm the day of planting.

Target ambient light (%)	*Actual achieved ambient light range (%)*
1	1 - 2
5	5 - 7
25	23 - 30
50	48 - 55
100	100

Table 4-2. *N. cristata* dry weight was measured at 2 and 4 weeks after planting. A t-test was used to compare 2 and 4 week means (n=10). Mean dry weight reported in grams.

Ambient light	*Mean dry weight (±SE)*[a]		*P-value*
%	2 weeks	4 weeks	
	--------------------------g-------------------------		
1	0.15(±0.007)a	0.18(±0.014)ab	0.173
5	0.19(±0.015)b	0.44(±0.023)cd	0.001
25	0.32(±0.015)c	2.09(±0.113)e	0.001
50	0.47(±0.022)cd	2.37(±0.167)e	0.001
100	0.65(±0.022)d	5.23(±0.427)f	0.001

[a]Means followed by the same letter within a row or column are not different (p = 0.05)



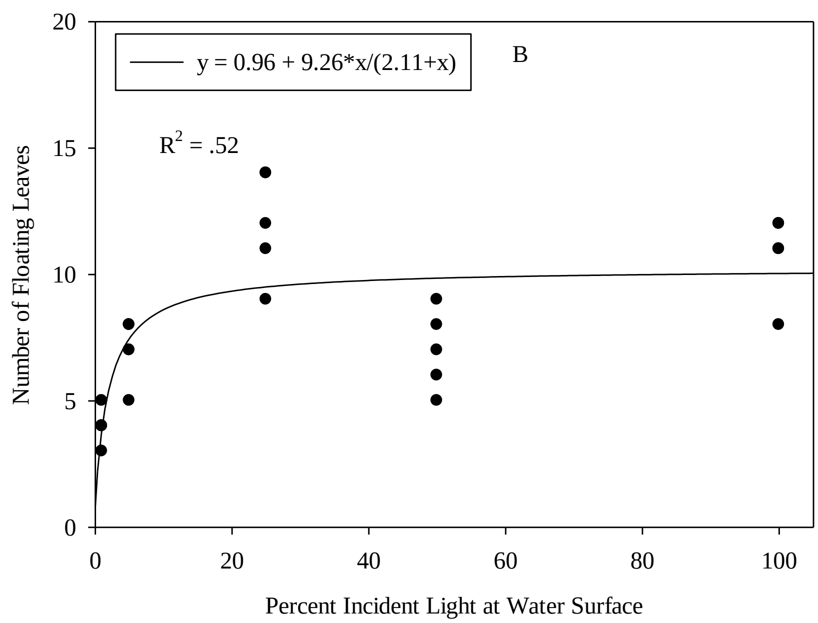


Figure 4-1. Number of floating leaves of *N. cristata* in response to light availability in the first study at. A) 2 week harvest. B) 4 week harvest.

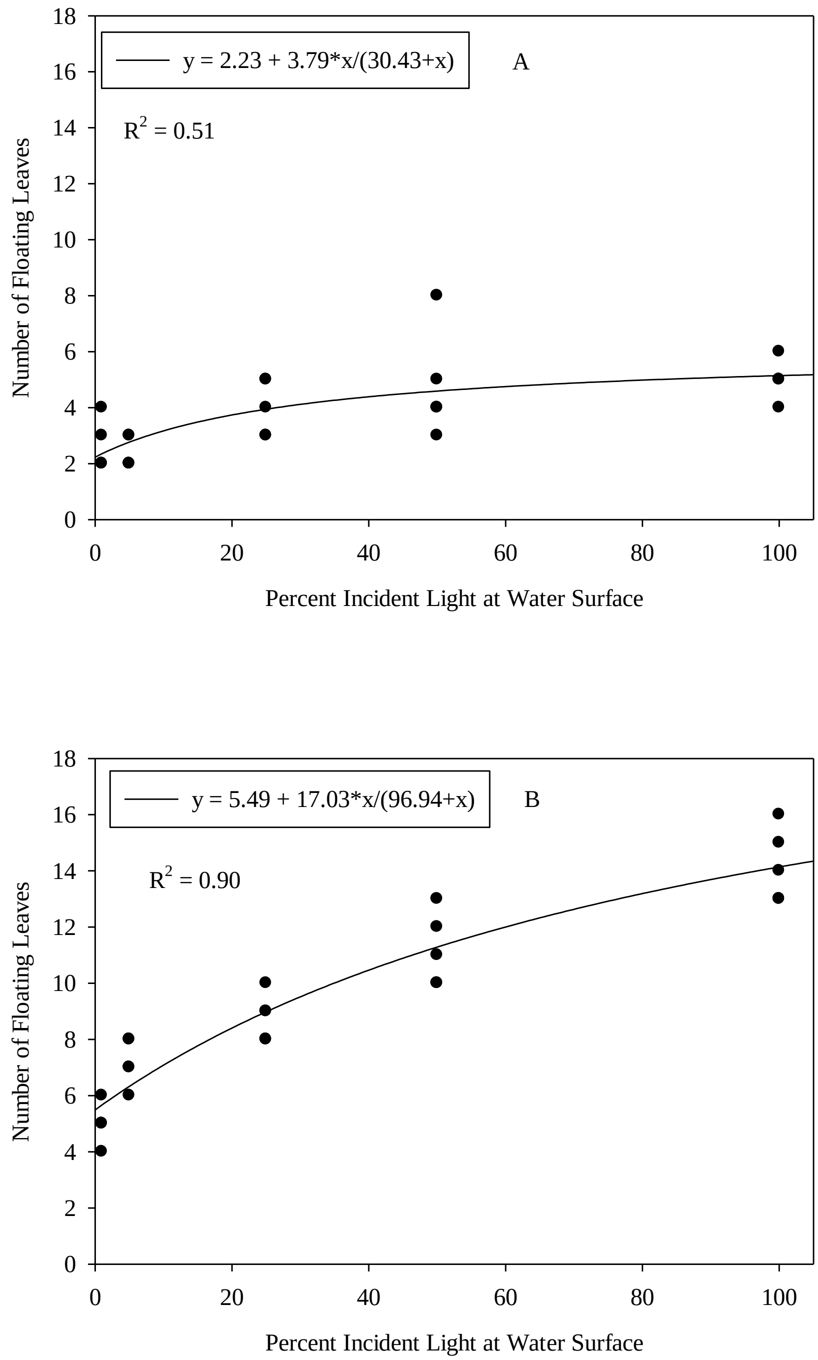
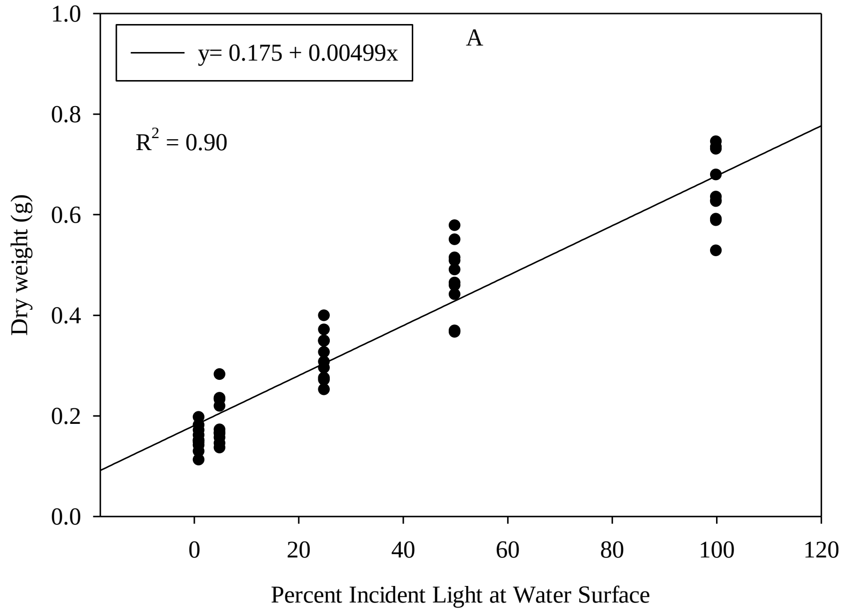


