Effects of lime addition on the growth of fanwort in softwater systems

WILLIAM F. JAMES*

ABSTRACT

Lime addition to softwater aquatic systems can shift inorganic carbon equilibrium to HCO_3^- dominance by temporarily elevating pH. For submersed aquatic macrophytes restricted to free CO_2 uptake for photosynthesis, the addition of lime may be an effective means of suppressing growth and propagation. Fanwort (*Cabomba caroliniana* Grey) is an invasive species to Midwestern and northeastern United States and Canada, predominantly found in low alkalinity, softwater systems, and could be susceptible to inorganic carbon limitation after lime application. Growth response of fanwort (invasive green phenotype) to hydrated lime addition was examined in replicate softwater (pH ~7; total alkalinity ~80 µM) experimental tanks to test this hypothesis. Modest lime concentrations of 55 and 160 µM were required to increase pH to 9 and 10, respectively, versus pH 7 in control tanks. Free CO_2 decreased from ~20 µM in the controls to ~0.1 and ~0.01 µM in tanks treated with 55 and 160 µM lime, respectively. Fanwort shoot biomass decreased to 36% and only 8% of the control mean biomass for tanks treated with 55 and 160 µM lime, respectively, indicating negative growth response to lime application. These patterns suggested that lime addition may be effective in suppressing fanwort growth.

Key words: *Cabomba caroliniana, C*arbon dioxide, dissolved inorganic carbon, fanwort, macrophytes, pH.

INTRODUCTION

Fanwort (*Cabomba caroliniana* Grey) is native to the southeastern United States but has become invasive to northeastern, Midwestern, and western North America. Phenotypic variations include a green type that has become invasive to northern portions of the United States and Canada, a red type that is native to southeastern United States, and a second invasive phenotype that is derived from the aquarium industry (Wain et al. 1983). It propagates rapidly via stem fragmentation and rhizomes (Ørgaard 1991) and displaces native species by forming dense stands (Reimer and Trout 1980). Although fanwort can be susceptible to herbicides (Westerdahl and Getsinger 1988, Nelson et al. 2002), Bultemeier et al. (2009) demonstrated differential phenotypic responses and resistance of the invasive green phenotype to a wide range of herbicides. The environmental niche for suc-

cessful invasion by fanwort seems to be specific to water bodies exhibiting low alkalinity (~150 to 300 µM), neutral pH (6 to 8), and low dissolved calcium (<250 µM; Jacobs and Macisaac 2009). These softwater attributes suggest that fanwort is restricted to free CO_2 as a source for photosynthetic assimilation and cannot use HCO_3^-. If so, shifting inorganic carbon equilibrium to HCO_3^- dominance and lowering free CO_2 below the carbon compensation point via lime application may be an effective means of selectively suppressing fanwort growth without impacting native submersed species that have evolved mechanisms for using HCO_3^- (i.e., elodea, northern milfoil, pondweeds; Maberly and Spence 1983, Madsen and Sand-Jensen 1991).

In hard water systems, addition of hydrated lime $(Ca(OH)_2)$ can both shift equilibrium toward HCO_3^- dominance by increasing pH and induce precipitation of CO_2 as calcite. Previous lime applications to hard water lakes have resulted in a change to the macrophyte community by suppressing certain species (Babin et al. 1992, Chambers et al. 2001, Prepas et al. 2001a and 2001b). Differential macrophyte growth responses observed in these studies were likely due to species-specific differences in the carbon compensation point (Allen and Spence 1981, Maberly and Spence 1983, Bowes and Salvucci 1989). In softwater systems, hydrated lime addition would result in a shift in inorganic carbon form without accompanying calcite precipitation, if these systems were undersaturated with respect to dissolved Ca. If free CO_2 concentrations can be driven below the carbon compensation point of fanwort (~3 µM CO_2; Smith 1938) by lime addition, this might be an effective means of suppressing growth with minimal impact on native submersed macrophytes that can use HCO_3^-. The objectives of this study were to quantify the growth response of fanwort to lime-induced shifts in pH and dominant inorganic carbon species and concentration in experimental softwater systems.

MATERIALS AND METHODS

Fanwort sprigs of the invasive green phenotype were grown in clear fiberglass tanks (1.2 m dia by 1.2 m height; 1400 L capacity) containing groundwater that had been pretreated with lime (~6.7 mM as $Ca(OH)_2$) to lower alkalinity and dissolved calcium (DCa), titrated with hydrochloric acid to adjust pH to 6.8 to 7.0, and bubbled with air through a diffuser stone to equilibrate the water with atmospheric CO_2. Apical tips (10 cm) were planted in 1.2 L polyethylene containers filled with sediment collected from a local lake (moisture content = 71%; bulk density = 0.29 g·mL^{-1}; total sediment N = 4.702 mg g^{-1}; porewater ammonium-N = 5.750 mg L^{-1}; total sediment P = 0.971 mg g^{-1}; porewater P = 0.359

*ERDC Eau Galle Aquatic Ecology Laboratory, W. 500 Eau Galle Dam Rd., Spring Valley, Wisconsin 54767 USA. 715-778-5896 (voice), 715-778-5897 (fax), E-mail: william.f.james@usace.army.mil.

mg L^{-1}) and allowed to grow for 45 d prior to treatment (22 June through 1 August 2009). Four tips were planted in each container. Natural lighting was regulated with a 30% shade cloth positioned 2 m above the tops of the tanks. The tanks were covered with clear plastic to prevent rain from altering chemistry. Pumps (Beckett Versa Gold G90AG; 0.34 m^3 min^{-1}) provided water circulation in each tank during the entire study. In addition, air was bubbled continuously through an air diffusing stone (Fisher Scientific; pore size = 60 µM; 2.5 cm dia) placed at the bottom of each tank to provide a CO_2 source to the plants.

