Effect of desiccation, burial duration, and daylength on ramet sprouting of crested floatingheart (*Nymphoides cristata*)

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ABSTRACT

Crested floatingheart is a state-listed noxious weed in Florida that reproduces primarily via *ramets*, rhizome clusters produced at leaf-petiole junctions. Little is known regarding the effect of desiccation, burial duration, and daylength on ramet viability. Fresh ramets were collected and placed on paper towels on a lab bench for 1 to 10 d (to mimic desiccation) or buried for 4 to 24 d under submersed conditions and then unearthed (burial duration). Daylength effects were evaluated by culturing fresh ramets under daylengths ranging from 9 to 15 h. All ramets were planted under submersed conditions after treatment and scored for sprouting for 12 wk after planting (WAP). A single day or more of desiccation greatly reduced ramet viability; the vast majority (97.5%) of fresh ramets had sprouted 4 WAP, whereas only one of the 480 ramets subjected to any desiccation had sprouted by 12 WAP. Burial also inhibited ramet sprouting; 98% of unburied ramets had sprouted 4 WAP, but no ramets buried for any duration sprouted by 12 WAP. Daylength had no effect on ramet sprouting; the vast majority (96.6%) of ramets had sprouted 4 WAP, and only two of the 560 ramets failed to sprout over the 12-wk evaluation period. These results suggest that sprouting of crested floatingheart ramets is unaffected by daylength but is negatively affected by desiccation and burial. These findings provide insight into reproductive strategies in this species and may be useful to resource managers. For example, because desiccation greatly hinders sprouting, resource users should be encouraged to adopt a clean–drain–dry protocol to reduce the spread of this noxious weed.

Key words: water garden plant, ornamental aquatic plant, aquatic weed, invasive plant, noxious weed, desiccation.

INTRODUCTION

Crested floatingheart is a newly emerging noxious weed that has colonized many of Florida's waters. The species, which is in the buckbean (Menyanthaceae) family, has a history of cultivation as an ornamental water garden plant (Burks 2002). Crested floatingheart owes its common name to physical characteristics: it has heart-shaped leaves that float on the surface of the water and bears flowers with five white petals that have a central crest. The Florida Department of Agriculture and Consumer Services (FDACS) added crested floatingheart to the state's Noxious Weed List in 2014 (FDACS 2014), meaning it is unlawful to introduce, multiply, possess, move, or release crested floatingheart without a permit issued by the state. Although crested floatingheart is tropical in origin, it also flourishes in temperate areas in the continental United States. For example, a nascent invasion of crested floatingheart in Lake Marion (Orangeburg County), South Carolina, occupied less than 10 ha when it was discovered in 2006 but had expanded to greater than 800 ha by 2009 (Westbrooks and McCord 2010). The U.S. Department of Agriculture Animal and Plant Health Inspection Service lists the species as a *high risk* based on factors that include its colonization potential and growth rate and suggests that the entire southeastern United States could be subject to invasion by crested floatingheart (USDA APHIS 2012).

Most reproduction in crested floatingheart occurs via daughter plants, tubers, and rhizomes (Burks 2002, Willey and Langeland 2011). Gettys et al. (2017) found that a single plant can produce more than 500 *ramets*—clusters of rhizomatous structures that form at the junction of each leaf and petiole—over a six-month period when grown under nutrient-rich (4 g of controlled-release fertilizer per liter of substrate) conditions. Although ramet production was greatest in heavily fertilized plants, even plants grown in pure sand with no nutrients yielded almost 100 ramets in a 6-mo period (Gettys et al. 2017).

Management of crested floatingheart is a high priority in invaded aquatic systems, and various research projects focus on identifying herbicide treatments that provide adequate control of existing plants. However, little is known regarding the viability and longevity of the prodigious ramets produced by this species, which serve as the propagules that drive colonization of new aquatic systems and could potentially repopulate areas where management of mature plants has been successful as well. Gettys et al. (2017) found that freshly collected ramets that were buried failed to sprout and suggested that ramets may require light to sprout. Gettys et al. (2017) also noted that daylength affected ramet production but did not evaluate the effect of this environmental factor on ramet sprouting. The objec-

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tives of this research were to further examine the effects of desiccation duration, burial duration, and daylength of crested floatingheart ramet sprouting.