Figure 4-2. Number of floating leaves of *N. cristata* in response to light availability in the second study at. A) 2 week harvest. B) 4 week harvest.



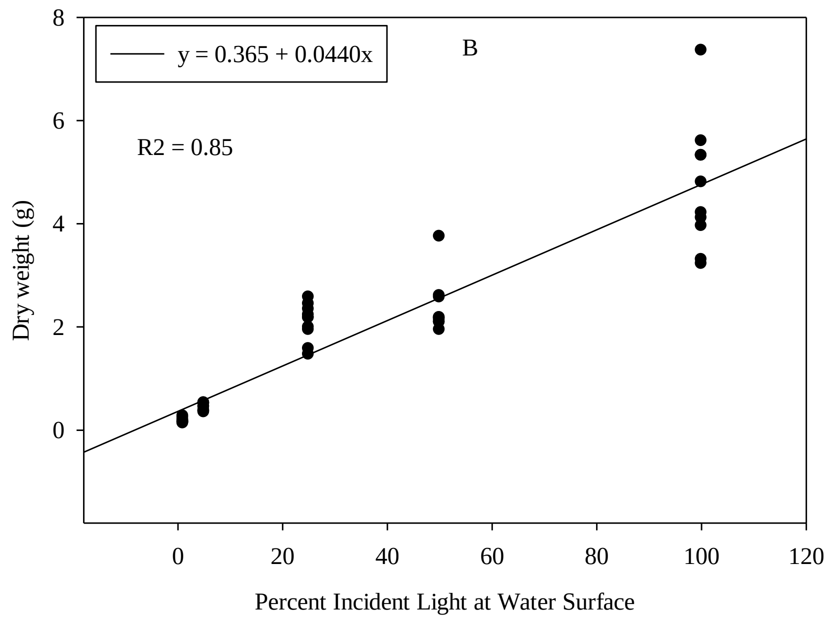


Figure 4-3. Dry weight of *N. cristata* in response to light availability at A) 2 week harvest and B) 4 week harvest. Data were combined from both trials (n=10).

CHAPTER 5
COMPETITION BETWEEN CRESTED FLOATING HEART AND HYDRILLA IN A REPLACEMENT SERIES TRIAL

Introduction

Evaluating the competitive ability of an exotic plant is an important step in assessing the community and ecosystem level impacts that may result if the plant becomes established in an area. In the last 20 years there has been a significant increase in the interest of understanding the competitive strategies of exotic species (Mony et al., 2007; Lonsdale, 1999; Williamson, 1999). However less of this effort has been on evaluating the interactions between exotic invasive species (Mony et al., 2007; Simberloff and Von Holle, 1999; Richardson, 2000). A previous study (Chapter 4), demonstrated that crested floating heart (*Nymphoides cristata* (Roxb.) Kuntze), hereafter referred to as *N. cristata* is tolerant of and responds to low light by producing rapid stem elongation to produce floating leaves at the surface as quickly as possible. This ability to tolerate and respond to low light conditions is an important factor in the success of an invasive aquatic plant. *N. cristata* also does not reproduce sexually, but spreads prolifically through production of ramets. These features suggest that *N. cristata* could be a highly competitive species; however, studies are needed to confirm this.

One way to evaluate the competitive ability of two different species is evaluating a replacement series in which two species are grown at a constant density but the ratio of each species changes. While there are many limitiations to the replacement series model, it is a valid way to compare species yield as a function of dry matter production and the ability of one species to accumulate greater mass than another (Joliffe, 2000).

Similarities between *N.cristata* and hydrilla (*Hydrilla verticillata*) include rapid elongation under low light, formation of dense canopies at the water surface, and production of copious small vegetative propagules that can promote spread and allow survival. The objective of this study was to assess the competitive abilities of *N. cristata* established from ramets and hydrilla (*Hydrilla verticillata*) established from apical sections as a function of dry weight accumulation in a replacement series study.

Materials and Methods

In January 2011, mature *N. cristata* plants were collected from the storm water treatment areas (STA) in South Florida. Ramets and root crowns were planted in 1 L pots filled with Margo® commercial potting soil (Chapter 1) with a mineral composition of 85.5% sand, 3.7% silt and 3.7% clay and an organic material content of 7% at the University of Florida, Center for Aquatic and Invasive Plants (CAIP) in Gainesville, FL. Pots were placed in 95 L tanks in a greenhouse from January 2011 through March 2011 for establishment and were then moved into outdoor 1,000 L culture tanks until prolific production of ramets was observed. Ramets were collected and used to perform the planned studies. Hydrilla tips (4 cm) were collected from plants growing in 1,000 L culture tanks. The first study was conducted from July 30, 2011 through September 10, 2011 and was repeated from August 10, 2012 through September28, 2012.

Studies were conducted outside under a shade canopy with 70% incident light. Twenty-one 95 L plastic tanks were filled with a 7.6 cm deep layer of potting soil amended with Osmocote® 15-9-12 at a rate of 1g kg^{-1} of dry soil. A layer of sand 0.5-1 cm deep was added over the potting soil as a cap to prevent turbidity and floating organic matter. Tanks were filled with well water and a small skim net was used to remove any debris after the tanks were filled. Tanks were allowed to set for 24 hours to allow the sediments to settle out of the water.