In experimental tanks, lime addition was intended to increase pH to either 9 or 10 from an initial pH of 7. Lime was added as a slurry at a concentration of either 55 or 160 µM to adjust pH to 9 and 10, respectively. The plants were allowed to grow for 24 d post-treatment (average water temperature = 22 C) and harvested for determination of shoot biomass after drying at 65 C for 3 d. Post-treatment biomass was compared with the biomass of additional replicate planted containers that were harvested on the day of lime application.

In situ temperature and pH were monitored in each tank at a minimum of 2 d intervals using a data sonde (Hydrolab Quanta System; Hach Company, Loveland, CO) calibrated against known buffers and Winkler titrations. Integrated water column samples were collected for the determination of inorganic carbon species and DCa. Total alkalinity of unfiltered water was determined via titration with 0.02 N sulfuric acid to an end-point of pH 4.8 using a 5 mL buret (APHA 2005). Free CO_2, bicarbonate (HCO_3^-), carbonate (CO_3^{-2}), and total CO_2 (TCO_2) were estimated by calculation based on pH, total alkalinity, and ionization constants (APHA 2005). DCa was determined using flame atomic absorption spectroscopy (Perkin-Elmer AA Analyst 100; Perkin Elmer Life and Analytical Sciences, Inc., Wellesley, MA) after filtration through a 0.45 µM syringe filter (APHA 2005). CO_2 flux (J_{CO2}; mmol·m^{-2} d^{-1}) between the atmosphere and tanks were estimated as:

$$Jco_2 = \frac{D}{z}k[(CO_{2water} - CO_{2air})]$$

where D is the gas diffusion coefficient (cm^2 s^{-1}), z is the boundary layer (m), k (dimensionless) is a chemical enhancement coefficient (assumed to be 1), and CO_{2water} and CO_{2air} are concentrations (µM) in the water and air, respectively. A boundary layer thickness of 150 µm was chosen because the tanks were bubbled with air.

A completely randomized block design that consisted of three replicate tanks per treatment and four planted containers per tank was used evaluate effects of lime addition on growth. Experiments were conducted at the Eau Galle Aquatic Ecology Laboratory located in west-central Wisconsin (W 44.85386°, N 92.24925°). Analysis of variance (ANOVA) was used to test for block versus treatment effects for shoot biomass. Changes in water chemistry over time were evaluated using ANOVA with repeated measures. Significant differences (P < 0.05) in water chemistry on individual dates were examined with ANOVA Duncan-Waller.

RESULTS AND DISCUSSION

Before lime application, mean pH was 6.93 (±0.06 Standard Error, SE) and mean free CO_2, HCO_3^-, CO_3^{-2}, total alkalinity, and DCa concentrations were 19 µM (±2 SE), 83 µM (±13 SE), 0.08 µM (±0.02 SE), 85 µM (±13 SE), and 315 µM (±9 SE), respectively, with no significant differences as a function of block. Fanwort shoot biomass after 45 d growth during the pretreatment period was similar for all tanks at 0.62 g (±0.02 SE). After lime addition, significant differences in water chemistry variables were attributed to time and treatment versus block effects. In general, they changed as a result of treatment and rebounded back toward control levels as a function of time (Figure 1). Mean pH gradually increased to ~7.4 over the 24 d post-treatment period in the control tanks. In experimental tanks, mean pH increased to target levels immediately after lime application. For tanks treated with 55 µM lime, pH declined from 9.26 (±0.15 SE) to control levels by day 12 (Figure 1a). Mean pH declined linearly with time (pH = -0.043x + 9.84; r^2 = 0.90) in tanks treated with 160 µM lime, but remained significantly higher than other treatments throughout the post-treatment period.

Mean free CO_2 declined substantially immediately after lime addition to experimental tanks (Figure 1b). It rebounded to control concentrations by day 14 in tanks treated with 55 µM lime; in 160 µM lime treatments, it increased gradually but was significantly lower than other treatments throughout the post-treatment period. Mean HCO_3^- increased in experimental tanks as a function of increasing lime application, particularly between days 7 and 24 post treatment, and concentrations were greatest in tanks treated with 160 µM versus the 55 µM lime (Figure 1c). Mean HCO_3^- gradually increased in the control tanks as well, coincident with an increase in pH, suggesting a slight shift in equilibrium toward HCO_3^-. Mean CO_3^{-2} increased substantially in tanks treated with 160 µM lime immediately after treatment and gradually declined by day 24 (Figure 1d). Mean concentrations of CO_3^{-2} increased to a much lesser extent in tanks treated with 55 µM lime and declined to control levels by day 14.

Mean TCO_2 and total alkalinity increased over time for all treatments, and concentrations were greatest for tanks treated with 160 µM lime > 55 µM lime > controls (Figure 1e-f). These patterns could be explained in large part by diffusion of atmospheric CO_2 into the tanks and conversion to HCO_3^- and CO_3^{-2}. Estimated J_{CO2} was near zero, 6.4, and 10.8 mmol m^{-2} d^{-1} for the control, 55 µM, and 160 µM lime treatments, respectively. These rates were comparable to measured changes in TCO_2 of 2.4, 7.2, and 9.1 mmol m^{-2} d^{-1}, respectively (Figure 1e). Post-treatment mean DCa concentrations were similar at ~0.40 µM for controls and tanks treated with 55 µM lime, but significantly higher (0.55 µM) for tanks treated with 166 µM lime (not shown).

