Note

Influence of sediment coverage on sprouting of crested floating-heart ramets and response of quiescent ramets to contact herbicides

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INTRODUCTION

Crested floating heart [*Nymphoides cristata* (Roxb.) Kuntze] (CFH) is a floating leaved aquatic plant native to Southeast Asia, and it is often considered to be a pest in rice fields (Burks 2002). It was introduced to North America through the water garden trade where it is readily available for purchase from numerous online aquatic plant distributors and aquarium stores. It is often marketed as water snowflake because it can cover a water surface in tiny white flowers, thus giving the appearance of snow throughout the long flowering season. CFH presently exists in expanding, invasive populations in many waterways in Florida as well as South Carolina, Texas (Center for Invasive Species and Ecosystem Health 2010), and Louisiana (Winslow 2013). The Florida Exotic Pest Plant Council (FLEPPC) lists CFH as a category 1 invasive species, indicating a nonnative species that has been observed altering native plant community structures, ecological functions, and is present in natural areas (FLEPPC 2009).

Despite prolific production of flowers, viable seeds are not produced. Reproduction and spread of CFH is facilitated via leaf, stem, and root fragmentation, which can be caused by contact with boat motors, wave action, and mechanical harvesting (Burks 2002). The main pathway for spread is facilitated through the production of small clonal reproductive structures called ramets (also referred to as daughter plants), which develop beneath the mature floating leaves and protrude from the stems of the plant as a tuber cluster with several small leaves. These quiescent propagules are somewhat unique among other invasive aquatic plants in that the propagule has a very similar appearance to the mature plants. Each mature plant has the potential to produce over 100 ramets in a growing season (Willey 2012). The ramets easily separate from the parent plant and can disperse to form new colonies or settle to the sediments and expand the parent colony (Burks 2002).

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Currently, limited information is available on the biology, longevity, or factors that influence sprouting of these vegetative propagules. Observations in culture tanks suggest that the majority of ramets remained quiescent at the sediment surface for over a year, while other ramets sprouted when algal growth covered the tuber cluster (L. N. Willey, pers. obs.). As the spring progressed and water temperature increased, some of the algae- and sediment-covered CFH ramets sprouted; however, this observation could have been a result of increasing day length or even a combination of temperature and day length, but these factors were not evaluated at the time of this study. The influence of light on sprouting of vegetative structures has been studied in other invasive aquatic species and has been observed to not stimulate sprouting of axillary turions of curlyleaf pondweed (*Potamogeton crispus* L.) (Netherland et al. 2000). Overall, the majority of the ramets remained quiescent throughout the growing season, after separating from the parent plant. Interestingly, when plants were required for herbicide trials, ramets could be removed from the culture tank and would sprout within days of planting. Once the ramet was covered, CFH stems could rapidly elongate and produce a surface leaf within 1 to 3 d in 60 cm of water during the summer months (Willey 2012). This observation suggested that physical disturbance of the ramets may play a significant role in sprouting, as has been observed with quiescent hydrilla tubers removed from the sediment (Netherland 1997). Hydrilla tubers can remain quiescent in the sediment for years; however, removal of the tuber results in rapid sprouting response when the plant is moved from anoxic to aerobic conditions (Netherland 1997). Similar environmental factors may also influence sprouting of CFH ramets.

Management of CFH infestations currently relies on chemical control, as previous research has found that mechanical harvesting is ineffective because of fragmentation and there are no known species-specific insect herbivores for biocontrol (Burks 2002). Furthermore it has been observed that sterile, triploid grass carp (*Ctenopharyngodon idella*) will not consume the plant (Van Dyke et al. 1984). Because of the issues created with mechanical harvesting, no viable biocontrol, and intensive labor requirement of physical removal, management-focused research has been directed toward controlling the mature

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TABLE 1. SUMMARY STATISTICS OF DATA COLLECTED ON THE NUMBER OF DAYS REQUIRED FOR CRESTED FLOATING-HEART RAMETS TO PRODUCE A LEAF AT THE SURFACE OF THE WATER. COVERAGE OF THE RAMET WAS BASED ON THE PROPORTION OF THE BASAL TUBER CLUSTER THAT WAS COVERED BY SEDIMENT. MEAN VALUES REPRESENT THE NUMBER OF DAYS TO FIRST LEAF EMERGENCE. MIN IS NUMBER OF DAYS TO LEAF EMERGENCE OF THE FIRST REPLICATE IN A TREATMENT GROUP. MAX IS NUMBER OF DAYS TO LEAF EMERGENCE OF THE LAST RAMET IN A TREATMENT GROUP.