The desired plant density was 23 plants m^{-2} or a total of 6 plants per tank. Sixty-three ramets of *N. cristata* of approximately equal size (0.52 ± 0.02 g fresh weight) and 63, apical tip sections of hydrilla were collected for planting. Hydrilla tips were clipped with scissors until the fresh weight was similar to that of the *N. cristata* ramets (0.49±0.04 g fresh weight). Each tank was randomly assigned a different planting ratio of *N. cristata*: *hydrilla* ranging from 0:6 to 6:0 changing the number of each plant in the mixture by 1 each time. Visual observations and photographs were taken on a weekly basis to document each species' progress in the various ratios throughout the 7 week experiment.

Planting ratios were assigned randomly to the 21 tanks and each ratio was replicated 3 times. Plants were harvested 7 weeks after planting. Plant shoots were cut at the surface of the sediment, removed and separated by species. Each species was then placed in a drying oven for 1 week at 76 C. Mean dry weight for each species and mean total combined plant dry weight were calculated for each planting ratio and plotted to determine comparative growth. An ANOVA test ($p \leq 0.05$) was performed using SigmaPlot® 11.0 comparing the mean dry weights in the first and second study. There were no treatment-by-experiment interactions found ($p=0.15$) and data were combined. Data were further analyzed with a Fisher's protected LSD ($p \leq 0.05$) test to detect significant differences in each species mean shoot dry weight for each planting ratio.

Results and Discussion

N. cristata was the dominant plant 7 weeks after planting (> 60% total plant biomass) in all treatments above a 1:5 (*N. cristata: hydrilla*) planting ratio (Figure 5-1). Dry weight and standard errors are listed in Table 5-1. Total plant shoot dry weight remained near 30 g across all planting ratios. Dry weight of both species was equal at a planting ratio of 1:5 (*N. cristata*: *hydrilla*). These results show that *N. cristata* was able to accumulate more biomass even at lower initial density than hydrilla and contribute more overall biomass, suggesting that *N. cristata* was the more competitive of the two species under these experimental conditions. Nonetheless, hydrilla was never excluded from the study system at any planting ratio. This finding is similar to the results reported by Wu et al. (2006), in which yellow floating heart (*Nymphoides peltata*), hereafter referred to as *N. peltata*, and produced more biomass than water chestnut (*Trapa bispinosa*). *T. bispinosa* was not excluded from the study system. Given the similar size of the initial propagules used for this study, the results were somewhat surprising based on reports of rapid summer growth rates of hydrilla (Glomski and Netherland, 2012).

It could be argued that the ramets of *N. cristata* had an advantage in growth due to the possibility of a dense carbohydrate reserve in tuber cluster at its base to initiate rapid growth, while the hydrilla tip fragment lacked this type of structure. However, it is important to note that the starting weights of the two species were the same. It was also observed that by 2 weeks after planting, *N. cristata* had not created a full canopy in the tanks and stems of hydrilla had grown to the water surface and begun to produce lateral branches (Figure 5-2 B). By 4 weeks after planting, *N. cristata* had formed a solid canopy over much of the water surface in the tanks and by 6 weeks had completely covered the water surface in all tanks with a layer of overlapping leaves (Figure 5-2 C and D), except those planted at the 1:5 ratio. Hydrilla was not visible at the water surface after canopy closure by *N. cristata* leaves. But at the time of harvest it was noted that hydrilla stems had apparently elongated under the canopy of *N. cristata* leaves. Stems of hydrilla reached lengths of 76 cm although the depth of the water in the tank was only 45 cm. This continued growth under the canopy demonstrates that even though the *N. cristata* had reached its capacity and shaded the water column beneath it, hydrilla, which is very tolerant of low light, was still able to grow and elongate below the floating leaves. The ability of hydrilla to continue to grow contributed to a synergistic effect of total plant biomass in the study system. This synergistic effect of plant biomass is evident because the total plant biomass exceeds the predicted biomass (Figure 5-1) (Gregory MacDonald, personal communication, 2012).

Studies reporting the competitiveness of *N. peltata* with the submersed invasive Eurasian watermilfoil (*Myriophyllum spicatum*) have shown that *N. peltata* accumulates most of its biomass in above ground parts first (Wu et al. 2006), then begins to partition mass to below ground structures. Given the short-term nature of the current competition trials, there was no focus on belowground biomass allocation. Larson (2006) reported that the effects of *N. peltata* on the submersed aquatic plants coontail (*Ceratophyllum demersum*), elodea (*Elodea canadensis*) and fan-leaved crow foot (*Ranunculus circinatus*) caused all three of these species to produce reduced growth when under any canopy coverage. However, these submersed species showed elongation of their nodes and stems in response to a limited light environment (Larson, 2006). While node length was not measured in hydrilla, it is possible the same response could have been present in the present studies. Longer term studies on the competitiveness of *N. cristata* will be useful in furthering our understanding of how this highly invasive species interacts with other species.

In summary, *N. cristata* is a highly competitive, invasive plant that can compete well with aggressive, low light tolerant, submersed invasives such as hydrilla. This information suggests that *N. cristata* ramets could serve as highly competitive structures in the presence or absence of submersed vegetation. The ability of *N. cristata* to become established in water up to 3 m deep suggests an ability to withstand low light conditions (Chapter 5) and this typically suggests a strong competitive ability for an aquatic species.

Table 5-1. Comparison of dry weight (± SE) of *N. cristata* and hydrilla after 7 weeks of competition. N=6. Different letters represent significant differences ($p \leq 0.05$) between the plant species.

Ratio	*Mean dry weight (±SE)*[a]	
	N. cristata	*hydrilla*
	-------------------------g----------------------------	
6:0	32.2(±3.6) a	0.0(±0) i
5:1	30.3(±1.4) ab	1.5(±0.3) i
4:2	27.9(±1.8) abc	4.2(±0.9) hi
3:3	23.9(±2.0) cd	8.9(±1.3) gh
2:4	20.9(±3.0) de	10.3(±1.7) g
1:5	15.6(±1.9) f	17.2(±1.5) ef
0:6	0.0(±0) i	26.4(±2.5) bc

[a] Different letters represent differences among the treatments determined by Fishers protected LSD ($p \leq 0.05$).