Significant decreases in fanwort mean shoot biomass were observed for tanks treated with lime versus controls (Figure 2). Mean shoot biomass nearly doubled in control tanks during the 24 d post-treatment period. In contrast, mean shoot biomass declined significantly in tanks treated with lime relative to initial mean shoot biomass at the time of lime application. Mean shoot biomass declined to 36% and only 8% of control means in tanks treated with 55 µM and 160 µM lime, respectively. Mi-
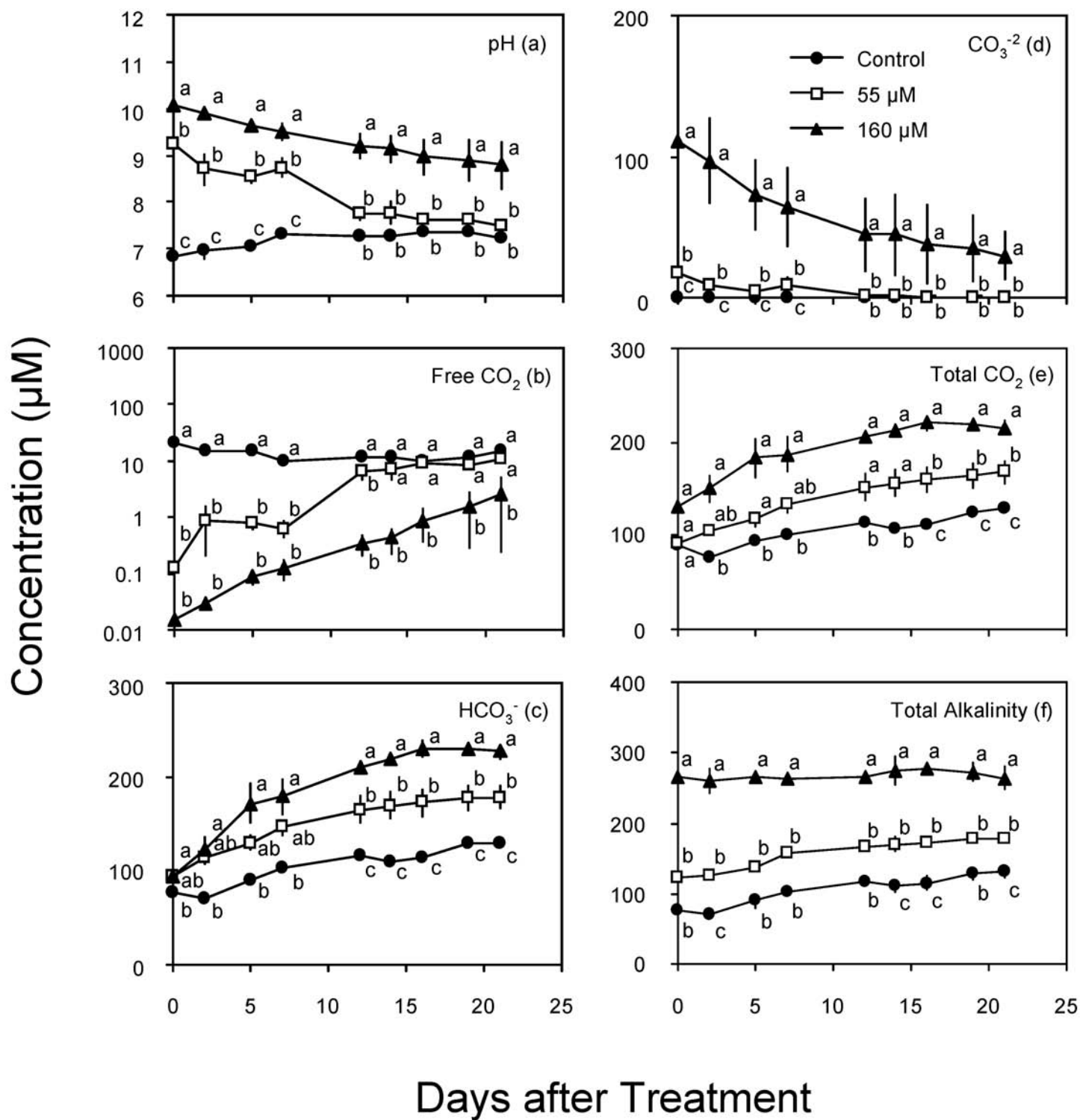
Figure 1. Variations in mean (a) pH, (b) free CO_2 (note log scale), (c) bicarbonate (HCO_3^-), (d) carbonate (CO_3^{-2}), (e) total CO_2, and (f) total alkalinity in control and experimental tanks treated with 55 or 160 µM lime (as $Ca(OH)_2$). Vertical lines represent ±1 standard error (n = 3). Different letters represent significant differences between treatments (P < 0.05) based on ANOVA.

nor fragmentation occurred in experimental tanks during the post-treatment period and accounted for some of the net biomass loss. These fragments settled to the bottom and decomposed by the end of the study.

