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Note

Importance of size and nitrogen content in establishment of Brazilian egeria (*Egeria densa*) fragments

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INTRODUCTION

The production of vegetative propagules (stem fragments, subterranean turions, axillary turions, stolons, and rhizomes) is important for perennation and dispersal of many aquatic macrophytes (Sculthorpe 1967, Capers 2003). The relative importance of vegetative structures in plant dispersal varies among species, and the distance vegetative propagules are dispersed is a function of propagule type (Spencer and Ksander 1991) and mechanism of dispersal (Henry et al. 1996, Riis and Sand-Jensen 2006).

Brazilian egeria (*Egeria densa* Planch.) is a submersed, dioecious perennial. Only male plants are found in the United States (Cook and Urmi-König 1984), where it is common in all but the upper midwestern states (USDA, NRCS 2015). Lack of sexual reproduction has resulted in an extremely low genetic diversity (Carter and Sytsma 2001). Dense growth of Brazilian egeria, which disrupts recreational activities and navigation, tends to form monotypic stands. Even in its native range of Brazil, Brazilian egeria is a nuisance in hydropower reservoirs (Thomaz et al. 2006, Mori et al. 2012). Brazilian egeria produces "bud nodes" (King 1943) or "double nodes" (Jacobs 1946, Getsinger 1982, Cook and Urmi-König 1984) every 10 to 11 internodes (Cook and Urmi-König 1984; T. G. Pennington, unpub. data). Because branches grow from these specialized meristematic regions, their frequency is critical to the morphology, dispersal, biomass production, and overwintering success of the plant.

We conducted a greenhouse experiment to improve our understanding of stem fragment establishment of Brazilian egeria. We evaluated whether fragment size and tissue nitrogen status influenced fragment establishment success.

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MATERIALS AND METHODS

Fragment establishment experiments were conducted between 13 October 2005 and 16 December 2005 under controlled greenhouse conditions on the Portland State University campus (Portland, OR). Brazilian egeria plant material was collected from the Chehalis River, WA, and was held for 1 wk in tap water under ambient greenhouse conditions before experimental use. All water used in the experiments was carbon-filtered (to remove chlorine), municipal water from the City of Portland. The city water supply, which was otherwise unfiltered, was supplied by Bull Run Reservoir. Bull Run Reservoir is oligotrophic reservoir with nitrate–nitrogen concentration ranging from 0.02 to 0.06 ppmv and approximately 34 mEq L^{-1} alkalinity.

Experiment 1: Effects of dissolved nutrients on fragment nutrient status

Two lengths of Brazilian egeria apical stem fragments were incubated under two nutrient regimes. Short fragments were 8 to 12 cm long, and long fragments were 18 to 22 long. Fragments were cut such that one double node was at the cut end of the stem to ensure the potential for rooting, and the fragment apex was characterized by healthy-looking, meristematic tissue.

Fragments were incubated in 0.03-m^3 containers (22 × 22 × 69 cm) in tap water (0×) or tap water amended with nutrients to produce one-fourth strength Hoagland's solution (1/4×) (Hoagland and Snyder 1933). $NaHCO_3$ was also added to increase the inorganic carbon supply for photosynthesis (Smart and Barko 1985). Each treatment was replicated three times. Temperature of the incubation medium was maintained near 18 C by a circulating water bath attached to a heater/chiller unit,[1] and aeration was provided to each incubation container.[2]

Initially, and after 2 wk of incubation, fragments from each treatment were dried at 70 C to constant weight and ground to pass through a 40-mesh screen. Tissue nitrogen concentration was measured using a PerkinElmer Instruments 2400 Series II CHN Analyzer[3] with acetanilide as the standard. Differences in the effects of nutrient media (0× or 1/4×) and incubation time (initial and 2 wk) on stem N

content (mg N g dry wt^{-1} [dw]) were determined using independent *t* tests on short stems and long stems. Significant differences were determined at $\alpha < 0.05$.