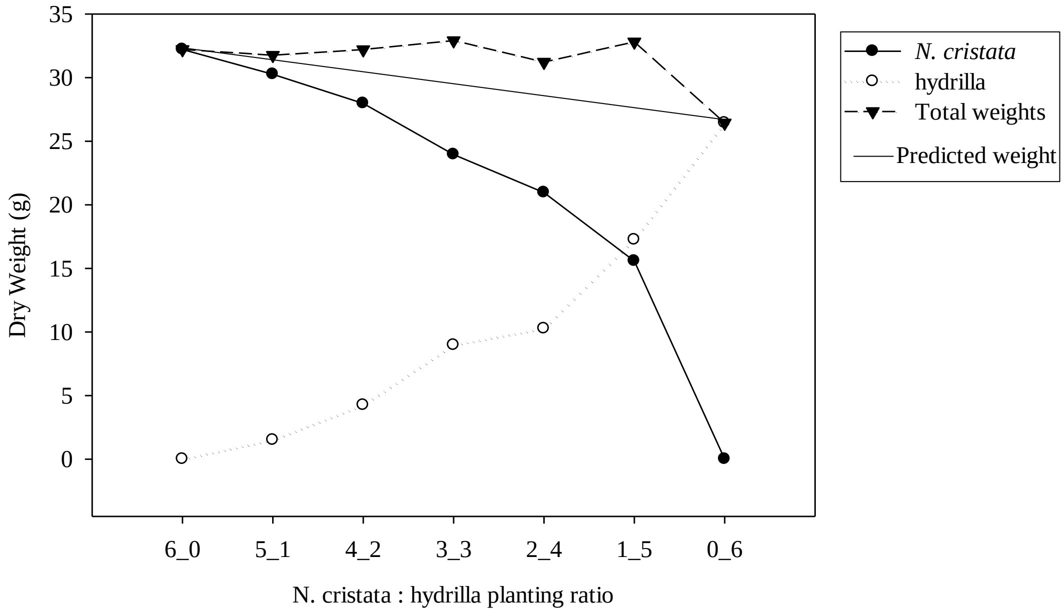


Figure 5-1. The dry weight of foliage of *N. cristata*, hydrilla, and total plant dry weight after 7 weeks of growth in a replacement series competition study.

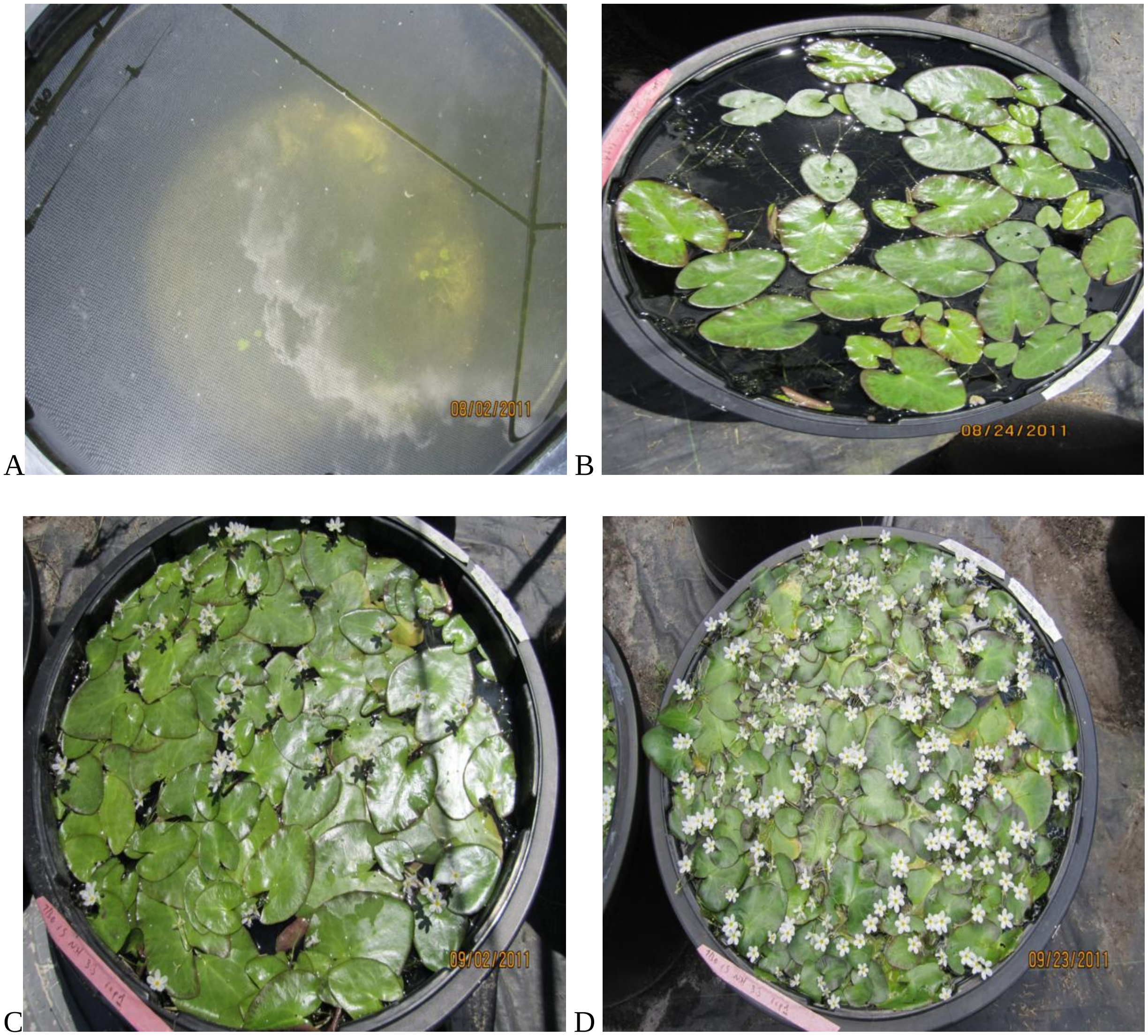


Figure 5-2. Pictures of study tanks at the 3:3 (N. cristata: hydrilla) planting ratio over the course of the study. A) Initial planting, B) 2 weeks after planting, C) 4 weeks after planting, D) 7 weeks after planting

CHAPTER 6
A COMPARATIVE ANATOMY OF CRESTED FLOATING HEART AND YELLOW WATER LILY LEAVES AND STEMS

Introduction

Crested floating heart (*Nymphoides cristata* (Roxb.) Kuntze), hereafter referred to as *N. cristata* a member of the Menyanthaceae family, is a recently expanding exotic invasive aquatic plant from Southeast Asia. Since it was positively identified in 1996, this plant has been observed completely blocking canals in areas throughout south Florida (Burks, 2002) and covering 2,428 ha of water surface on the Santee Cooper reservoir in South Carolina (Wesrbrooks et al. 2012). Aquatic invasive species have been reported to cause several ecological problems including formation of dense surface mats, displacement of native vegetation and reduction of biodiversity (Janes et al. 1996). *N. cristata* grows rooted in submersed sediments and produces floating leaves at the end of long stems (a nymphaeid morphology), and has been observed to form large monocultures. The overlapping leaves can shade out microscopic algae and other aquatic plants which can cause reductions in dissolved oxygen due to the reduction in photosynthetic productivity below the water surface.