Changes in inorganic carbon chemistry after lime application provided insight into probable factors contributing to suppression of fanwort growth. Unlike hardwater systems with higher alkalinity and DCa concentrations, lime addition
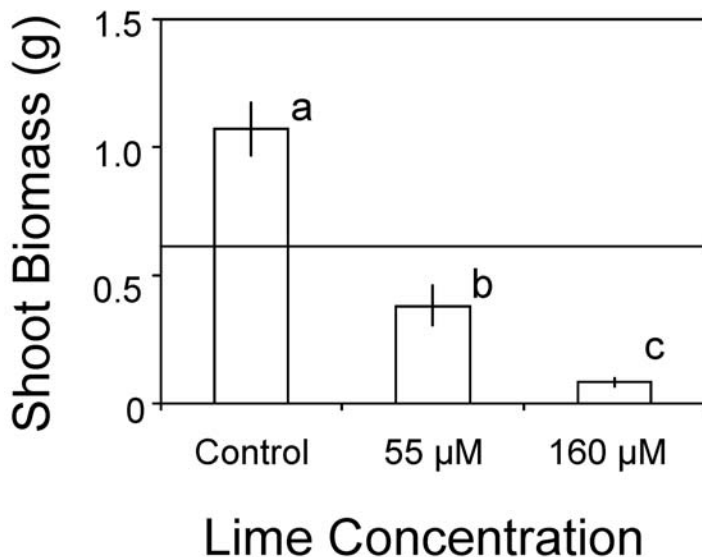
Figure 2. Variations in mean (±1 standard error; n = 12) post-treatment shoot biomass as a function of lime application for tanks treated with 55 or 160 μM lime (as $Ca(OH)_2$). The horizontal line denotes fanwort biomass at the time of treatment. Different letters represent significant differences between treatments (P < 0.05) based on ANOVA.

equilibrium in this study, it could be added more gradually in field situations to prevent rapid increases in pH that might impact other biota. Modest increases in pH to 8 or 9 may be adequate to shift equilibrium and drive free CO_2 concentrations below the compensation point. Many species native to temperate North America can tolerate lower free CO_2, use HCO_3^- for photosynthetic assimilation, and thus thrive in more alkaline waters. In addition, James (2008) found that natives such as elodea (*Elodea canadensis* Michx.) and sago pondweed (*Stuckenia pectinata* L.) tolerated lime treatment and grew despite reductions in both free CO_2 and HCO_3^-. Judicious treatment with lime to control fanwort may result in a shift in species assemblage to more desirable natives that can utilize HCO_3^-. In contrast, species restricted to free CO_2 uptake from the water column such as whorled-leaf milfoil (*Myriophyllum verticillatum* L.), bladderworts (*Utricularia* spp.), swaying bulrush (*Schoenoplectus subterminalis* (Torr.) Soják), and pickerelweed (*Pontederia cordata* L., Maberly and Madsen 2002, Pagano and Titus 2007) may be susceptible to shifts in equilibrium to HCO_3^- dominance caused by lime addition.

With the exception of bubbling air through diffuser stones, the experimental tanks used in this study were essentially closed systems with respect to inorganic carbon flux with the atmosphere. Lime impacts on inorganic carbon chemistry were, thus, probably exaggerated compared to field conditions where CO_2 inputs from the atmosphere, benthic sources, and hydraulic exchanges would be expected to dilute or buffer lime-induced perturbations to free CO_2. More research is needed on the effects of exposure time to low CO_2 on growth, length of growth suppression after treatment, and changes in species assemblage under field conditions open to inorganic carbon fluxes to better evaluate the effectiveness of lime addition on submersed aquatic macrophyte growth.

did not result in oversaturation of Ca and precipitation of CO_2 as calcite. Rather, lime-induced increases in pH were associated with a shift in equilibrium to HCO_3^- (for 55 μM lime) or HCO_3^- and CO_3^{-2} (for 160 μM lime) dominance and a corresponding 2 to 3 order of magnitude decrease in free CO_2 after treatment. Free CO_2 remained at or below ~1 μM in tanks treated with 55 or 160 μM lime over a 7 and 16 d period, respectively, suggesting concentrations were limiting to growth. Even though CO_2 diffusion into tanks was enhanced in treated systems due to low aqueous CO_2 relative to atmospheric concentrations, it was converted to HCO_3^- and CO_3^{-2} because pH was >8, resulting in overall increases in TCO_2 and total alkalinity in lime-treated systems. Thus, CO_2 availability for assimilation was still low despite enhanced atmospheric diffusion.

Overall patterns of decline in shoot biomass in treated tanks suggested that fanwort could not utilize HCO_3^- for growth. In addition, biomass loss coincided with free CO_2 concentrations of <1 μM. This threshold concentration was similar to the carbon compensation point of 3 μM estimated for fanwort by Smith (1938), derived from laboratory experiments that quantified photosynthetic activity versus CO_2 concentration in the surrounding water. This suggested that free CO_2 concentrations were below the compensation point for net growth in lime-treated tanks due to a shift in equilibrium to HCO_3^- dominance. More research is needed over much longer post-treatment time periods and after inorganic carbon chemistry has rebounded to nominal levels to assess regrowth potential after initial suppression.

