

# Asexual reproduction and ramet sprouting of crested floatingheart (*Nymphoides cristata*)

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## ABSTRACT

Crested floatingheart is widely cultivated as a water garden ornamental and reproduces primarily via *ramets*—rhizome clusters produced at leaf–petiole junctions. Little is known regarding the effect of substrate composition and fertility on ramet production. Young plants were cultured in one of five artificial substrates (ranging from 100% sand to 100% organic matter) amended with one of four fertility rates (0 to 4 g fertilizer L<sup>-1</sup> of substrate), and ramet production was evaluated for 6 mo. Experiments were replicated four times, with four replicates of each treatment combination examined in each experiment. Fertility strongly influenced ramet production—highest ramet counts were produced by plants cultured with 4 g L<sup>-1</sup> of fertilizer—but planting season and substrate composition had lesser effects on ramet production. The effect of substrate composition and burial depth on ramet sprouting was also evaluated. Ramets were buried at one of five depths (ranging from placed on the surface to covered with 4 cm of substrate) for 8 wk in the previously described substrates. Experiments were replicated four times, with eight replicates of each treatment combination examined. Sprouting was not affected by planting season or substrate composition, but burial depth had a profound effect. Sprouting ranged from 60 to 20% in ramets that were placed on the substrate surface, half-buried, or barely covered with substrate but was negligible in ramets covered with 2 or 4 cm of substrates. These results suggest that ramet production by crested floatingheart is greatest in nutrient-rich conditions and that ramet sprouting may require light.

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

## INTRODUCTION

Crested floatingheart [*Nymphoides cristata* (Roxb.) Kuntze] is an Asian native in the buckbean (Menyanthaceae) family that was introduced to North America via the water garden industry (Burks 2002). The species is widely cultivated as an ornamental in water gardens, koi ponds, and other artificial aquatic systems. Crested floatingheart has a nymphaeid growth form; it is rooted in the substrate and produces floating cordate leaves on petioles that may be 2 m (6.56 ft)

or more in length, depending on water depth. Leaves are up to 25 cm (9.85 in) long and are bright green with a deep-red margin. The flowers of crested floatingheart measure approximately 2 cm across and have five white petals with a central ridge or crest.

The first report of the species' escape from cultivation in Florida occurred in the mid 1990s, when crested floatingheart was discovered in a Collier County lake in southwest Florida (Burks 2002). The species quickly moved into nearby lakes and canals through connected waterways and had invaded waters in Sarasota, FL—more than 160 km north of the Collier County population—within a few years of its initial discovery. Around the same time, crested floatingheart was vouchered on Florida's southeast coast in Palm Beach County as well, and by 2002, the species was documented in two lakes near Orlando in central Florida. Burks (2002) reported, "The present distribution suggests that the scattered populations likely represent multiple introductions, quite possibly from dumping of cultivated plants." Crested floatingheart has continued to expand its invaded range in state and out of state. Although crested floatingheart is of tropical provenance (i.e., India, Taiwan, Vietnam, and the southern provinces of China), it grows quite well in more temperate areas in the continental United States. For example, the species was found in an 8.09-ha cove in Lake Marion (Orangeburg County), SC, in August 2006. Despite droughts and freezing temperatures, crested floatingheart had expanded its coverage to more than 809.37 ha by 2009 (Westbrook and McCord 2010).

Although crested floatingheart sometimes produces seeds, most of its reproduction occurs through vegetative means (i.e., the production of daughter plants, tubers, and rhizomes) (Burks 2002; Willey and Langeland 2011), a strategy that is common in species of *Nymphoides*. (Sivarajan and Joseph 1993). Crested floatingheart spreads rapidly to form mats of overlapping floating leaves; in fact, untreated populations can expand to cover almost 3.64 ha in less than 1 mo (Burks 2002). This dense surface coverage results in significant light attenuation through the water column, reductions in dissolved oxygen by interfering with the air–water interface, and impeded water flow (Burks 2002; Willey and Langeland 2011).