MATERIALS AND METHODS

Fresh unsprouted ramets of crested floatingheart were collected from stock plants maintained in culture at the University of Florida Institute of Food and Agricultural Sciences Fort Lauderdale Research and Education Center in Davie (Broward County), Florida (hereafter FLREC). Ramets were selected for uniformity (all measured between 2 and 2.5 cm in diameter) and were subjected to the treatments described below. Treated ramets were then placed on the surface of 2 L containers without holes that were filled with unamended coarse masonry sand. Four replicates of 10 ramets each were prepared for each treatment. All planted containers in the desiccation and burial studies were maintained in a 1,700 L mesocosm (surface area 4.6 m^2) filled with well water to a depth of 30 cm.

Desiccation

Ramets were subjected to one of seven storage durations: 0 (fresh), 1, 2, 3, 4, 5, or 10 days of desiccation (DOD). Fresh ramets were collected at the beginning of each prescribed storage period so that all ramets were planted on the same day. For example, ramets that received 10 DOD were collected 10 d before planting (DBP), those receiving 5 DOD were collected 5 DBP, and so on. Dry-stored ramets were placed on paper towels on a lab bench with temperature maintained at 23 C, whereas 0 DOD (fresh) ramets were collected immediately before planting. These experiments were repeated and ramets were moved to 2 L containers in the mesocosm as described above on 16 June 2016 (Run 1) and 9 July 2016 (Run 2). All treatments were monitored for sprouting three times per week for 12 weeks after planting (WAP). Percent sprouting data were subjected to arcsine transformation to normalize data; then transformed data were analyzed using the general linear model procedure in SAS 9.3 (SAS Institute, Cary, North Carolina, USA[1]), and treatment means were separated by least significant differences with an α of 0.05.

Burial

Ramets were subjected to one of seven burial durations: 0 (unburied), 4, 8, 12, 16, 20, and 24 days of burial (DOB). Unburied ramets were planted on 27 June 2016 (Run 1) and 22 May 2017 (Run 2), and all ramets receiving any DOB were buried on the same day. Burial treatments were accomplished by filling 10 L dishpans to a depth of 6 cm with unamended coarse masonry sand. A single layer of standard insect screening was placed on the surface of the sand, and the ramets were placed on the screening. A second piece of screening was placed on top of the ramets; then a 2.5 cm deep layer of sand was gently added to each dishpan to cover the ramets. Screening was used to ensure that ramets remained at the depth where they were buried and to facilitate locating ramets to move them from burial to

planting. Once the prescribed burial durations were accomplished, ramets were moved to 2 L containers in the mesocosm as described above. All treatments were monitored for sprouting three times per week for 12 WAP. Percent sprouting data were subjected to arcsine transformation to normalize data, then transformed data were analyzed using the general linear model procedure in SAS 9.3 (SAS Institute, Cary, North Carolina, USA), and treatment means were separated by least significant differences with an α of 0.05.

Daylength

Ramets were collected and planted in 2-L containers as described above, but planted containers were placed in 68-L mesocosms filled with well water to a depth of 30 cm that were enclosed in blackout chambers. Each chamber measured 0.7 by 0.7 by 1 m (length by width by height) and consisted of a wood frame made from 2 by 2″ pine that was wrapped in 6-ml black plastic to exclude all external light. An opening was cut into the top of each chamber to allow the placement of a light fixture housing an incandescent floodlight that delivered 1,200 lumens to the enclosed mesocosm. All blackout chambers were maintained in an air-conditioned room (temperature 24 ± 2 C) at the FLREC. Ramets were subjected to one of seven daylengths: 9, 10, 11, 12, 13, 14, and 15 h of light (HOL). These experiments were conducted twice with all ramets planted on 25 July 2016 (Run 1) and 12 September 2016 (Run 2). All treatments were monitored for sprouting three times per week for 12 WAP. Monitoring events took place during the light period to avoid light contamination during the dark period. Percent sprouting data were subjected to arcsine transformation to normalize data; then transformed data were analyzed using the general linear model procedure in SAS 9.3[1], and treatment means were separated by least significant differences with an α of 0.05.