The apparent susceptibility of fanwort growth to lime application has potential management implications. Relatively small additions of lime to temporarily raise pH, lower free CO_2 concentrations, and shift equilibrium to HCO_3^- dominance may be effective in suppressing fanwort growth *in situ*. Although lime was added to promote an immediate shift in

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Impact of invertebrates on three aquatic macrophytes: American pondweed, Illinois pondweed, and Mexican water lily

JULIE G. NACHTRIEB, M. J. GRODOWITZ, AND R. M. SMART*

ABSTRACT

The objective of this study was to investigate the impact of invertebrates on three native macrophytes: American pondweed (*Potamogeton nodosus* Poir.), Illinois pondweed (*P. illinoensis* Morong), and Mexican water lily (*Nymphaea mexicana* Zucc.). Biomass production of the three plant species was measured and compared under two conditions: one with an uncontrolled population of herbivorous invertebrates and one in which most herbivorous invertebrates were removed by an insecticide treatment. The insecticide effectively removed most plant-feeding insects, including those in orders Coleoptera, Diptera, Trichoptera, and Lepidoptera, but did not remove one invertebrate group likely to impact plants, Hemiptera (aphids). Differences in plant biomass due to feeding and nonconsumptive damage by remaining invertebrates were variable and dependent upon plant species. Nontreated samples of Mexican water lily exhibited high levels of insect damage (primarily herbivory), as well as case making and egg deposition, but biomass differences between treatments were not detected. The impacts of invertebrate herbivory and nonconsumptive damage were more pronounced in both pondweed species as nontreated biomass was significantly less than biomass of insecticide-treated pondweeds. Biomass of American and Illinois pondweed was reduced by 40 and 63%, respectively, due to invertebrate herbivory. Invertebrate herbivory, once thought to be insignificant to aquatic macrophytes, was shown to cause substantial biomass reductions in two of the three plant species studied.

Key words: herbivory, *Nymphaea mexicana*, *Potamogeton illinoensis*, *Potamogeton nodosus*.

INTRODUCTION

Native aquatic macrophytes are a valuable component of aquatic habitats. They provide important fish and wildlife habitat (Savino and Stein 1982, Heitmeyer and Vohs 1984, Dibble et al. 1996), improve water clarity and quality, and reduce rates of shoreline erosion and sediment resuspension (Smart 1995). Native plants, such as wild celery (*Vallisneria americana* Michx.), have also been shown to compete effectively against invasive macrophytes, thereby providing sustainable management of aquatic ecosystems (Smart et al. 1994, Smart 1995, Ott 2005, Owens et al. 2008).

Understanding the importance of native aquatic plants has prompted their use in an increasing number of revegetation projects. However, herbivory can negatively impact the establishment of plant founder colonies, consequently decreasing the success of revegetation projects (Lodge 1991, Dick et al. 1995, Doyle and Smart 1995, Doyle et al. 1997).

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*First and third authors: US Army Engineer-Lewisville Aquatic Ecosystem Research Facility, 201 East Jones St., Lewisville, TX 75057; second author: US Army Engineer Research and Development Center, 3909 Halls Ferry Rd., Vicksburg, MS 39180. Corresponding author's E-mail: jnachtrieb@laerf.org. Received for publication July 28, 2010 and in revised form October 13, 2010.

Cages can be constructed to protect plants from larger herbivores such as turtles, nutria, and crayfish, but excluding invertebrates is nearly impossible. Knowledge of the complex interactions between invertebrate herbivores and native macrophytes can aid in revegetation by improving plant species selection and timing and location decisions.

Although typically beneficial, plants can exhibit weedy growth, not only outside but also within their native range, prompting the need for control methods. Macrophytes such as American lotus (*Nelumbo lutea* [Willd.] Pers.), cattails (*Typha* spp.), and coontail (*Ceratophyllum demersum* L.) are commonly problematic within their native range of North America. Fanwort (*Cabomba caroliniana* A. Gray) can be weedy within its native range of North America as well as in Australia where it forms monospecific stands and is listed as one of Australia's 20 Weeds of National Significance (Schooler et al. 2006). Several species of spatterdock, waterlilies, and pondweeds native to North America are regarded as weeds in Holarctic countries (Sculthorpe 1967). Also, wetlands in the United Kingdom, the Netherlands, and Australia are being threatened by floating marshpennywort (*Hydrocotyle ranunculoides* L. f.), a species believed to be native to North America (EPPO 2006). A greater understanding of invertebrate impacts on native macrophytes could lead to the discovery of natural enemies with potential use as classical biological control agents in other areas of the world.

Little information is available that quantifies the impact of invertebrate herbivores on native macrophyte biomass in North America. Early research indicated that while macrophytes were useful as a substrate for invertebrates and epiphytic growth, they provided little if any nutritional value (Shelford 1918). This same viewpoint was recently supported by Jolivet (1998); however, additional studies have shown importance of macrophytes as a food source for invertebrates. Among those, Soszka (1975) reported pondweeds can lose 50 to 90% of their leaf area from insect herbivory and non-consumptive destruction, mostly from lepidopterans, trichopterans, and dipterans. Leaf area damage as high as 56%, depending on plant species and locality, was documented by Sand-Jensen and Madsen (1989) and attributed to herbivory mostly by trichopterans and dipterans. Newman (1991) later identified five insect orders, Trichoptera, Diptera, Lepidoptera, Coleoptera, and Hemiptera, as containing most herbivores associated with aquatic macrophytes. Live macrophytes were also found to be engaged in aquatic food webs, sometimes to the extent that macrophyte biomass, productivity, and relative species abundance are dramatically changed by grazers (Lodge 1991). Finally, Cronin et al. (1998) determined that freshwater macrophyte herbivory is similar to that reported for terrestrial plants. This viewpoint differed widely from the early idea that macrophytes offered surface substrates only (Shelford 1918).

Although evidence has been collected to prove the existence of invertebrate herbivory of aquatic plants, the significance of this interaction is difficult to quantify. This study attempted to quantify invertebrate herbivory by comparing differences in biomass between grazed and ungrazed populations of macrophytes native to North America and commonly used in the southeastern United States for revegetation and invasive species exclusion efforts.