The Florida Exotic Pest Plant Council (FLEPPC) first listed crested floatingheart as a *Category II plant*, defined as an invasive exotic that has increased in abundance or frequency but has not yet altered Florida plant communities, in 2005 (FLEPPC 2005). FLEPPC promoted the species to a *Category I plant*, defined as an invasive exotic that alters native plant communities by displacing native species, changing community structures or ecological functions, or

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hybridizing with natives, in 2009 (FLEPPC 2009). FLEPPC has no regulatory or legal standing in Florida, but the Florida Department of Agriculture and Consumer Services (FDACS) does; FDACS added crested floatingheart to the state 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. In addition, the United States Department of Agriculture, Animal and Plant Health Inspection Service (USDA APHIS 2012) has given crested floatingheart a “high risk” rating based on a number of factors, including its colonization potential and growth rate, and conservatively estimates that the potential invasion range of the species includes the entire southeastern United States.

It is clear that populations of crested floatingheart can cause a number of serious problems to humans and the ecosystem. The species interferes with navigation, recreation, flood control, and other water-based, anthropogenic activities throughout the southeastern United States and has grown to problematic levels in most areas in which it has become established (Westbrook and McCord 2010; Willey and Langeland 2011). Crested floatingheart is intensively managed to preserve or restore the function of invaded water bodies, and chemical control methods are being studied to optimize management of this species. Identifying products that provide effective control of crested floatingheart is a high priority, but little is known regarding the biology and reproductive capacity of this species. It is important to understand the conditions that foster growth and colonization of crested floatingheart because those factors can have a profound effect on management efforts. For example, rhizome clusters (referred to as *ramets* by researchers studying crested floatingheart) form at the junction of every leaf and petiole and represent a significant source of propagules to drive population expansion. However, there is scant information describing how vegetative reproductive capacity in this species is influenced by environmental conditions (e.g., substrate type and nutrient level). In addition, little is known regarding how environmental conditions affect sprouting and growth of ramets. Understanding the biology of ramet production, sprouting, and growth may allow resource managers to more accurately predict the optimal time to begin management efforts. Therefore, the objectives of this research were twofold: 1) to examine the effects of substrate composition and nutrients on ramet production; and 2) to evaluate sprouting of crested floatingheart ramets after burial at different depths in a variety of substrates.

## MATERIALS AND METHODS

All experiments took place at the University of Florida Institute of Food and Agricultural Sciences Fort Lauderdale Research and Education Center in Davie (Broward County), Florida (hereafter, FLREC). Trials were conducted in 1,000-L concrete vaults (surface area 1.66 m<sup>2</sup>), which were filled with well water, and water depth was maintained at 60 cm throughout the duration of the experiments. These experiments were repeated four times; Runs 1, 2, 3 and 4 were

initiated on 22 September 2014, 20 December 2014, 23 March 2015, and 24 June 2015, respectively.

### Ramet production

Plants of crested floatingheart were collected from stock material maintained in culture at FLREC. All plants selected for use in these experiments had well-developed root systems, four to seven leaves and petioles that were between 20 and 25 cm long. Each plant was planted in an 8-L dishpan filled with one of five substrate mixes that was amended with one of four nutrient levels. Substrate mixes were measured, volumetric combinations of coarse masonry sand and composted manure as follows: 1) 100% sand; 2) 75% sand + 25% manure; 3) 50% sand + 50% manure; 4) 25% sand + 75% manure; and 5) 100% manure. Nutrients were supplied in the form of controlled-release fertilizer<sup>1</sup> at 0, 1, 2, or 4 g of fertilizer L<sup>-1</sup> of substrate (0, 8, 16, or 32 g per dishpan, respectively). Four replicates were prepared for each combination of treatments during each run of this five by four factorial. Dishpans were placed in a completely randomized design in one of ten concrete vaults (described above), and plants were cultured for 6 mo after planting (MAP). Eight planted dishpans were housed in each vault, so dividers made from polyvinyl chloride (PVC) pipes and insect screening were constructed to ensure that ramets produced by a given plant remained within that plant's culture area. Ramets were counted twice per month for the duration of these experiments; once counts were recorded, all ramets were removed from the vault. Data were analyzed using the general linear model procedure in SAS software,<sup>2</sup> and treatment means were separated by least significant differences with  $P = 0.01$ .