Experiment 2: Effects of plant N content and fragment length on establishment

At the same time that stems were removed for nitrogen analysis in Experiment 1, 18 fragments from each treatment were randomly selected for use in Experiment 2. We had anticipated that the incubations in Experiment 1 would result in stems of differing N tissue concentration. Nine fragments were either planted approximately 4 cm deep or were floated above a 175-cm^3 pot filled with modified UC Mix (Spencer and Anderson 1986). Pots were placed into a 1,000-L^3 mesocosm containing approximately 45 cm of carbon-filtered tap water, amended to increase alkalinity, as previously described. Each 175-cm^3 pot was encircled with a 60-cm-high cage made of plastic, 1-cm^2 construction mesh to allow water circulation but to maintain fragment position over the container. Hobo temperature loggers in submersible cases[4] were used to monitor temperature every 90 min. Water temperature in the incubation containers averaged 18.1 ± 0.06 C, and alkalinity averaged 0.5 ± 0.01 mEq L^{-1}. Light (approximately 600 µmol m^{-2} s^{-1} at the water surface) was provided by four, 96-W, compact fluorescent light bulbs (6,700 K) on 10 h : 14 h (light : dark) cycle. Experiment 2 was harvested after 11 wk of incubation, and the lengths of stems and roots were measured and dried at 70 C to constant weight. Tissue N concentration was measured as previously described.

Significant differences in the N concentration between initial and final fragment lengths and planted or floated status within size classes were determined using independent *t* tests ($\alpha < 0.05$). Changes in stem length between floated or planted short stems and between floated or planted long stems were determined with *t* tests at $\alpha < 0.05$. Differences in the number of adventitious roots (per centimeter of stem), length of adventitious roots (in centimeters), root growth (in centimeters per day), and root dry weight (per centimeter of stem) between floated and planted short stems and between floated and planted long stems were also determined using *t* tests at $\alpha < 0.05$. The statistical information will be referenced in the text as t(n), the number of data points used in the t-test, followed by the corresponding P-value. All statistical analyses were performed with SPSS 13.0 software.[5]

RESULTS AND DISCUSSION

Experiment 1: Effects of dissolved nutrients on fragment nutrient status

Initial tissue N concentration was 36.6 ± 1.4 mg N g dw^{-1} in short-stem fragments and 38.0 ± 2.5 mg N g dw^{-1} in long stems (3.6% ± 0.14 and 3.8% ± 0.25, respectively) with no significant differences between lengths; $t(2)$, $P = 4.30$. Gerloff and Krombholz (1966) reported critical N concentrations in several submersed angiosperms at approximately 1.3%, suggesting that our initial fragments contained tissue concentrations of N well above critical concentrations for growth and that additional N uptake was unnecessary for plant health.

After 2 wk of incubation, short stems contained significantly higher tissue N concentrations (38.8 ± 0.6 mg N g dw^{-1}) than long stems did (36.0 ± 0.5 mg N g dw^{-1}); $t(34) = 2.03$, $P = 0.0009$. This difference was likely due to concentrations of N in Brazilian egeria tips (Pennington and Sytsma 2009), which comprised a greater proportion of the stem biomass in short stems than it did in long stems. The incubation in 1/4× Hoagland's did not result in a difference in tissue N concentration; therefore, fragments from the high- and low-nutrient treatments were combined for use in Experiment 2.

Experiment 2: Effects of fragment length on establishment

After 11 wk of growth, there were no significant differences in tissue N concentration between planted and floated or between short (38.2 ± 5.58 mg N g dw^{-1}) and long stems (40.29 ± 21.1 mg N g dw^{-1}); $t(17) = 2.11$, $P = 0.11$. Thus, fragments in this experiment were unlikely stressed for N, even after floating for a combined 13 wk (11 wk exclusively in low-nutrient tap water).

Duration of floating time for the aquatic plant stem fragments is an important determinant of dispersal ability. None of the floating stems in Experiment 2 sank to the sediment surface during the 11-wk incubation period. Stems floated on the water surface without visible signs of stress, and all but three stems, regardless of initial length, produced adventitious roots. One-half of the floated Brazilian egeria stems established root contact with the sediment. Adventitious root growth on floating fragments thus prepares Brazilian egeria stem fragments for rapid establishment when they are transported into shallow-water habitats and expands the range of depths in which fragments can become established.