Despite an increased emphasis on management of this species, control measures have proven inconsistent. Currently there are no known species specific insect herbivores that will consume *N. cristata*, the plant is not consumed by the herbivorous grass carp (*Ctenopharyngodon idella*), and mechanical and cultural control methods have also proven ineffective (Van Dyke et al. 1984; Singh et al. 1966; Middleton 1990). This suggests that chemical control may be the only current option for slowing the spread of this rapidly expanding invasive plant.

Many chemical control options and techniques have been ineffective for controlling *N. cristata*. The lack of reported efficacy could potentially be due to anatomical and physical features of the plant. The leaves and stems are very flaccid, and are easily disturbed by light wind and wave action. This makes herbicide contact time requirements difficult to achieve especially during foliar herbicide applications from a boat or helicopter as any disturbance causes the limp structures to 'dip' under the water surface and washes off any applied herbicide. Yellow water lily (*Nymphaea mexicana*), hereafter referred to as *N. mexicana* is very similar to *N. cristata* in appearance. Both plants have small surface leaves and long slender stems. However, *N. mexicana* has much more rigid and stiff leaves and stems and may not be affected as much by wind and wave action.

The objective of this study was to compare anatomical and physical differences between *N. cristata* and the native plant *N. mexicana.* While these species have numerous similar morphological characteristics, it is possible that more detailed anatomical differences could explain potential differences in herbicide efficacy.

Materials and Methods

Leaf Sections

Plant material from both species used in this study was collected from two different sources. *N. cristata* plants were collected from South Florida Water Management District (SFWMD) ponds in south Florida in January, 2011. Populations established at the University of Florida Center for Aquatic and Invasive Plants (CAIP) in Gainesville, FL. *N. cristata* plants were planted in Margo® potting soil with a mineral composition of 85.5% sand, 3.7% silt and 3.7% clay and an organic content of 7% that was amended with Osmocote® (15-9-12) at 1 g kg^{-1} dry soil then set in 1,000 L culture tanks filled with well water. Tissue samples were taken at the time of flower production. *Nymphaea mexicana* samples were collected from plant cultures growing in 95 L tanks at the CAIP. Four stems and 4 leaves were collected from each species. Leaves were left whole, and loosely rolled and placed in vials of formalin acetic acid (FAA) for preservation for 72 hours. Tissue samples were then moved into vials of 70% ethanol for storage (Stern et al., 1997).

Half of the leaf tissue for each species was then cut into 1cm squares and placed in new vials for dehydration in a tertiary-butyl alcohol series, embedded in paraffin and sectioned on a rotary microtome (Kuo-Huang et al. 1994). Microtome sections of 15µm were permanently mounted on glass slides. Slides were then emersed in a safranin solution to stain sclerified and lignified tissues. Once prepared, slides were examined under light microscopes of magnification varying from 4x to 40x to count the number of vascular bundles and sclereids in each species. Leaf thickness was measured using an ocular micrometer calibrated to 4x magnification.

Leaf sections for analysis were selected randomly from the preserved sample and sections were replicated 12 times. Data were analyzed using a student's t-test ($p \leq 0.05$) to detect differences in the means between the 2 species.

Stem Sections

Stems were cut into 4 cm sections and placed in vials of FAA for 72 hours and then transferred to vials of 70% ethanol for storage. A notch was cut out in one half of a 6.4 cm^2 block cut from a carrot to hold the stem without crushing it when the other half was placed over top of it and locked into the sliding microtome. Blocks of carrot were used because the orange-colored tissue of the carrot provided a color contrast against the green stem section (compared to using two cork sections to hold the sample) which made recovery of sections easier after cutting with a sliding microtome. The block of carrot containing the stem was placed in a sliding microtome and set to cut 30µm sections and then prepared for permanent mounting following the procedure used by Stern and Carlsward (2006). In addition to the sliding microtome sections, hand sections were cut using disposable razor blades to cut thin cross sections of tissue. For some species of aquatic plants these thicker hand sections are preferable to the thinner microtome sections because they can display larger portions of vascular tissues, and can minimize damage to the microstructure during handling of the tissue sample (Schneider and Carlquist, 2009).

Stem tissue was evaluated for diameter, arrangement of vascular tissues and number of sclereids. Tissue samples were photographed under the microscope using a Canon PowerShot CD1400 IS[1] digital camera. Stem sections for analysis were selected randomly from the preserved sample and sections were replicated 12 times. Data were analyzed using a student's t-test to detect differences between the two species in each measured parameter.

Results and Discussion

Leaf Sections

Leaf thickness was greater in *N. mexicana* than in *N. cristata* (450 ± 26 µm and 289 ± 5 µm, respectively) (Table 6-1). The palisade (upper) layer contributed to approximately half of the total leaf thickness in *N. cristata*, while the aerenchymatous (lower) leaf of *N. mexicana* made up over half of its leaf thickness (Figure 6-1 A, B).

The mean number of vascular bundles present in leaf tissue samples was not different between species (Table 6-1). The vascular bundles of *N. cristata* were located in the very center of the leaf (Figure 6-1 C) whereas the vascular bundles in *N. mexicana* leaves were located in the lower portions of the leaf surrounded by the aerenchyma (Figure 6-1 D).

Asterosclereids are crystal-like structures founds in plants. In aquatic plants they are strengthening structures (Huang et al. 2000) (Figure 6-1 E, F). The mean number of asterosclereids differed between the two species with *N. Mexicana* having more than 2 times the number that were present in *N. cristata* (Table 6-1). Sclereids were arranged randomly throughout the leaf tissue of both species.