### Ramet sprouting

Single, unrooted ramet clusters were collected from stock materials maintained at the University of Florida Center for Aquatic and Invasive Plants in Gainesville, FL, and transported to FLREC for Run 1; ramets used in Runs 2 through 4 were collected from stock materials maintained at FLREC. All leaves were removed from each ramet, and ramets were then sorted by size to ensure that clusters used in these experiments were uniform (2 to 2.5 cm diam). Ramets were planted in 2-L containers without holes, filled with one of the five substrates described above; however, all substrate mixes were amended with 1 g L<sup>-1</sup> (2 g container<sup>-1</sup>) of controlled-release fertilizer. Ramets were planted with the base at one of five depths as follows: 0 cm (cluster on the surface/no burial), 1 cm (ramet base 1 cm below the surface/clusters approximately half-buried), 2 cm (ramet base 2 cm below the surface/cluster barely covered), 4 cm (ramet base 4 cm below the surface/cluster covered with approximately 2 cm of substrate), and 6 cm (ramet base 6 cm below the surface/cluster covered with approximately 4 cm of substrate). Eight replicates were prepared for each substrate type-planting depth combination during each run of this five by five factorial. Planted containers were placed in one of eight concrete vaults (described above) and were arranged in a randomized block design, with replicate

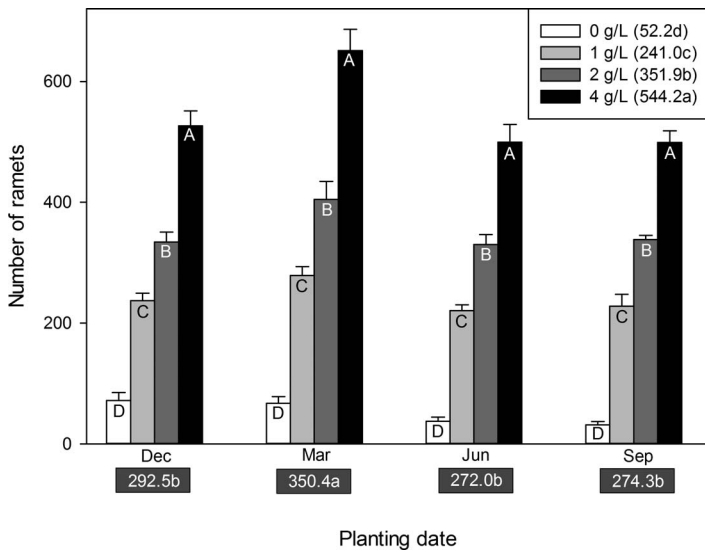


Figure 1. Effect of fertilizer rate and planting date on ramet production by crested floatingheart during a 6-mo culture period. Bars are the mean of 20 replicates, and error bars represent 1 standard error from the mean. Means of planting date as a main effect are provided in grey boxes under substrate descriptions; each boxed value is the mean of 80 replications, and means followed by the same lowercase letter are not different at  $P=0.01$ . Means of fertilizer rate as a main effect are provided in the legend; each value is the mean of 80 replications, and means followed by the same lowercase letter are not different at  $P=0.01$ . Differences among fertilizer rate means within substrate at  $P=0.01$  are indicated by uppercase letters. The interaction between fertilizer rate and planting date was not significant at  $P=0.01$ .

(vault) as the block. All treatments were monitored for sprouting 3 times  $\text{wk}^{-1}$  for 8 wk 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 version 9.3 software, and treatment means were separated by least significant differences with  $P=0.01$ .