RESULTS AND DISCUSSION

Desiccation

No difference was seen between Runs 1 and 2, so data were pooled before analysis. These studies revealed that a single day of desiccation has a profound effect on sprouting of crested floatingheart ramets (Table 1). An average of 87.5 and 95% of fresh ramets had sprouted 2 and 4 WAP, respectively, whereas only a single ramet subjected to 1 DOD sprouted during the same period. No additional sprouting was recorded more than 6 WAP, and no sprouting was recorded for ramets that were desiccated for more than 1 day.

Burial

As with the desiccation studies, no difference was seen between Runs 1 and 2 of the burial experiments, so data were pooled before analysis. These studies revealed that as few as 4 days of burial prevented sprouting of crested floatingheart ramets (Table 2). More than 92 and 97.5% of

TABLE 1. EFFECT OF DESICCATION ON SPROUTING OF CRESTED FLOATINGHEART RAMETS. VALUES ARE THE MEAN % SPROUTING OF EIGHT REPLICATES (TWO RUNS, FOUR REPLICATES PER RUN) ± STANDARD DEVIATION. TREATMENTS WITHIN AN EVALUATION PERIOD (WEEKS AFTER PLANTING; WAP) CODED WITH THE SAME LETTER ARE NOT DIFFERENT BASED ON TUKEY'S LEAST SIGNIFICANT DIFFERENCE WITH an α OF 0.05.

	Days of Desiccation and Percent Ramet Sprouting						
	0	1	2	3	4	5	10
4 WAP	95 ± 9.26 a	1.3 ± 3.54 b	0 ± 0 b	0 ± 0 b	0 ± 0 b	0 ± 0 b	0 ± 0 b
6 WAP	95 ± 9.26 a	1.3 ± 3.54 b	0 ± 0 b	0 ± 0 b	0 ± 0 b	0 ± 0 b	0 ± 0 b

unburied ramets had sprouted 2 and 4 WAP, respectively, and no new sprouting was recorded more than 6 WAP, whereas none of the ramets buried for any duration had sprouted 12 WAP. In fact, ramets that were subjected to 8 or more DOB had rotted by the time they were removed from dishpans and transferred to 2 L containers.

Daylength

As with the desiccation and burial studies, data from Runs 1 and 2 were not different and were pooled before analysis. Daylength had no effect on sprouting of ramets, and only two of the 560 ramets used in these studies failed to sprout during the 12-wk evaluation period (Table 3). The vast majority (96.6%) of ramets had sprouted by 4 WAP, and 99.6% of ramets had sprouted by 6 WAP, with no additional sprouting recorded for the remainder of the evaluation period.

These experiments revealed that storage conditions such as desiccation and burial might have profound effects on viability of crested floatingheart ramets. Desiccation has been shown to reduce vegetative propagule viability in some aquatic species. For example, Doyle and Smart (2001) reported that 90% of freshly collected hydrilla (*Hydrilla verticillata*) tubers were viable, but that there was a 2% loss in viability for every 1% decline in moisture content. However, Bruckerhoff et al. (2015) found that curly-leaf pondweed (*Potamogeton crispus*) turions were able to sprout after 28 d of desiccation. We found that a single day of desiccation resulted in near-total loss of crested floatingheart ramet viability, which is particularly important information from a management perspective since ramet production is the primary mode of reproduction for this species. Gettys et al. (2017) reported that crested floatingheart is extremely productive, a single plant growing under nutrient-rich conditions can produce more than 500 ramets in 6 mo, and 50 to 60% of unburied ramets could be expected to sprout, causing substantial recruitment in a relatively short period of time. Sprouting of fresh, unburied ramets in our experiments was significantly higher than reported by Gettys et al. (2017) (97.5 to 99.6% of all fresh, unburied

ramets in our studies vs. 50 to 60% in Gettys et al.), which suggests that ramet-driven population expansion could be even greater than previously thought. Anglers and other users of aquatic resources that are infested with crested floatingheart should be encouraged to clean, drain, and dry boats, trailers, and other devices to foster ramet desiccation, thus reducing viability.