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[1] Canon U.S.A., Inc. Lake Success, NY 11042

Leaves of *N. mexicana* were 1.5 times thicker than the leaves of *N. cristata* (Table 6-1). The leaves of *N. mexicana* also stained with safranin more readily than those of *N. cristata*. While this could have been due to the time exposure of the sample to safranin not being long enough, it could also indicate a lack of sclerified tissue as well as less lignified tissue in *N. cristata*. This is important to note because with these structures and compounds lacking, it could be difficult for foliar herbicide application to be effective due to the resulting flaccid nature of the leaf. While not measured, the spongy mesophyll layer of the leaf appeared to account for a greater portion of leaf thickness in *N. cristata*. In the lower aerenchymatous layer of the leaf, *N. cristata* had numerous small aerenchyma while *N. mexicana* had fewer, larger aerenchyma. It was observed that the aerenchymatous layer contained most of the sclereids in the leaf of both species. Since *N. mexicana* had a larger aerenchymatous layer this could account for the greater number of sclereids and therefore the leaf being more rigid than the leaf of *N. cristata*. The lack of sclerified and lignified leaf tissue in *N. cristata* may also play a role in the vegetative reproduction of the plant. The leaf composed of mostly soft tissue could allow for the development of roots from leaf fragments (Kenneth Langeland, personal communication, 2011). This type of vegetative growth was observed during this research. Cuticle thickness was not measured since it has been reported that species with floating leaves do not vary greatly in cuticle thickness but rather more in the length of carbon chains that make up the waxy layer (Amaral et al. 1990).

The number of vascular bundles present in each leaf sample did not differ between the 2 species which could be due to the small physical size of each sample which limited the sampling region to about a 1cm or smaller piece of leaf tissue. In the past, however, most studies on the vasculature of the order Nymphaeaceae have concentrated on the stem rather than the leaves (Weidlich, 1976).

N. mexicana leaf samples contained 2.3 times more sclereids per leaf section than *N. cristata*. These sclereids are arranged randomly, but occur mostly around air channels (Kuo-Huang et al. 1994). These sclereids in *N. mexicana* are known to have cell walls consisting of prismatic crystals of calcium oxalate (Kuo-Huang et al. 1994) but this composition has not been reported in *N. cristata*. Leaf sclereids of *N. cristata* averaged 148.34µm long, while in blue water lily (*Nymphaea nouchali*) sclereid length averaged 302.15µm (Khatun and Mondal, 2011). Fewer and smaller sclereids would likely contribute to in the flaccid nature of *N. cristata* leaves.

Stem Sections

Stems of *N. mexicana* were 1.7 times greater in diameter than *N. cristata* (Table 6-1) and there were also visible differences in the size and arrangement of the gas tubes in the center of the stems (Figure 6-5 A, B). The gas channels in *N. cristata* were arranged radially around a central vascular bundle with 1 or 2 smaller vascular bundles located to the outside of the stem. In *N. mexicana* 2 gas channels occupied most of the center of the stem with the other cells and with 6-8 vascular bundles located on the outside of the gas channels. There were also areas of highly sclerified tissue located in the epidermis of *N. mexicana* which were absent in *N. cristata*.

N. mexicana had twice the number of vascular bundles compared to *N. cristata* (Table 6-1). In *N. mexicana* the number of vascular bundles in a stem was 8, with the phloem in the middle surrounded by a ring of xylem (Weidlich, 1976). The arrangement of these bundles was also different between the species. In *N. mexicana* the bundles were arranged toward the outside of the stem, while in *N. cristata* they were in the center of the stem (Figure 6-5 C, D). The individual bundles in *N. cristata* were larger than the individual bundles in *N. mexicana*.

Sclereid counts were higher in *N. mexicana* and ranged from 11 to 25 while in *N. cristata* these counts ranged from 5 to 17 (Table 6-1). In general, for both species, these structures tended to be distributed along bands of connecting cells (Figure 6-5 E, F). Similar to the leaves, these structures were arranged around the air channels. While types of different sclereids were not identified, previous work has found three different types of sclereids present in *N. cristata* i.e. astero, osteo, and trichosclereids (Khatun and Mondal, 2011).

The differences between these species in terms of supporting structures and cell wall composition provide strong evidence that anatomical and physical differences may play a role in the efficacy of foliar applied herbicides on each of these plants. The lack of lignified and sclerified structures in leaves of *N. cristata* may be a factor that results in the flaccid nature of the leaf which allows the leaf to easily submerge below the water surface in the slightest boat wake or ripple on the water surface. This would likely wash any herbicide off of the leaf surface before sufficient contact for control has been achieved. In contrast, the presence of greater numbers of those structures and compounds may allow a longer contact time to be achieved in *N. mexicana*. The difference in vascular bundle numbers in the stem could also play a role in the apparent difference in herbicide efficacy. The number of vascular bundles was found to be the same in the leaves of the two species but less in the stems of *N. cristata* compared to *N. mexicana*. This reduction in the number of vascular bundles present from leaf to stem could cause a bottleneck effect for herbicide translocation. A bottleneck effect could cause a build-up of herbicide in the leaves before it can be loaded and moved into the stems and translocated to the roots. This could explain why many foliar treatment results in quick necrosis of the foliage but do not damage the root systems (see Chapter 2). Water depth (stem length) also may play a role in the ability of a herbicide to translocate from leaf to root. It has been noted from field applications that when plants are in shallow water (<1 m), greater control has been achieved than when plants in deeper water receive the same treatment (Chip Davis, personal communication, 2011). This difference could be because, even though a bottleneck effect may exist, in shallow water (short stems) the herbicide has a shorter distance to travel from the leaf to the root system, where it may provide longer and more complete control.

Table 6-1. Features measured on *N. cristata* and *N. mexicana* for anatomical and physical comparisons. Data reported as mean value ± standard error.

Feature measured	*Mean (±SE)*[a]	
	N. cristata	*N. mexicana*
Leaf thickness (µm)	289.7(±5.48)a	450.6(±26.46)b
Vascular bundles (leaf)	2.6(±0.28)	2.5(±0.39)
Sclereids (leaf)	3.5(±0.28)a	8.1(±1.10)b
Stem diameter (mm)	1.5(±0.04)a	2.4(±0.08)b
Vascular bundles (stem)	3.3(±0.13)a	6.8(±0.16)b
Sclereids (stem)	12.1(±0.96)a	18.8(±1.19)b

[a] Different letters next to the means indicate differences between the 2 species within the specific feature evaluated. A student's t-test was used to compare means.