## RESULTS AND DISCUSSION

### Ramet production

Although these experiments were not initially designed to examine planting date, preliminary analyses revealed that planting date had a significant effect on ramet production. Therefore, this discussion includes planting date as a main effect. All three main effects (nutrient level, substrate type, and planting date) had significant effects on ramet production by crested floatingheart, but nutrient level was clearly the most important factor in these experiments. Ramet production was consistently lowest in plants cultured with  $0 \text{ g L}^{-1}$  of fertilizer and greatest in plants cultured with  $4 \text{ g L}^{-1}$  of fertilizer (Figures 1 and 2).

Ramet production was influenced by planting date, with plants started in March producing the greatest number of ramets (Figures 1 and 3). Average day length (i.e., hours of sunlight) throughout a 6-mo run seems to have had a role in ramet production in these studies. For example, plants started in March were exposed to the longest average day length (13 h) and were most productive. Day length in

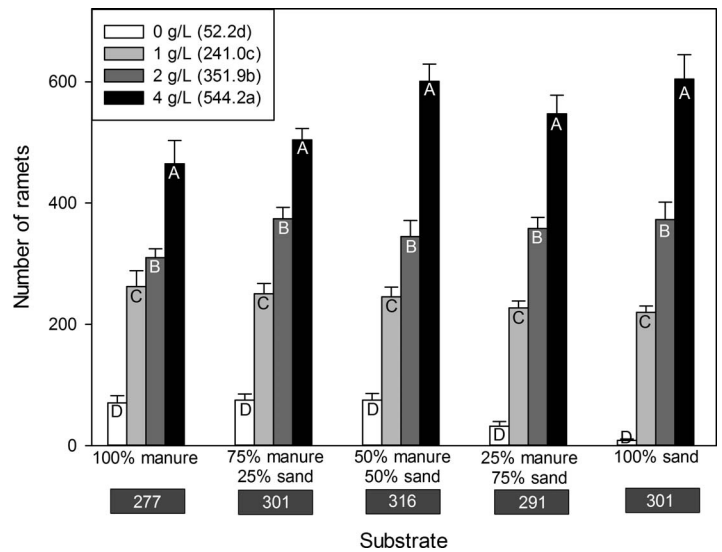


Figure 2. Effect of fertilizer rate and substrate on ramet production by crested floatingheart during a 6-mo culture period. Bars are the mean of 16 replicates, and error bars represent 1 standard error from the mean. Means of substrate as a main effect are provided in grey boxes under substrate descriptions; each boxed value is the mean of 64 replications, and means were not different at  $P=0.01$ . Means of fertilizer rate as a main effect are provided in the legend; each value is the mean of 80 replications, and means followed by the same lowercase letter are not different at  $P=0.01$ . Differences among fertilizer rate means within substrate at  $P=0.01$  are indicated by uppercase letters. The interaction between fertilizer rate and substrate type was not significant at  $P=0.01$ .