Coverage	Mean[1]	Standard Error	Min	Max
0	0 a	0	0	0
¼	0 a	0	0	0
½	13.2 b	1.420	7	20
¾	10.9 b	1.187	6	20
Full	11.6 b	1.343	6	20

[1]Data from both trials were combined, $n = 10$. Means followed by the same letter were not significantly different ($P \geq 0.05$). Treatments with a 0 value showed no signs of growth through the duration of the trial.

growth stages of CFH with herbicides, as this is likely to be the most effective option for long-term control. The most effective herbicide control options include submersed applications of diquat and endothall, foliar applications of imazapyr and imazamox, and foliar-applied combinations of flumioxazin and glyphosate (Glomski et al. 2014; Willey et al. 2014). The effects of herbicides on the ramets of CFH have not been directly evaluated.

Knowledge about potential dormancy and sprouting of these structures can be useful to managers when considering the response of the plant to management, longevity of management, and timing of management strategies. The objective of this study was to evaluate the response of CFH ramets to disturbance (removal and transfer from stock tank to study system) and coverage of the basal tuber cluster in sediment. We also evaluated the response of quiescent ramets to various exposures of the contact herbicides that were active on mature CFH plants.

MATERIALS AND METHODS

Experiments were conducted at the University of Florida, Center for Aquatic and Invasive Plants in Gainesville, FL in 2013. In June 2012 two mature CFH plants of similar size growing in 1-L containers were transplanted in two 95-L containers filled half way with Margo Professional Topsoil[1] (92% sand, 4% silt, 4% clay), amended with fertilizer (Osmocote® 15–9–12)[2] at 1 g kg^{-1} of soil, and capped with a 4-cm layer of sand. These containers were placed in a single 1,000-L concrete tank filled with well water (pH 8.5). These culture plants were used to produce ramets for additional studies, including this one. The plants were producing large numbers of ramets by August 2012 and by October 2012, these ramets had begun to separate from the parent plants and settle to the bottom of the stock tank. In March 2013, it was observed that very few of the ramets had sprouted. The few that did sprout had a dense covering of filamentous algae growth around the tuber cluster or had settled into the sediments.

The first trial was initiated on 17 April 2013 and ran until all plants had produced leaves at the water surface, or it was believed that the plants that had not yet produced leaves at the water surface would not produce leaves inside a greenhouse. The study system used was a series of five 95-

L high-density polyethylene (HDPE) tanks. The study was repeated in space and time on 30 May 2013 in 1,000-L outdoor tanks and run under the same observational growth parameters as the first trial. Light and temperature data were not collected, as these factors were not being considered in this study. In both trials, five coverage amounts based on the proportion of the tuber cluster covered with sediment were selected: 0 (uncovered), ¼, ½, ¾, and fully covered. Each coverage proportion was replicated five times. In each study, 25 ramets, each measuring 2.5 ± 0.5 cm across, were selected. Ramets were placed in 1-L (10.2 cm by 10.2 cm by 10.2 cm) containers filled with the previously mentioned potting soil and fertilizer mixture. Each 1-L container was randomly assigned a coverage proportion and the ramet was planted accordingly.

Daily visual observations for both sprouting studies included notes on stem elongation and root formation. Data were collected in both trial runs for the response variable of the number of days required for the first leaf of each replicate to reach the water surface, 35.5 cm in trial 1 (greenhouse) and 60 cm in trial 2 (outdoor tanks). Data were analyzed using SigmaPlot 11.0.[3] ANOVA found no interaction of the response variable by trial run so data were pooled. Pair-wise t tests were used to compare treatment means by coverage proportion.

A third study was initiated in July 2013 to evaluate the effects of selected herbicides on quiescent ramets. Five ramets of approximately the same size (0.06 ± 0.006 g dry weight) were placed into each of 12 95-L HDPE tanks without being planted into the sediment and allowed to acclimate for 1 wk. After the acclimation period, the tanks were treated with dipotassium endothall[4] (concentration of 3.0 mg ae L^{-1}), amine endothall[5] (0.3 mg ae L^{-1}), and diquat[6] (0.37 mg ai L^{-1}). An untreated control was also included. One ramet was removed from each treatment tank at 24, 48, and 96 h after treatment and planted in a 1-L pot filled with the same potting soil and fertilizer mixture and placed in a herbicide-free tank for the remainder of the trial. Plants were harvested 4 wk after treatment (WAT) when control plants began to produce ramets. Whole plants including roots were harvested, washed, then dried for 1 wk in a forced-air oven set to 76 C. Dry weight data were analyzed using ANOVA ($P < 0.05$) and a post hoc Fisher's LSD test was used if differences by treatment were found.