Like King (1943), we observed natural abscission points above double nodes in field-collected Brazilian egeria. This suggests that naturally produced stem fragments contain a terminal double node and may be more likely to resist flooding of the aerenchyma in the internodes and to retain buoyancy. The buoyancy characteristics of aquatic plant fragment are species specific. Like Brazilian egeria, marestail (*Hippuris vulgaris* L.) fragments floated for up to 10 wk in the laboratory, but common elodea (*Elodea canadensis* Michx.) fragments sank within hours (Barrat-Segretain et al. 1998). Riis and Sand-Jensen (2006) found similar results for common elodea under field conditions. The differences among species may relate to the structure of nodal diaphragms and to resistance to flooding (Soukup et al. 2000, Yang et al. 2011).

Fragment length (main stem plus branches) increased in both floated and planted fragments during the 11-wk study period. There was no significant difference in growth between floated (32. ± 2.9 cm) and planted short fragments (36.1 ± 2.3 cm); $t(31) = 2.03$, $P = 0.41$. However, floated long fragments (52.8 ± 3.7 cm) grew more than planted long fragments did (45.2 ± 2.7 cm); $t(33) = 2.03$, $P = 0.05$. Floated fragments grew at the surface and experienced greater light

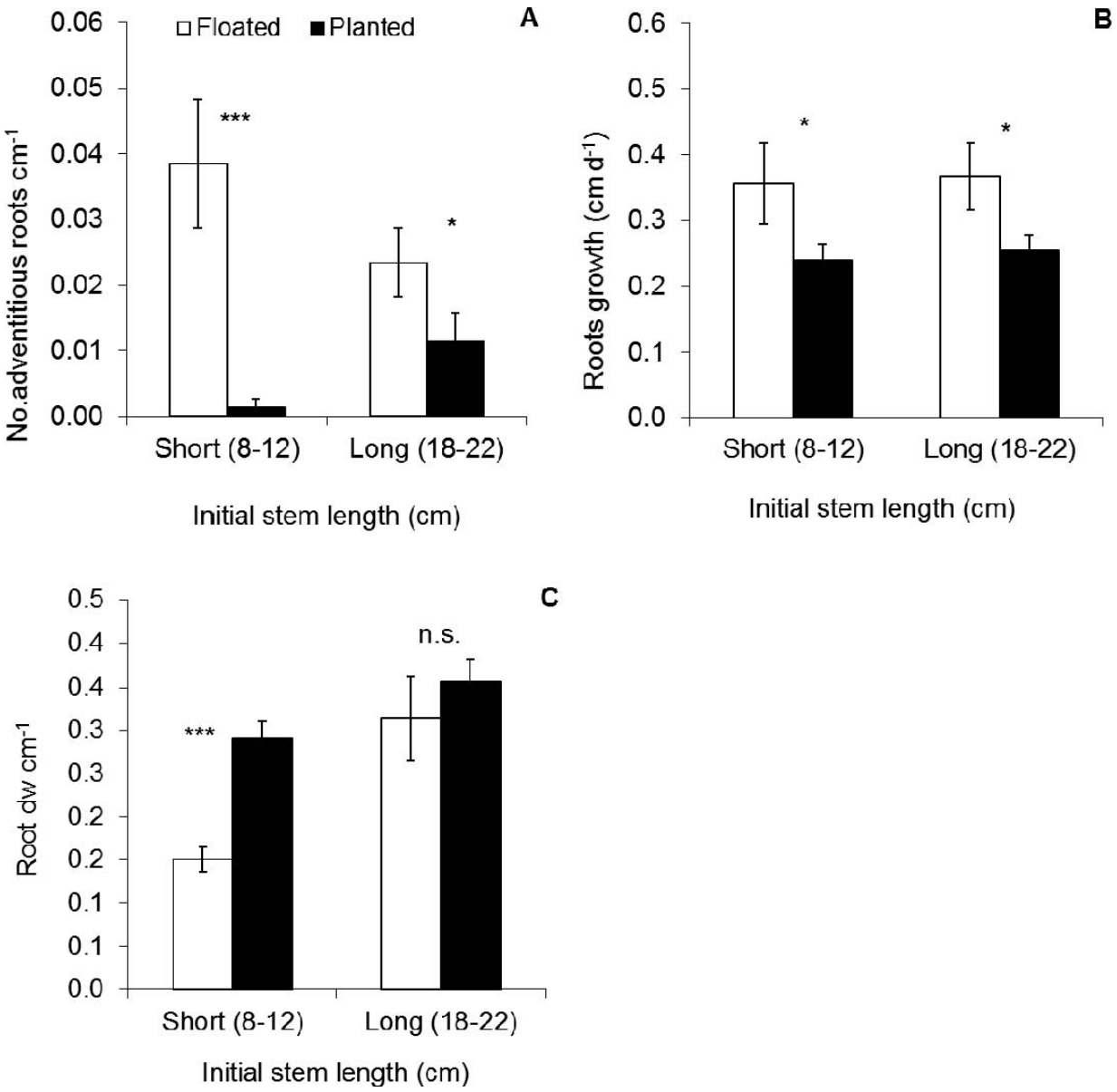

Figure 1. (A) Number of adventitious roots per centimeter of stem. (B) Root growth rate (centimeters per day). (C) Root dry weight per centimeter for short and long stems that were either floated (white) or planted (black) above sediment. Values indicate mean ± SE. Significant differences between floated and planted stems within lengths were determined by t test, where $***\alpha < 0.0001$, $*\alpha < 0.1$, and n.s. = not significant.

intensity than did planted fragments, which may have contributed to the greater growth rate of floated fragments. It is unclear why short, floated stem fragments did not grow appreciably more than short, planted stems; however, less overall photosynthetic capacity is suspected.

The characteristics of roots were markedly different among treatments, particularly for floated vs. planted fragments. The number of adventitious roots was significantly greater on floated stems compared with planted stems for both stem lengths (Figure 1A). Sediment roots on planted fragments originated primarily from the terminal double node. Only on rare occasions did adventitious roots form on the double nodes above the planted fragment that was in the water column. Floated fragments formed roots at the basal double node, and more commonly, they formed adventitious roots at double