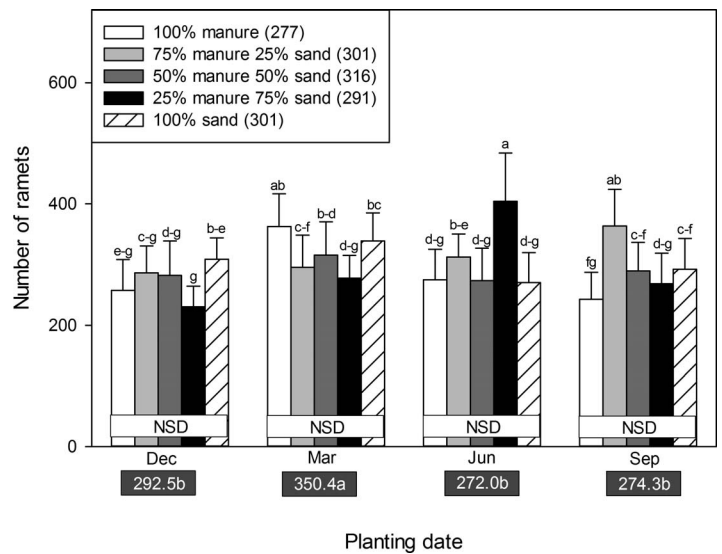


Figure 3. Effect of substrate and planting date on ramet production by crested floatingheart during a 6-mo culture period. Bars are the mean of 16 replicates, and error bars represent 1 standard error from the mean. Means of planting date as a main effect are provided in grey boxes under planting date descriptions; each boxed value is the mean of 80 replications, and means followed by the same lowercase letter are not different at  $P=0.01$ . Means of substrate as a main effect are provided in the legend; each value is the mean of 64 replications, and means were not different at  $P=0.01$ . There was no difference among substrate means within planting date at  $P=0.01$ . Planting date by substrate interactions coded with the same lowercase letter are not different at  $P=0.01$ .

March plantings was parabolic in nature, with the shortest durations at the beginning and end of the run, and the longest duration (13 h, 46 min) 3 MAP on 23 June. In addition, day length was never less than 12 h, 4 min, throughout the course of the March planting. In contrast, plants started in September were exposed to the shortest average day length (11 h, 20 min) and were significantly less productive than those planted in March. Day length in September plantings was also parabolic in nature, but the relationship was inverted compared with March plantings, with the longest durations at the beginning and end of the run, and the shortest duration (10 h, 30 min) 3 MAP on 23 December. Day length was never greater than 12 h, 14 min, throughout the course of the March planting. The day length for plants started in December averaged 12 h, 11 min, and daily values increased in a linear fashion from 10 h, 30 min, at the time of planting to 13 h, 46 min at 6 MAP, whereas the day length of plants started in June averaged 12 h, 5 min, with a linear decrease in day length from 13 h, 46 min, at the time of planting to 10 h, 30 min, at 6 MAP. Because these experiments were not designed to evaluate planting date as a main effect, other environmental factors, such as air and water temperature, were not recorded, and their effect on these studies is unknown. Although the effect of planting date was significant, it is important to note that an average of approximately 275 ramets were produced by single plants cultured during the least-productive 6-mo period evaluated in these experiments.

The effect of substrate, although significant in some analyses, was less profound than the effects of nutrient level and planting date. Plants cultured in substrate with 0% sand (e.g., 100% composted manure) produced fewer ramets than plants grown in other substrate mixes through 3 MAP, although there was no difference between 0% sand and 25% sand treatments 1 and 3 MAP (data not shown). The effect of substrate was not significant in later evaluations and did not contribute as a main factor to cumulative ramet production (Figures 2 and 3).

There were a number of interactions between the main effects of nutrient level, substrate type, and planting date, but the three-way interaction among the effects was significant only in a few evaluation periods (Table 1). The most influential interaction in these experiments occurred between planting date and substrate type, but that interaction was only significant in counts recorded 1, 2, and 6 MAP (Table 1). Plants that were started in June and cultured in 75% sand produced more ramets than any other starting date–substrate type combination at 1 MAP. These plants were still most productive at 2 MAP, but there was no difference between these plants and plants started in June and grown in substrate with 0 or 100% sand, those started in March and grown in 25% sand, and those planted in September and cultured in substrate with 25 or 50% sand. Ramet production at 6 MAP was greatest in plants started in September and cultured in substrate with 25% sand, those started in March and grown in substrate with 0% sand, and those planted in June and grown in substrate with 75% sand (Figure 3). A significant interaction between substrate type and nutrient level was detected at 1 MAP (Table 2), but that interaction appears to be due to plants grown in 100% sand