RESULTS AND DISCUSSION

There were no differences in the number of days required for ramets to develop the first surface leaf (in 35.5 or 60 cm of water depth) among the burial depths of ½, ¾, and fully buried (Table 1) ($P > 0.05$ in all pair-wise comparisons). Although the water depth differed, the 60-cm tank, used in the second trial, was exposed to warmer air temperatures and likely higher-intensity light because it was outside and those factors probably contributed to a higher growth rate than in the first trial. Thus, plants were able to send leaves to the water surface in the same amount of time, despite the water being deeper. There were also no differences between uncovered and ¼-covered ramets (P
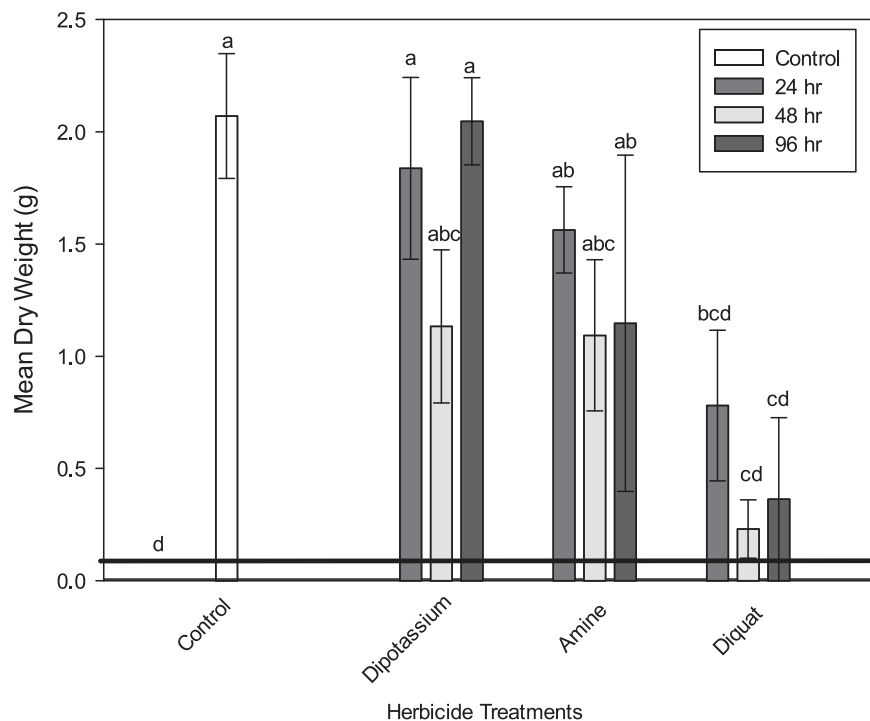
Figure 1. Mean (± standard error) dry weight of crested floating-heart ramet response to 24-, 48-, and 96-h exposures to dipotassium endothall (3.0 mg ae L^{-1}), amine endothall (0.3 mg ae L^{-1}), and diquat (0.37 mg ai L^{-1}). Bars with differing letters were found to be different from each other according to Fisher's LSD ($P < 0.05$). Horizontal black line represents the pretreatment dry weight labeled as d in the lower left for analysis comparison.

= 1.00). However, there were differences between ½, ¾, and fully covered ramets when compared with the uncovered and ¼-covered ramets ($P < 0.001$ in all pair-wise comparisons). In both trial runs, root formation at the crown of the tuber cluster was observed within 2 to 4 d after planting (DAP) in ½-, ¾-, and fully covered ramets. Stems had begun to elongate noticeably by 4 DAP and all ramets had small white roots forming from the crown penetrating into the sediment (similar to brace roots in corn (*Zea mays* L.). Ramets that were not covered and ¼ covered did not have emergent leaves present by 30 DAP. Moreover, these plants did not develop roots or elongated stems during the 30-d trial, indicating that the ramets remained quiescent. On the basis of observations of uncovered ramets remaining quiescent for up to a year in the bottom of stock tanks, it is believed that allowing this study to continue for additional time would not have shown sprouting in the uncovered and ¼-covered ramets.

