Assessing vegetative growth potential of exotic *Rotala rotundifolia* (Roxb.) Koehne (roundleaf toothcup), in comparison with *Alternanthera philoxeroides* (Mart.) Griseb. (alligator weed), a known successful invader

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Summary

Rotala rotundifolia (Roxb.) Koehne (Lythraceae) is native to south and southeast Asia, but has been recorded in the United States in canals of south Florida and one pond in Alabama. The potential for further invasion of this species in the US was evaluated by comparing growth of *Rotala* with *Alternanthera philoxeroides* (Mart.) Griseb. (Amaranthaceae), a widespread exotic invasive aquatic plant in the southeastern US and other regions of the world. Results of growth chamber experiments demonstrated that *Rotala* was as capable of vegetative regeneration as was *Alternanthera*. In fact, *Rotala* exhibited a higher shoot regenerative capacity than did *Alternanthera*. *Rotala* consistently demonstrated higher shoot regrowth across three temperature regimes, from 21ºC to 30ºC, while the observed advantage in *Alternanthera* root regrowth was observed only at the lowest temperature. These experimental results, in combination with observations from field and greenhouse-grown *Rotala rotundifolia*, indicate that this species may pose a serious invasion risk in wetlands of the southeastern US.

Introduction

Rotala rotundifolia (roundleaf toothcup) is native to south and southeast Asia from India to Japan (Cook, 1979). In its native range, this *Rotala* species is reported to occur primarily in mountainous areas and at altitudes of more than 2600m. However, *Rotala* has been recorded in two areas of the United States: canals in southern Florida (USGS FISC, 2006) and a single pond on the University of Alabama campus in Tuscaloosa, Alabama (Reese and Haynes, 2002). Despite its presently limited distribution, this exotic species is considered a possible threat as an invasive species in southeastern US wetlands, especially in southern Florida (Jacono and Vandiver 2007). Furthermore, the Alabama pond in which it was detected in 2001 was drained during 2005, with no precautions taken to prevent the spread of this species into the immediately adjacent Black Warrior River. It subsequently was observed along the stream connecting the pond with the Black Warrior, and all individuals observed were removed (R. G. Westbrooks and V. Maddox, personal communications).

During August following drainage of the pond, *Rotala* was observed persisting in patches of gravel throughout the former pond shoreline, including a southward facing slope of the former shoreline, more than 15m from any remaining water. In May 2004, *Rotala* was observed growing in a dense mat on the pond surface, extending more than one meter from shore around most of the pond perimeter. Thus, this species has the potential to grow in a broad range of conditions (aquatic mats to sun-exposed gravel) and appears capable of persisting in US climates from roughly 25ºN to 33.25ºN latitude. Furthermore, *Rotala* also is capable of producing a heterophyllous growth form (different form for aerial vs. submersed leaves) in greenhouse tanks used to grow plants for the present work, and thus seems highly capable of persisting even in submersed conditions (also documented by Burks et al., 2003). The present study aimed to evaluate, in controlled laboratory conditions, the relative growth potential of *Rotala* in comparison with a presently widespread non-native plant species in the southeastern United States.

Methods

Plant materials

Growth of *Rotala rotundifolia* was compared with specimens of *Alternanthera philoxeroides* (alligator weed) collected in September 2005 from a backwater of the Tennessee-Tombigbee Waterway (US Army Corps of Engineers) in east-central Mississippi, near State Highway 50 (33.572ºN, 88.450ºW). Stock plants were maintained in hydroponic culture (tap water amended with nutrient solution as described below) in the laboratory prior to and during the experiments. *Rotala* was collected in August 2005 on the campus of the University of Alabama, in Tuscaloosa from the site identified by Reese and Haynes (2002; 33.218ºN, 87.547ºW). Plants of *Rotala* initially were transplanted into tanks fed by well water in a greenhouse at the R.R. Foil Plant Research Facility (Mississippi State University), but cuttings were transferred into the laboratory at least one week prior to use in experiments, in an effort to expose both test species to similar conditions just prior to experimental use.