MATERIALS AND METHODS

This study was conducted in three 0.3 ha earthen ponds (40 m by 60 m) at the Lewisville Aquatic Ecosystem Research Facility located in Lewisville, Texas, (33E04'45"N, 96E57'30"W). Preparation of the study ponds included draining, mowing, rototilling, and installing a barrier to separate each pond lengthwise into two congruent sides. The barrier consisted of 5 cm by 10 cm mesh welded-wire fencing covered by pond liner (45 Mil EPDM Firestone pond liner, AZ Ponds and Supplies, Inc. Birdsboro, PA), creating two treatment areas per pond, an insecticide treatment, and an untreated herbivory area. The height of the fence was adjusted to fit the pond's contour, and the liner was measured to fit the height of the fence plus one meter. The extra meter of liner was buried in pond sediment to seal pond sides. Pond water was gravity-fed from Lake Lewisville, Lewisville, Texas, and supplied evenly to both sides of each pond.

On 27 May 2005 each treatment area was planted with five replicates each of three native macrophytes: American pondweed, IIllinois pondweed, and Mexican water lily. Each replicate was enclosed in a 91 cm dia by 120 cm tall cylinder (cage) constructed from 5 cm by 10 cm mesh welded-wire fencing anchored with 120 cm lengths of concrete reinforcing bar. Cages provided plant protection from disturbances such as turtles or ducks. Cages were spaced at equidistant intervals and positioned at equal depths by following the pond's contour. Amount of plants determined suitable for a cage varied by species due to plant size and growth rate. Each cage was planted with one of the following: three 1 L pots of American pondweed or Illinois pondweed, or one 1 L pot of Mexican water lily. Plants were removed from pots and planted directly into sediment. Each plant species was randomly placed within each treatment area. Ponds were maintained at a depth of approximately 1 m.

An insecticide, temephos (O,O'-(thiodi-4, 1-phenylene) O,O,O',O',-tetramethyl phosphorothioate) (Abate® 4-E, Clarke Mosquito Control Products, Inc. Roselle, IL), was applied once per week as an emulsifiable concentrate to one-half of each pond at a rate of 0.24 µL Abate formulation/L pond water. The temephos application system was constructed of 1.3 cm dia irrigation hose attached to the top of each cage within each temephos treatment area. One 3.78 Lph drip emitter was attached to the irrigation hose in the center of each cage so that temephos was directly applied to plants within the cage. One end of the hose was capped shut and the other end left open. Temephos was applied by attaching the open end of irrigation hose to a gas powered sprayer (FIMCO, No. Sioux City, SD), which forced the temephos solution into the hose and out through drip emitters.

To evaluate end of growing season differences in plant biomass due to invertebrate—plant interactions, all five replicates of each plant species per treatment area were selected and harvested for plant biomass at 4 months after planting (16 September 2005). One replicate was randomly selected, and invertebrates were harvested as well as biomass. Aboveground plant biomass within each cage was harvested and immediately placed into a plastic bag. Plant material was rinsed with water to remove sediments and algae, and dry weights were obtained by separating into species and drying

to constant weights in an oven at 55 C for a minimum of 48 h. Replicates harvested for invertebrates were rinsed over a bucket to collect dislodged invertebrates. Internally feeding organisms were not expected to be recovered by these methods. Buckets were emptied into 710 µm sieves and all invertebrates collected were preserved in 70% ethanol. Invertebrates were later identified in the laboratory to the following taxonomic levels: Annelids to class, and Gastropoda and Insecta to genus (except for family Chironomidae, which was identified to subfamily).

Statistical Analyses

A one-way analysis of variance (ANOVA) was performed to differentiate treatment effects on total number of invertebrates collected. Nine invertebrate groups were analyzed separately including; Ephemeroptera, Oligochaeta, Coleoptera, Diptera, Trichoptera, Hemiptera, Lepidoptera, Odonata, and Gastropoda. Invertebrate effects on aquatic plants were quantified by comparison of plant dry biomass between treated and nontreated samples. Differences in plant biomass between treatments were analyzed with a one-way ANOVA for each plant species. Experimental data were analyzed at a significance level of $p < 0.05$ using STATISTICA version 8.0 (StatSoft, Inc., 2008, Tulsa, OK).

RESULTS AND DISCUSSION

Invertebrate Collections

Differences due to treatment (insecticide vs. no insecticide) in collected number of invertebrates varied based on invertebrate group (Figure 1). Two invertebrate groups, Ephemeroptera and Oligochaeta, were not analyzed because

an average of fewer than three individuals was collected. Numbers of collected invertebrates from five orders (Coleoptera, Diptera, Trichoptera, Lepidoptera, and Odonata) were significantly reduced in treated areas (Figure 1) by 94 to 100% depending on invertebrate order. In two other groups, Gastropoda and Hemiptera, no significant differences were detected (Figure 1). The failure of temephos to eliminate these invertebrates precluded our ability to ascertain their effects on biomass of any of the plant species in this study. However, the 2 snail genera collected, *Physa* sp. and *Helisoma* sp. (Gastropoda: Physidae and Planorbidae, respectively), both feed primarily on epiphytic growth or detritus without damaging aquatic plants (Brown 2001, Smith 2001), and no obvious plant damage attributable to snail grazing was noted. Hemiptera samples consisted of 90% aphids in the genus *Rhopalosiphum* in nontreated and treated samples alike. *Rhopalosiphum* spp. can be damaging to plants and could therefore affect biomass results in treated areas (Blackman 1974).