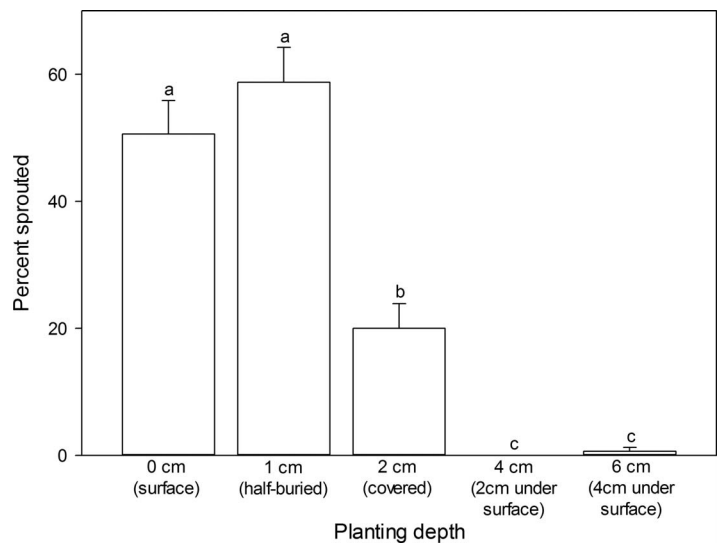


Figure 4. Effect of planting depth on ramet sprouting in crested floatingheart over an 8-wk culture period. Bars are the means of 20 replicates, and error bars represent 1 standard error from the mean. Treatments coded with the same letter are not different at  $P = 0.01$ .

amended with 2 g L<sup>-1</sup> of fertilizer, which produced fewer ramets than expected and was not evident in later evaluation periods. Finally, the interaction between planting date and nutrient level had a significant effect on ramet production at 2 and 3 MAP (Table 1). Plants started in June or September and fertilized with 4 g L<sup>-1</sup> of fertilizer produced the greatest number of ramets at 2 MAP, whereas those planted in March or June and fertilized with 4 g L<sup>-1</sup> of fertilizer produced the greatest number of ramets at 3 MAP. No other interactions were detected in these experiments.

### Ramet sprouting

The main effects of substrate type and planting date did not influence ramet sprouting, and there were no significant interactions between main effects. In contrast, the effect of planting depth was strong and had a substantial effect on sprouting of ramets of crested floatingheart. Ramets that were planted at 0 or 1 cm (cluster on the surface/no burial or approximately half-buried, respectively) sprouted at the highest rates, whereas sprouting occurred in only 1 of the 80 ramets that were planted 4 or 6 cm under the surface of the substrate (clusters covered with approximately 2 or 4 cm of substrate, respectively) (Figure 4).

A total of 800 ramets were evaluated in these experiments, and 216 of these sprouted during the 8-wk culture periods. Virtually all sprouting occurred within 3 WAP, and new sprouting was observed beyond 4 WAP only in Runs 3 and 4 (started in March and June, respectively). Most (191 of 216) of the sprouted ramets topped out and produced floating leaves by 6 WAP, and only 10 of the 216 sprouted ramets failed to top out by 8 WAP. These results suggest that ramets that are uncovered, partially buried, or barely covered by substrate have the potential to sprout quickly and to produce leaves that reach the surface of the water. In addition, because 94% of sprouted ramets topped out by 8