Growth assays

Fragments consisting of one or of two nodes were used to compare vegetative regeneration capacity of *Rotala* with that of *Alternanthera*. These fragments included either one or two nodes, along with the associated proximal internode segment(s). The internodes included in these assays were those growing proximally from the targeted nodes because it was found in earlier assays that the distal internode senesced during similar growth assays. Plant fragments were incubated in plastic containers (Rubbermaid Takealongs®), in 500 mL plant growth media consisting of commercial plant food (Sam's Choice™ Deep Feeding® all-purpose plant food; 15% total nitrogen, 30% P_2O_5, 15% soluble K_2O) supplemented with $MgSO_4$ (because the plant food did not contain a source of magnesium). This solution was mixed at a rate of 7g all-purpose plant food and 420mg $MgSO_4$ in 14L ultrafiltered water (Millipore Synergy® 185 filtration system). When substantial water loss was noted, ultrafiltered water was used to refill to the initial fill line.

Two plant fragments were placed in each container, for a total of eight fragments per species per fragment size; these were incubated in a Lab-Line Biotronette® Mark II plant growth chamber (model 844). A full set of these assays was performed at each of three temperature regimes: 23ºC (day)/21ºC (night), 27/25ºC, and 30/28ºC. Light levels, as recorded on each shelf of the growth chamber on the first and last day of each run of these assays, ranged from a minimum of 41±0.7 µmol m^{-2} s^{-1} (lowest shelf) to a maximum of 78±2 µmol m^{-2} s^{-1} (upper shelves) within the growth chamber over the three sets of experiments. Plants were maintained on a 15h light: 9h dark photoperiod for 12 days during each assay, and containers were allocated randomly to two or three shelves within the growth chamber and rotated within each shelf periodically during the 12-d growth assay to minimize effects attributable to location within the chamber.

Each plant fragment was blotted dry and weighed at the beginning and end of each 12d growth period. Initial fragments consisted only of one or two nodes, internodes, and associated leaves. Any roots present were clipped at the beginning of the assay so that new root growth could be monitored. Because all new leaves/shoots were produced from axils, and no terminal shoot sections were used, all new growth during these assays was readily observed. After 12 days of growth, all new shoots, nodes, and roots were counted prior to blotting and reweighing the fragments.

Parameters used for comparison between the species and initial size of plant fragments (one vs. two nodes and internodes) were: number of new shoots, number of new nodes, and number of new roots produced, along with relative growth rates. Relative growth rates were calculated for the full 12-d growth period as: [natural log(final mass) – natural log(initial mass)] per 7d (units $g \cdot g^{-1} \cdot week^{-1}$). Data were analyzed within each of the three experiments (temperature regimes) by ANOVA using SYSTAT (version 11.0; Systat Software, Inc., Richmond, CA) after examination for normality using the Kolmogorov-Smirnov test. All variables met the normality assumption based on this test (all $P>0.05$), and an $\alpha = 0.05$ was used to determine statistical significance in these analyses.

Results

At 30/28ºC, three one-node plants of *Rotala* died during the course of the 12-d assay; all other plants survived the experiments. Relative growth rates of *Alternanthera* and *Rotala* did not differ between the two species or between plants of the two initial sizes (Figure 1, page 6).

The number of new shoots produced by *Rotala* was greater than that of *Alternanthera* at both 23/21ºC ($F_{1,28} = 15.7$, $P < 0.001$) and 27/25ºC ($F_{1,28} = 11.5$, $P = 0.002$), and the number of new nodes produced per plant was greater for *Rotala* at all three temperatures (23/21ºC: $F_{1,28} = 18.4$, $P < 0.001$; 27/25ºC: $F_{1,28} = 27.0$, $P < 0.001$; 30/28ºC: $F_{1,25} = 12.7$, $P = 0.001$; Figure 1). *Alternanthera* fragments, on the other hand, produced a greater number of new roots than *Rotala* at the lowest temperature (23/21ºC: $F_{1,28} = 4.9$, $P = 0.04$; Figure 1). The only instance where the initial plant size affected growth significantly was in the number of new shoots produced per fragment at 27/25ºC ($F_{1,28} = 4.1$, $P = 0.05$; Figure 1).