nodes higher up on the fragment. Adventitious roots that formed on floating fragments, regardless of fragment length, grew more rapidly (0.36 ± 0.06 cm d^{-1}) than did roots that formed in the sediment on planted fragments (0.25 ± 0.02 cm d^{-1}); $t(70) = 1.99$, $P = 0.009$ (Figure 1B) and were finer than the roots were that developed in the sediment on planted stems (Figure 1C). Differences in morphology between water column roots of aquatic plants and roots that form in the sediment may be due to accumulation of suberin as an apoplastic barrier to oxygen loss and toxins in anoxic sediments (Watanabe et al. 2013) and to mechanical impedance of root elongation by the sediment matrix (Bengough et al. 2006).

Seventy-eight percent of long fragments and 22% of the short fragments produced adventitious roots that reached the sediment. Because adventitious root formation is important for fragment establishment and double node frequency (hence frequency of potential root primordia) appears to be a fixed, genotypic characteristic of Brazilian egeria, long fragments should be able to produce more adventitious roots to establish more successfully than do short fragments.

In summary, nutrient conditions in the incubation media evaluated in this study did not influence subsequent nutrient concentrations in the stem fragments. Fragment length, however, contributed to greater growth rates in long, floated stems compared with planted stems. Root growth and conditions were significantly different between floated and planted stems, which subsequently influenced fragment establishment. Root formation is important in the establishment of aquatic plant fragments and their dispersal (King 1943; Kimbel 1982; Sytsma and Anderson 1993). Adventitious root formation is regulated by mother-plant nutrition and carbohydrate status and complex hormone interactions (Rasmussen et al. 2012, da Costa et al. 2013, Sun et al. 2015). A better understanding of the physiology of root formation in aquatic plant fragments could aid in development of chemical treatments that reduce fragment establishment success.

SOURCES OF MATERIALS

[1]Heater/chiller unit, Pacific Coast Imports, P.O. Box 378, Woodburn, OR 97071.

[2]Sweetwater Linear II Air Pump, Pentair Aquatic Eco-systems, 2395 Apopka Boulevard, Apopka, FL 32703.

[3]PerkinElmer 2400 Series II CHN Analyzer, PerkinElmer Instruments, 7812 Kempwood Drive, Houston, TX 77055.

[4]HOBO Water Temp Pro v2, Onset Computer Corporation, 470 MacArthur Boulevard, Bourne, MA 02532.

[5]SPSS 13.0 software, IBM Corporation, 1 New Orchard Rd, Armonk, NY 20504-1722.

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