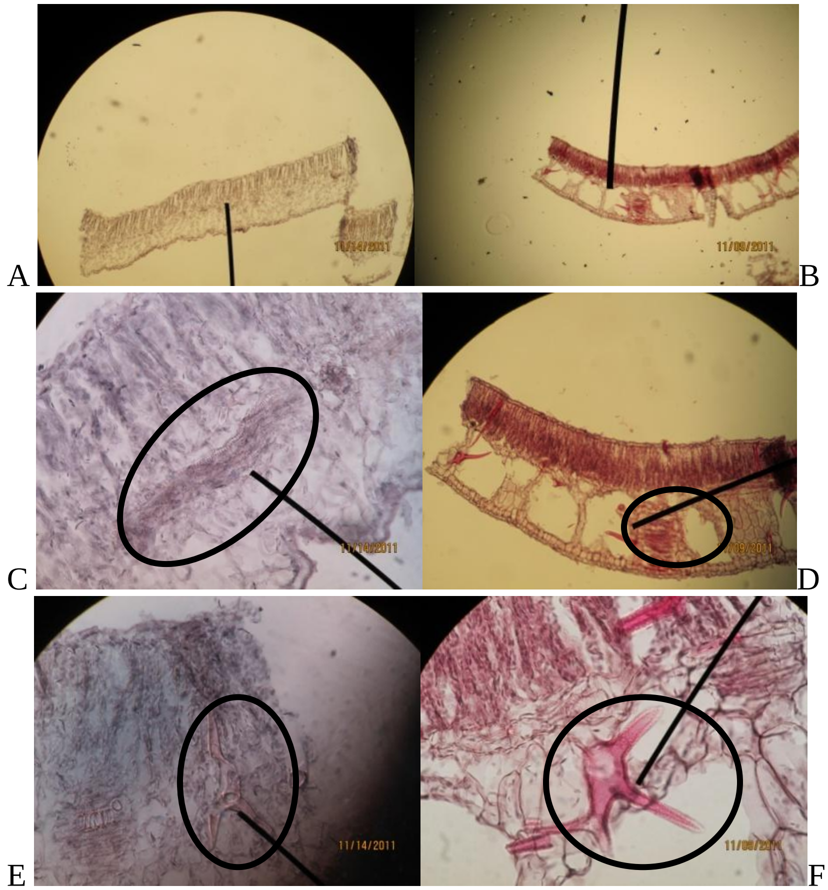

Figure 6-1. A) Leaf tissue sample under 4x magnification of *N. cristata*, B) *N. mexicana* leaf section under 4x magnification, C) Leaf vascular bundles in *N. cristata* under 10x magnification, D) *N. mexicana* leaf vascular bundle under 10x magnification, E) Asterosclereid in *N. cristata* under 40x magnification, F) Asterosclereid in *N. mexicana* under 40x magnification.

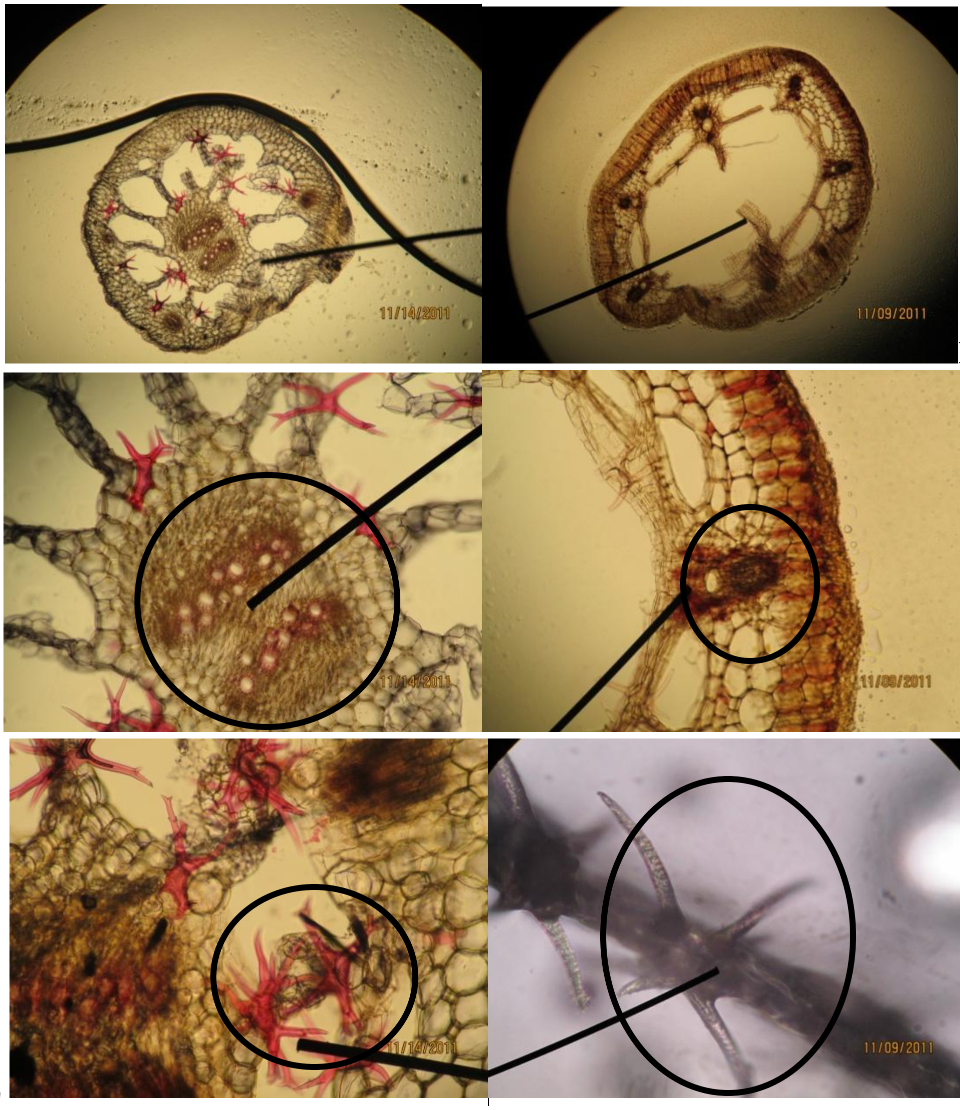

Figure 6-2. A) Stem section of *N cristata* under 4x magnification, B) *N. mexicana* stem section under 4x magnification, C) Vascular bundle in stem of *N. cristata* under 10x magnification, D) vascular bundle of *N. mexicana* under 10x magnification, E) asterosclereids in stem of *N. cristata* under 40x magnification, F) asterosclereids in *N. mexicana* stem under 40x magnification.

CHAPTER 7
CONCLUSIONS AND RECOMMENDATIONS

The information gained from these studies is important since there is very limited peer-reviewed literature pertaining to the basic biology and management of *N. cristata*. *N. cristata* was found to be tolerant of most aquatic herbicides; however the herbicides diquat, endothall (amine salt), imazamox, imazapyr and several combinations of these herbicides reduced dry weight of *N. cristata* below their respective pre-treatment reference samples. It was also determined that application method and formulation did not have an impact on the efficacy of most herbicides. Exceptions to this were imazamox which had greater efficacy as a foliar application compared to subsurface application and endothall (amine salt) was more effective in a liquid formulation than a granular formulation. The lack of strong endothall (dipotassium salt) activity on *N.cristata* in these assays is in contrast to findings from previous research; however, multiple trials with the dipotassium salt of endothall suggested that prolonged exposure periods (essentially days of static exposure) were required to reduce dry weight. Results of these herbicide efficacy studies require further field verification.