Discussion

The data presented here suggest that *Rotala rotundifolia* is at least as capable of vegetative regeneration as is *Alternanthera philoxeroides*, the latter of which is a serious aquatic invader in North America and other regions of the globe (e.g., China – Ye et al., 2003). In fact, shoot regenerative capacity of *Rotala* was consistently higher than that of *Alternanthera* across a relatively broad temperature gradient.

These experimental results, in combination with observations from field and greenhouse-grown *Rotala rotundifolia* indicate that this species may indeed pose a serious threat to wetlands of the southeastern US. *Rotala* has been observed growing in dry, sun-exposed gravel in Tuscaloosa, Alabama, growing as dense mats atop the water's surface at the same location, and exhibited heterophylly when growing submersed in greenhouse tanks at the R.R. Foil Plant Research Facility at Mississippi State University. Such observations suggest substantial plasticity in habitat tolerances of *Rotala rotundifolia*. Indeed, in a separate set of growth assays, conducted with several exotic species in a greenhouse, *Rotala* managed to survive air temperatures up to 45ºC, with accompanying water temperatures of 37ºC (Ervin et al., unpublished). In those assays, growth of *Rotala* fragments with an initial size of one to four nodes was comparable to that of *Alternanthera philoxeroides*, *Myriophyllum aquaticum* (Vell.) Verdc., and *Salvinia minima* Baker (three widely recognized aquatic invasives).

The invasiveness of exotic aquatic plant species results in large part from their ability to spread rapidly, in many cases via vegetative regeneration. Fragmentation is one key mechanism of vegetative dispersal and one which can occur naturally (Cronk and Fennessey, 2001, Smith et al. 2002) or as the result of human disturbance of plant canopies (Madsen and Smith, 1999). In fact, for such exotic plant populations as southeastern US *Hydrilla verticillata* (L. f.) Royle, which are entirely dioecious and unisexual, vegetative dispersal is the only means available (Cronk and Fennessey, 2001). Plant fragments, by virtue of being well developed clones of the parent plant, can establish easily in areas where a sexually produced propagule might encounter unfavorable microhabitat conditions. Fragments also can be dispersed easily in water bodies, providing rapid distribution purely by vegetative means. Thus, capacity for vegetative regeneration as was indicated for *Rotala* in the present study is a serious indicator of potential invasiveness in non-native aquatic plant species.

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References

Burks K.C., Hall D.W., Vandiver V.V., Jr., Jacono C.C. 2003. *Rotala rotundifolia* (Lythraceae) new to Florida. *Sida* 20: 1765-1769.

Cook, C.D.K. 1979. A revision of the genus *Rotala* (Lythraceae). *Boissiera* 29: 1-155.

Cronk, J.K. and Fennessey, M.S. 2001. *Wetland Plants: Biology and Ecology*. Lewis Publishers, Boca Raton, FL.

Jacono, C.C. and V.V. Vandiver, Jr. 2007. *Rotala rotundifolia*, purple loosestrife of the south? *Aquatics* 29: 4-9.

Madsen, J.D. and Smith, D.H. 1999. Vegetative spread of dioecious hydrilla colonies in experimental ponds. *Journal of Aquatic Plant Management* 37: 25-29.

Reese, N.L., Haynes, R.R. 2002. Noteworthy collections: Alabama. *Castanea* 67: 216.

Smith, D.H., Madsen, J.D., Dickson, K., Beitinger, T.L. 2002. Nutrient effects on autofragmentation of *Myriophyllum spicatum*. *Aquatic Botany* 74: 1-17.

United States Geological Survey, Florida Integrated Science Center. 2006. *Rotala rotundifolia*. Accessed on April 10, 2006 from http://cars.er.usgs.gov/Nonindigenous_Species/Rotala_rotundifolia/rotala_rotundifolia.html.

Ye, W.H., Li, J., Cao, H.L., Ge, X.J. 2003. Genetic uniformity of *Alternanthera philoxeroides* in South China. *Weed Research* 43: 297-302.