In the third study, differences were found in ramet response to three contact herbicides ($P = 0.002$) (Figure 1). Although there were differences between herbicide treatments, there were no differences among exposure times within a specific herbicide (Figure 1). Ramets exposed to diquat developed symptoms of chlorosis in developing leaves at all exposure times. Visual recovery of diquat-treated plants was variable as plants in all exposure times exhibited weak recovery or became fully necrotic by 4 WAT. There were no differences in biomass between pretreatment plants and diquat-treated plants. This suggests that the quiescent ramets were absorbing diquat and the photosynthetic apparatus is active within CFH ramets. Some

endothall symptoms after the amine treatments were noted in recovering plants; however, the activity was reduced compared with diquat-exposed ramets. Interestingly, Willey (2012) observed that when mature CFH plants were treated with amine endothall at 0.25 and 0.5 mg ae L^{-1}, ramets that were still attached to the parent plant were controlled 100%. In this study, ramets separate from the parent plant were treated with a concentration of endothall intermediate of those tested by Willey (2012) and ramets survived. This finding suggests that the plant mechanism affected by endothall exhibits greatly reduced activity when it becomes detached from the parent plant. Dipotassium endothall also had no impact on the ramets, as all exposure times recovered to control levels (Figure 1).

Our data suggest that disturbance and subsequent burial (coverage) of the ramet tuber cluster plays a role in stimulating sprouting. This situation would be likely in areas where boat propellers, mechanical harvesting operations, or wave action may cause ramets to separate from the parent plant and settle to the bottom where they may gradually settle into the sediments or algae growth may cover the basal tuber clusters. A similar scenario is also likely at the Santee Cooper reservoir system in South Carolina, where winter temperatures are cool enough for mature plants to become dormant, which would release the ramets from the stems of the parent plant.

Although we did not determine the specific environmental factors that stimulate sprouting, it is unlikely that the change in the light environment of the tuber cluster played a key role, as the ½-buried ramets exposed to light sprouted in the same time as those completely covered. The burial of

the propagules would likely result in a change in oxygen availability. Production of subterranean turions by hydrilla and burial of axillary turions of curlyleaf pondweed results in these propagules inhabiting a largely anoxic environment. Previous work suggests that removal of these propagules into an aerobic environment can stimulate rapid sprouting (Netherland 1997, Netherland et al. 2000). In the case of CFH, it is possible that moving the plant from an aerobic to a more anoxic environment stimulates sprouting. Although specific environmental factors that trigger sprouting need to be determined, this work demonstrates that simple disturbance did not influence sprouting. A combination of factors such as light availability/exposure, anoxic or hypoxic sediment conditions, and water temperature are all likely important in determining sprouting response of the CFH ramets. Another important factor that requires additional research relates to the longevity of ramets. There is no published data on how long these quiescent propagules can remain viable. Knowledge of those conditions that cause the ramets to sprout and initiate growth may allow managers to better coordinate management activities during sprouting events (plants actively growing) or to manipulate environments to either encourage or suppress sprouting.

Evaluation of the response of ramets to contact herbicides suggests that the metabolic activity of nonsprouted ramets that have separated from the parent plant was low or that the ramets were not actively growing at the time of treatment, as most contact herbicide treatments did not affect subsequent viability or growth from sprouted ramets. We noted an increase in posttreatment biomass compared with pretreatment biomass (0.06 ± 0.006 g dry weight) of 5× to 40× after various herbicide exposures. The increased activity of diquat over amine endothall is in contrast to observations on trials with mature plants (Willey et al. 2014). Additional studies evaluating the effects of systemic herbicides on ramets would be useful to evaluate the activity of those various physiological processes within the ramet and also to assess those herbicides as a management tool for reducing potential regrowth from benthic ramet banks. The observation in previous literature (Willey 2012) that noted ramets attached to parent plants were easily controlled, whereas in the present study, separated ramets were not easily controlled, suggests that activity of some physiological processes may be reduced after separation. Specifically, this was noticeable by the lack of activity by amine endothall in this study, although some processes like photosynthesis may be reduced to a maintenance mode (active when needed) to maintain sufficient energy reserves as suggested by diquat activity in this study (limited recovery, but not total plant death in all replicates). The results of these studies add new considerations into the physiology and management of an already difficult-to-control invasive species. Furthermore, these data highlight the importance of early-detection rapid-response management programs as well as herbicide application timing, development of alternative and integrated management strategies, and development of innovative herbicide use patterns.

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