Macrophyte Biomass

Dry weights of Mexican water lily were not statistically different between treatments (Figure 2 A); however, herbivory and nonconsumptive damage from the adult coleopteran *Donacia cincticornis* Newman, larval lepidopteran *Synclita* sp., aphids of *Rhopalosiphum* sp., and odonate egg deposition were apparent on nontreated Mexican water lily. Changes in leaf density within cages were less obvious because new leaves were continuously emerging while highly damaged leaves were decaying. Undocumented observations from this study imply that leaf turnover rate increased in nontreated Mexican water lily plants, which were subjected to various types of invertebrate damage. This would make it difficult to determine biomass differences (i.e., measure herbivory) between treatments and could result in underestimates of the impact of invertebrates on Mexican water lily. Other researchers experienced similar difficulties in measuring invertebrate damage levels on other aquatic plant species. For instance, Wallace and O'Hop (1985) documented that leaf turnover rate of spatterdock (*Nuphar luteum* [L.] Sibth & Sm.) was higher at a site that experienced herbivory by the waterlily leaf beetle (*Pyrrhalta nymphaeae* [L.]) in contrast to where beetles were absent. At the herbivore site, leaves died faster but were replaced quickly as if plant growth was compensating for herbivory losses.

In contrast, treated Mexican water lily plants were mostly void of any signs of invertebrate damage other than that due to odonate egg deposition and *Rhopalosiphum* sp. Two anisopteran families, Aeschnidae and Petaluridae, as well as most zygopterans, are known to oviposit in aquatic plant tissue, which can leave holes in plants once the larva emerge. This endophytic trait can result in excessive damage to plant tissue by large numbers of females (Westfall and Tennessen 1996). Near harvest time, aphids were present in both treatment areas in large enough numbers to completely cover the floating leaves of Mexican water lily. While not problematic in small numbers, large aphid colonies are capable of removing enough of the plant's nutrients so that the plant prematurely breaks down plant tissue to replenish its nutrient
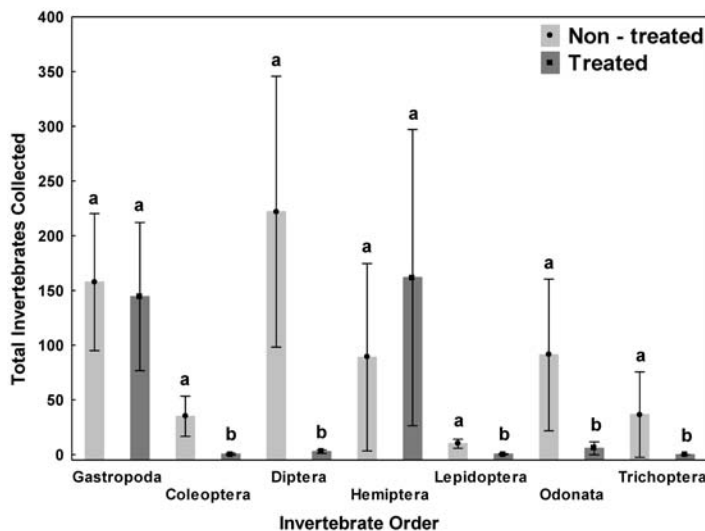

Figure 1. Total number of invertebrates collected per invertebrate order and treatment. Within each order, means with the same letter are not statistically different. One-way ANOVAs (DF = 1, 16): Gastropoda: p = 0.745, F = 0.109; Hemiptera: p = 0.310. F = 1.098; Coleoptera: p = 0.000, F = 18.861; Diptera: p = 0.001, F = 16.710; Trichoptera: p = 0.046, F = 4.673; Lepidoptera: p = 0.000, F = 27.831; and Odonata: p = 0.012, F = 7.990.
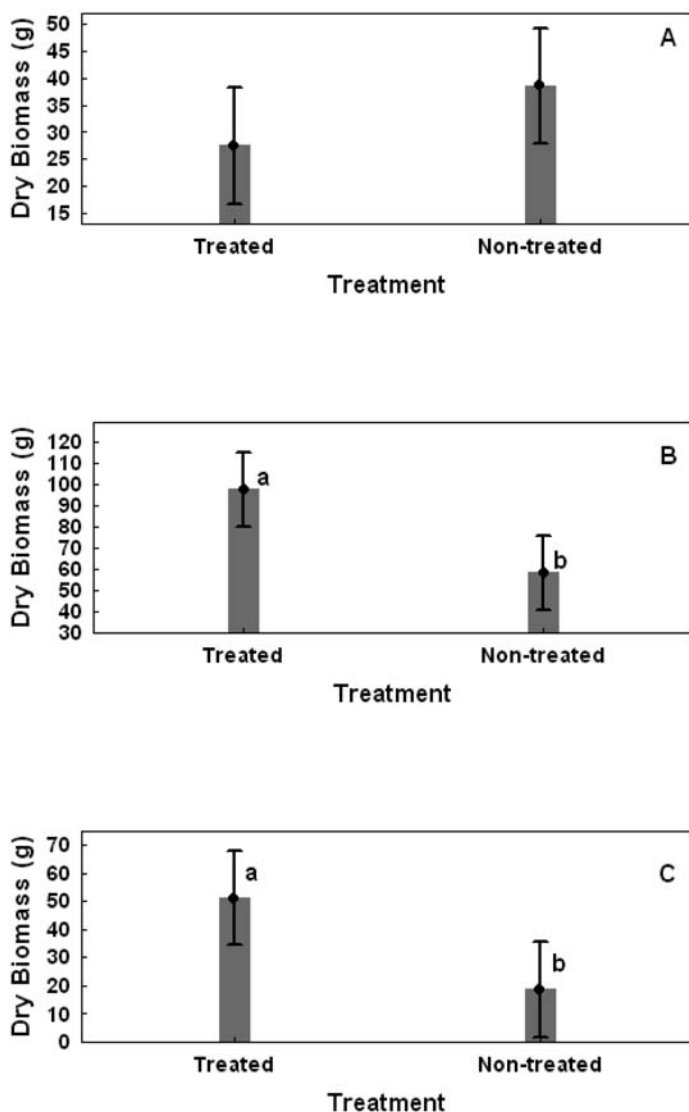
Figure 2. Mean (±0.95 confidence interval) dry biomass (g) of Mexican water lily (A), American pondweed (B), and Illinois pondweed (C) collected per treatment area. Means with the same letter are not statistically different. One-way ANOVA. Mexican water lily (A): p = 0.144, F = 2.258, DF = 1, 28; American pondweed (B): p = 0.003, F = 10.568, DF = 1, 28; and Illinois pondweed (C): p = 0.010, F = 7.668, DF = 1, 28.