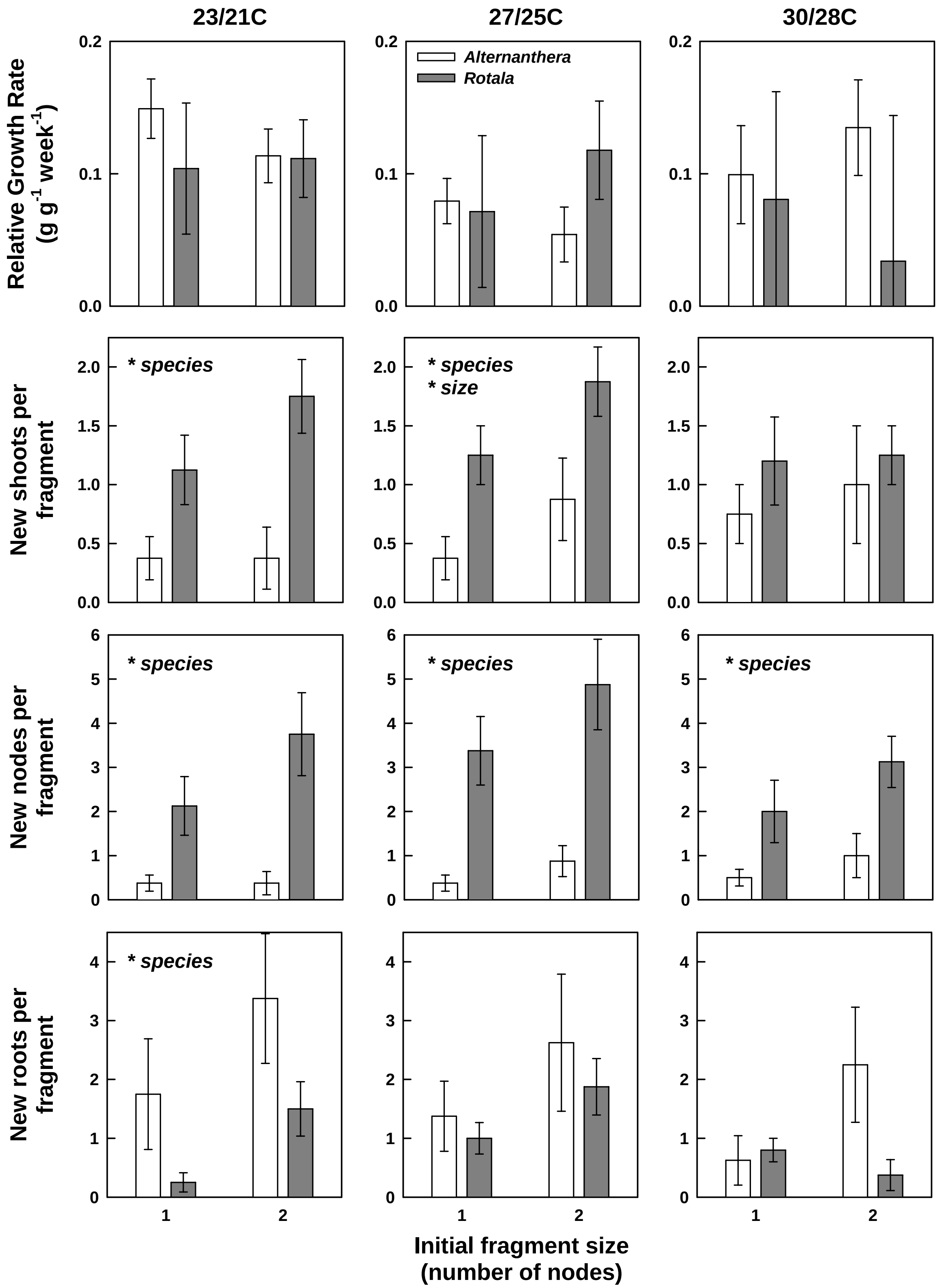

Figure 1. Growth responses of *Rotala rotundifolia* and *Alternanthera philoxeroides* under controlled conditions at three temperature regimes. Temperatures for each set of growth assays (day/night), 12d in duration, are given at the top of each column of graphs. The italicized term(s) in individual panels indicate the factor(s) that correlated significantly with fragment regrowth, at $\alpha = 0.05$.