Vegetative propagules of many aquatic plants are unharmed by burial. For example, buried hydrilla turions remain viable for a year or more (Van and Steward 1990), whereas buried hydrilla tubers may be able to survive for a decade or more (Hofstra et al. 1999). However, this is not the case for crested floatingheart. Gettys et al. (2017) reported that burial eliminated nearly all sprouting of crested floatingheart ramets, and our results are similar. We found that ramet viability was eliminated by 4 d of burial, the shortest duration evaluated in these experiments, and ramets buried for 8 or more days were rotted when unearthed. The reason for this is unknown, but these findings may be due to reduced oxygen levels in the substrate versus on the surface of the substrate. However, it is also possible that ramet viability is affected by prolonged (> 48 h) exposure to darkness. If ramets do require light to sprout, it may be possible to reduce or eliminate recruitment using benthic barriers or similar strategies, but more investigation is needed to determine whether this would be a useful way to inhibit ramet sprouting.

Daylength is known to influence growth and vegetative reproduction in some aquatic plants. For example, hydrilla biomass and individual tuber weight is greater under long-day (14 h light) conditions, but tuber production is also influenced by interaction between daylength and temperature (Haller et al. 1976, McFarland and Barko 1990). Spencer and Anderson (1987) reported that the pondweed species *Potamogeton nodosus* and *Potamogeton pectinatus* produced more buds or tubers under short (10 or 12 h light) versus long (14 or 16 h light) days, but none of these authors evaluated the effects of daylength on sprouting of vegetative propagules. Gettys et al. (2017) reported that average daylength over a 6-mo culture period affected crested

TABLE 2. EFFECT OF BURIAL ON SPROUTING OF CRESTED FLOATINGHEART RAMETS. VALUES ARE THE MEAN % SPROUTING OF EIGHT REPLICATES (TWO RUNS, FOUR REPLICATES PER RUN) ± STANDARD DEVIATION. TREATMENTS WITHIN AN EVALUATION PERIOD (WEEKS AFTER PLANTING; WAP) CODED WITH THE SAME LETTER ARE NOT DIFFERENT BASED ON TUKEY'S LEAST SIGNIFICANT DIFFERENCE WITH AN α OF 0.05.

	Days of Burial and Percent Ramet Sprouting						
	0	4	8	12	16	20	24
4 WAP	97.5 ± 9.60 a	0 ± 0 b	0 ± 0 b	0 ± 0 b	0 ± 0 b	0 ± 0 b	0 ± 0 b
6 WAP	100 ± 0 a	0 ± 0 b	0 ± 0 b	0 ± 0 b	0 ± 0 b	0 ± 0 b	0 ± 0 b

TABLE 3. EFFECT OF DAYLENGTH ON SPROUTING OF CRESTED FLOATINGHEART RAMETS. VALUES ARE THE MEAN % SPROUTING OF EIGHT REPLICATES (TWO RUNS, FOUR REPLICATES PER RUN) ± STANDARD DEVIATION. NO DIFFERENCES WERE DETECTED AMONG DAYLENGTHS USING TUKEY'S LEAST SIGNIFICANT DIFFERENCE WITH AN α OF 0.05.

| | Hours of Light and Percent Ramet Sprouting | | | | | | |
	9	10	11	12	13	14	15
4 WAP	100 ± 0	87.5 ± 13.89	95 ± 10.69	100 ± 0	98.8 ± 3.54	100 ± 0	95 ± 5.35
6 WAP	100 ± 0	100 ± 0	97.5 ± 7.07	100 ± 0	100 ± 0	100 ± 0	100 ± 0

floatingheart ramet production, which was the impetus for including daylength experiments in the studies we report here. In our studies we evaluated the effects of daylength on ramet sprouting only—not on ramet production—and we found that daylength had no discernable effect on crested floatingheart ramet sprouting. Most sprouting occurred in the first 4 wk after planting, which is similar to findings reported by Gettys et al. (2017), and only two of the 560 ramets used in the daylength studies failed to sprout, resulting in an overall sprouting rate of 99.6% under daylengths ranging from 9 to 15 h.

These experiments are valuable additions to the body of knowledge regarding vegetative propagation of crested floatingheart, but more work should be done, especially to address some of the questions resulting from these experiments. For example, we show that a single day of complete desiccation on a lab bench greatly reduced ramet viability, but it would be useful to evaluate whether field desiccation (which ramets might be exposed to on a boat trailer or during a drawdown, where moist but not flooded conditions could be present) yields similar results. Also of interest would be an evaluation of the effects of oxygen deprivation and/or light attenuation (the latter of which could be achieved by dying the water column or deploying benthic barriers) on ramet viability.

SOURCE OF MATERIALS

[1]SAS Version 9.3, SAS Institute, Cary, NC, USA.

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