TABLE 1. SUMMARY STATISTICS FOR RAMET PRODUCTION BY CRESTED FLOATINGHEART DURING A SINGLE MONTH.<sup>1,2</sup>

Independent variable	1 MAP <sup>a</sup>	2 MAP	3 MAP	4 MAP	5 MAP	6 MAP
Planting date	$F_{3,240} = \mathbf{65.67}$ $p < 0.0001$	$F_{3,240} = 73.14$ $p < 0.0001$	$F_{3,240} = 44.32$ $p < 0.0001$	$F_{3,240} = 55.38$ $p < 0.0001$	$F_{3,240} = 290.11$ $p < 0.0001$	$F_{3,240} = 208.54$ $p < 0.0001$
Nutrient level	$F_{3,240} = 26.18$ $p < 0.0001$	$F_{3,240} = \mathbf{230.15}$ $p < 0.0001$	$F_{3,240} = \mathbf{377.91}$ $p < 0.0001$	$F_{3,240} = \mathbf{315.83}$ $p < 0.0001$	$F_{3,240} = \mathbf{366.01}$ $p < 0.0001$	$F_{3,240} = \mathbf{252.88}$ $p < 0.0001$
Substrate	$F_{4,240} = 6.97$ $p < 0.0001$	$F_{4,240} = 8.30$ $p < 0.0001$	$F_{4,240} = 2.10$ $p = 0.0811$	$F_{4,240} = 3.17$ $p = 0.0147$	$F_{4,240} = 9.10$ $p < 0.0001$	$F_{4,240} = 6.63$ $p < 0.0001$
Planting date x substrate	$F_{12,240} = 2.96$ $p = 0.0007$	$F_{12,240} = 3.08$ $p = 0.0004$	$F_{12,240} = 0.96$ $p = 0.4872$	$F_{12,240} = 0.49$ $p = 0.9188$	$F_{12,240} = 5.65$ $p < 0.0001$	$F_{12,240} = 3.53$ $p < 0.0001$
Planting date x nutrient level	$F_{9,240} = 5.03$ $p < 0.0001$	$F_{9,240} = 12.11$ $p < 0.0001$	$F_{9,240} = 7.45$ $p < 0.0001$	$F_{9,240} = 4.26$ $p < 0.0001$	$F_{9,240} = 23.46$ $p < 0.0001$	$F_{9,240} = 25.67$ $p < 0.0001$
Nutrient level x substrate	$F_{12,240} = 2.15$ $p = 0.0146$	$F_{12,240} = 4.25$ $p < 0.0001$	$F_{12,240} = 2.84$ $p = 0.0012$	$F_{12,240} = 1.99$ $p < 0.0257$	$F_{12,240} = 1.12$ $p = 0.3467$	$F_{12,240} = 2.00$ $p = 0.0253$
Date x nutrient x substrate	$F_{36,240} = 1.59$ $p = 0.0229$	$F_{36,240} = 2.18$ $p = 0.0003$	$F_{36,240} = 1.10$ $p = 0.3260$	$F_{36,240} = 1.60$ $p = 0.0215$	$F_{36,240} = 1.53$ $p = 0.0344$	$F_{36,240} = 1.75$ $p = 0.0076$

<sup>a</sup>MAP = months after planting

<sup>1</sup>Shaded cells indicate no significant difference at  $P = 0.01$ .

<sup>2</sup>Bold type indicates the most important variable within a given evaluation period.

WAP, it seems likely that most sprouted ramets grow into full plants within 2 mo of sprouting.

These experiments revealed that substrate composition had little effect on ramet production and sprouting of crested floatingheart. The species is highly productive year-round in southern Florida, even under nutrient-poor conditions. Single plants grown without fertilizer produced an average of > 50 ramets during a 6-mo culture period, whereas those fertilized with 4 g of controlled-release fertilizer L<sup>-1</sup> of substrate yielded an average of 544 ramets during the same time period. These studies also revealed that uncovered or barely covered ramets sprouted at much higher rates than those covered with ≥ 2 cm of substrate, which suggests that light has a beneficial effect on ramet sprouting of crested floatingheart.

Taken together, these results paint a troubling picture for bodies of water that are at risk for invasion by crested floatingheart. For example, assume that an aquatic system has a moderate level of substrate fertility—e.g., equivalent to this experiment's 2 g of controlled-release fertilizer L<sup>-1</sup> of substrate treatment. These experiments showed that a single plant growing under those conditions would be expected to produce around 350 ramets during a 6-mo period. If those ramets were deposited on the surface of the substrate,

around 40% would be expected to sprout, which would create a population of around 140 plants. If each of those first-generation clonal progeny performed in a manner similar to that of the original parent plant, the new population would collectively yield around 49,000 ramets of their own over a 6-mo period—a time frame equivalent to the 1-year anniversary of the introduction of the first single plant into the system. If that level of production continued for several generations, theoretically, one could expect

- 19,600 second-generation clonal plants (40% sprouting of 49,000 ramets), collectively producing 6,860,000 ramets;
- 2,744,000 third-generation clonal plants (40% sprouting of 6,860,000 ramets), collectively producing 960,400,000 ramets.