N. cristata ramets are tolerant of low light conditions. When exposed to 1% available light at the water surface the small propagules responded with rapid petiole elongation and quickly developed a canopy of leaves on the surface of the water. Once at the water surface, these leaves are in full sunlight and photosynthetic output is no longer restricted. There was a linear relationship between biomass and light availability, but a non-linear relationship with regard to leaf production. Leaf variegation was noted in full sunlight and believed to be a response to high light intensity.

N. cristata exhibited the ability to grow and establish in a wide range of sediment types as well as different levels of sediment fertility. The plant grew best in sand and muck sediments compared to clay or commercial potting soil. Growth was generally enhanced in the more fertile sediments; however, increased fertility did not greatly enhance growth in the clay or commercial potting soil. Sediment pH was linked to *N. cristata* growth and biomass accumulation. Plants grew best in low pH (acidic) sediments compared to high pH (basic) sediments and growth showed a strong, negative linear response to increasing the pH of a sand-based sediment.

N. cristata demonstrated that it is a competitive plant by accumulating more dry weight than hydrilla in a replacement series competition study. Even when there was 5 times more hydrilla than *N. cristata* planted, *N. cristata* still accumulated the same dry weight as when planted alone. *N. cristata* petioles quickly elongate from submersed ramets and reach the water surface before hydrilla. *N. cristata* formed a complete canopy over the hydrilla within a few weeks; however hydrilla continued to elongate beneath the surface mat. The copious production of ramets, rapid stem extension to form a canopy in water up to 3 m in depth, ability to grow under a wide range of sediment types, and tolerance to low light conditions suggest *N. cristata* has several invasive characteristics.

The anatomy of the leaves and petioles of *N. cristata* offer some insight as to possible mechanisms for the plants' reduced susceptibility to most foliar applied herbicides when compared to the more easily controlled *N. mexicana*. A reduced number of vascular bundles from the leaf to the petiole could cause a bottleneck effect for herbicide translocation. *N. cristata* also contains less lignified and sclerified structures than several other plants of similar life form which could cause herbicides to 'burn' off foliage before enough herbicide is taken up to translocate to the roots. This lack of supporting structure in the leaf also offers a mechanism of reduced contact time from foliar applications since the flaccid nature of the leaf allows it to easily dip beneath the water surface in the wake of a spray boat or air disturbance from aerial application.

Additional studies to evaluate translocation of foliar applied herbicides and the potential impact of water depth (petiole length) would be helpful to management efforts since it is currently thought that the plant is more susceptible to herbicides in shallower (<1.5m) water. The large numbers of ramets that are produced by each plant also present a challenge to management efforts. Research to better understand the factors that induce ramet sprouting and quiescence could be important to future control programs. The wide availability of *N. cristata* through the ornamental trade and well established populations in south Florida and North and South Carolina (as well as nascent populations in Louisiana and Texas) suggest high potential for continued spread of this plant. Management options are currently limited and further emphasis on herbicide application timing and response of ramets to herbicides would be valuable to resource managers.

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APPENDIX
ANOVA TABLES FROM CHAPTER 3 STUDY

Table A-1. 2-way ANOVA table for 6 week floating leaf data from trial 1 of the sediment type x nutrient availability study.

Source of variation	*DF*	*SS*	*MS*	*F*	*P*
soil	2	628.133	314.067	8.212	<0.001
Fertilizer	2	14358.933	7179.467	187.726	<0.001
Soil x fertilizer	4	1909.333	477.333	12.481	<0.001
Residual	36	1376.8	38.244		
Total	44	18273.2	415.3		

Table A-2. 2-way ANOVA table for 6 week dry weight data from trial 1 of the sediment type x nutrient availability study.

Source of variation	*DF*	*SS*	*MS*	*F*	*P*
Soil	2	208.780	104.39	10.082	<0.001
Fertilizer	2	2429.420	1214.71	117.322	<0.001
Soil x fertilizer	4	700.378	175.095	16.911	<0.001
Residual	36	372.732	10.354		
Total	44	3711.311	84.348		

Table B-1. 2-way ANOVA table for 6 week floating leaf data from trial 2 of the sediment type x nutrient availability study with addition of clay sediment.

Source of variation	*DF*	*SS*	*MS*	*F*	*P*
Soil	3	6916.078	2305.359	33.001	<0.001
Fertilizer	2	11367.394	5683.697	81.362	<0.001
Soil x fertilizer	6	10383.089	1730.515	24.772	<0.001
Residual	42	2934.00	69.857		
Total	53	30825.5	581.613		

Table B-2. 2-way ANOVA table for 6 week dry weight data from trial 2 of the sediment type x nutrient availability study with addition of clay sediment.

Source of variation	*DF*	*SS*	*MS*	*F*	*P*
Soil	3	0.159	0.0529	2.154	0.108
Fertilizer	2	8.973	4.487	182.698	<0.001
Soil x fertilizer	6	4.53	0.755	30.745	<0.001
Residual	42	1.031	0.0246		
Total	53	14.865	0.28		

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BIOGRAPHICAL SKETCH

Born in 1988 in Seymour, Indiana, Leif is the son of Michael and Lora Willey. Leif grew up in Seymour, where his father owned a nursery and landscape construction company. He attended Seymour Senior High School and graduated with Academic Honors in June, 2006. After graduating, he pursued his Bachelor of Science degree at Purdue University in West Lafayette, Indiana majoring in fisheries and aquatic sciences.

While at Purdue, he was a member of FarmHouse Fraternity and was active in various student organizations. Leif was a member and later president of the Recreational Fishing Club as well as the Purdue University Trap and Skeet Club; he also was a member of the Purdue University Student Chapter of the American Fisheries Society. During his undergraduate career, Leif worked for Aquatic Control, Inc. during the summers assisting the applicators and leading vegetation survey crews. Through his summer work experiences here, he developed an interest in aquatic plant management. After completion of his bachelor's degree, Leif moved to Gainesville, Florida to pursue a Master of Science degree in the field of aquatic plant management. In his second year at the University of Florida, Leif co-founded the Florida Gators Trap Skeet and Sporting Team which became largely successful during their first competitive season. Upon completion of his degree, he plans on continuing to work in the field of aquatic plant management in the private sector.