Clearly, those numbers represent a best-case (for the plants) or worst-case (for managers) scenario. It is unlikely that any aquatic system would be able to supply the resources needed to sustain a population of nearly a billion plants of crested floatingheart, and the actual invasion would be significantly smaller. However, if only 1% of that projection were to occur, the resulting population would still comprise almost 10 million plants. This makes clear the

TABLE 2. SUMMARY STATISTICS FOR CUMULATIVE RAMET PRODUCTION BY CRESTED FLOATINGHEART THROUGHOUT THE 6-MONTH CULTURE PERIOD.

Independent variable	1 MAP <sup>a</sup>	2 MAP	3 MAP	4 MAP	5 MAP	6 MAP
Planting date	$F_{3,240} = 65.67$ $p < 0.0001$	$F_{3,240} = 90.27$ $p < 0.0001$	$F_{3,240} = 77.40$ $p < 0.0001$	$F_{3,240} = 42.99$ $p < 0.0001$	$F_{3,240} = 27.22$ $p < 0.0001$	$F_{3,240} = 19.34$ $p < 0.0001$
Nutrient level	$F_{3,240} = 26.18$ $p < 0.0001$	$F_{3,240} = 201.88$ $p < 0.0001$	$F_{3,240} = 379.29$ $p < 0.0001$	$F_{3,240} = 502.58$ $p < 0.0001$	$F_{3,240} = 567.40$ $p < 0.0001$	$F_{3,240} = 613.33$ $p < 0.0001$
Substrate	$F_{4,240} = 6.97$ $p < 0.0001$	$F_{4,240} = 10.87$ $p < 0.0001$	$F_{4,240} = 6.04$ $p = 0.0001$	$F_{4,240} = 4.03$ $p = 0.0025$	$F_{4,240} = 2.78$ $p = 0.0274$	$F_{4,240} = 2.48$ $p = 0.0449$
Planting date x substrate	$F_{12,240} = 2.96$ $p = 0.0007$	$F_{12,240} = 3.92$ $p < 0.0001$	$F_{12,240} = 2.64$ $p = 0.0025$	$F_{12,240} = 2.43$ $p = 0.0054$	$F_{12,240} = 2.59$ $p = 0.0029$	$F_{12,240} = 2.78$ $p = 0.0014$
Planting date x nutrient level	$F_{9,240} = 5.03$ $p < 0.0001$	$F_{9,240} = 12.24$ $p < 0.0001$	$F_{9,240} = 9.99$ $p < 0.0001$	$F_{9,240} = 6.40$ $p < 0.0001$	$F_{9,240} = 3.94$ $p = 0.0001$	$F_{9,240} = 2.70$ $p = 0.0053$
Nutrient level x substrate	$F_{12,240} = 2.15$ $p = 0.0146$	$F_{12,240} = 4.46$ $p < 0.0001$	$F_{12,240} = 3.99$ $p < 0.0001$	$F_{12,240} = 4.54$ $p < 0.0001$	$F_{12,240} = 4.37$ $p < 0.0001$	$F_{12,240} = 4.57$ $p < 0.0001$
Date x nutrient x substrate	$F_{36,240} = 1.59$ $p = 0.0229$	$F_{36,240} = 2.18$ $p = 0.0003$	$F_{36,240} = 1.71$ $p = 0.0103$	$F_{36,240} = 1.83$ $p = 0.0042$	$F_{36,240} = 1.64$ $p = 0.0168$	$F_{36,240} = 1.61$ $p = 0.0200$

<sup>a</sup>MAP = months after planting.

<sup>1</sup>Shaded cells indicate no significant difference at  $P = 0.01$ .

<sup>2</sup>Bold type indicates the most important variable within a given evaluation period.

need to prevent introductions of crested floatingheart and to employ early detection–rapid response protocols to eradicate nascent invasions of this noxious weed before it can establish a foothold in susceptible aquatic systems.

### SOURCE OF MATERIALS

<sup>1</sup>Osmocote Plus 15–9–12 (N–P–K), Everris, Dublin, OH 43017.

<sup>2</sup>SAS Software Version 9.3, SAS Institute, Cary, NC 27513.

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