supply (Blackman 1974), which can halt plant growth and ultimately cause death. Without aphids in treated samples, biomass may have increased at a rate greater than nontreated plants. Therefore, even though plant conditions from the two treatments were clearly different, combined effects of possible increased leaf turnover rate in nontreated plants and aphid herbivory in treated plants made it difficult to identify biomass differences due to the impact of invertebrates on Mexican water lily. To document leaf turnover rate in future studies, leaves should be marked at emergence and days to leaf death should be noted to compare leaf turnover rate between treatments.

Both pondweeds followed similar trends throughout the study (Figures 2B and 2C). Treated plants were rarely damaged by herbivores other than *Rhopalosiphum* sp. and odonate egg deposition, while nontreated plants sustained damage mostly from a combination of *Rhopalosiphum* sp., lepidopteran larvae of *Synclita* sp. and *Paraponyx* sp., and dipteran larvae of *Hydrellia discursa* Deonier and *H. bilobifera* Cresson. Biomass differences between treatments for both pondweeds were significant (Figures 2B and 2C). Nontreated dry weights of American and Illinois were reduced by 40 and 63%, respectively, when compared to treated dry weights. Because aphids were present in large quantities in both treatments, differences in plant biomass were most likely attributable to the lepidopterans and dipterans identified above. Unlike Mexican water lily, an increase in leaf turnover rate was not observed for the pondweeds. Invertebrate herbivory and nonconsumptive damage were shown to significantly impact both pondweeds.

Future research should focus on invertebrate herbivory on other native species of aquatic plants in controlled research settings as well as natural conditions in water bodies. The impacts invertebrate herbivory may have on revegetation efforts (e.g., reduced competitive potential against nuisance species, reduction of tolerance to species-selective herbicide applications) merit investigation. Studies should be designed to include comparisons of leaf turnover rates in the presence and absence of herbivorous insects. More important, individual plant and insect combinations should be studied to further our knowledge of possible host-specific biological control agents for native plants for use in areas where they become problematic.

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Impact of two herbivores, *Samea multiplicalis* (Lepidoptera: Crambidae) and *Cyrtobagous salviniae* (Coleoptera: Curculionidae), on *Salvinia minima* in south Louisiana

S. TEWARI AND S. J. JOHNSON*

ABSTRACT

A field study was conducted in 2005 and 2006 to evaluate the impact of the herbivores *Cyrtobagous salviniae* Calder and Sands and *Samea multiplicalis* (Guenée) on common salvinia (*Salvinia minima* Baker) in south Louisiana. Our study revealed that treatments consisting of *C. salviniae* and *S. multiplicalis* feeding both independently and together significantly reduced plant biomass of common salvinia. The lowest biomass was recorded for the treatment with both *C. salviniae* and *S. multiplicalis* feeding on common salvinia in October during 2005 and 2006. Biomass showed a significant linear trend for the treatment consisting of feeding by both *C. salviniae* and *S. multiplicalis* in 2005 and significant treatment by month interaction in both 2005 and 2006. Percentage terminal-damage and percentage mat-green showed significant treatment effect in 2005 and 2006.

Key words: common salvinia, herbivores, interaction, invasive.

INTRODUCTION

Nonindigenous weeds invade about 700,000 ha of wildlife habitat per year (Babbitt 1998) in the United States, and the annual management costs for nonindigenous aquatic weed species is approximately $100 million (OTA 1993). Common salvinia (*Salvinia minima* Baker) is a free-floating aquatic fern that occurs in nature as a sporophyte. It consists of a horizontal rhizome lying just below the surface of the water with a pair of floating leaves (Jacono 2005) and a highly dissected submerged third leaf, which is believed to function as a root (Nauman 1993). Common salvinia is native to South America and was probably introduced to North America during the late 1920s and early 1930s (Jacono et al. 2001). As of April 2005, common salvinia has been recorded in more

*First author: Department of Plant, Soil, and Insect Sciences, Fernald Hall, 270 Stockbridge Road, University of Massachusetts Amherst, Amherst, MA 01003; second author: Department of Entomology, LSU AgCenter, Room no. 404, Life Sciences Bldg., Baton Rouge, LA 70803. Corresponding author's E-mail: stewari@psis.umass.edu. Received for publication October 29, 2009 and in revised form October 25, 2010.

than 690 locations in 89 freshwater drainage basins of Florida, Georgia, Louisiana, Alabama, Texas, South Carolina, Mississippi, and Arkansas (USGS 2005a).

Common salvinia is considered sterile and reproduces asexually through fragmentation at a fast rate, covering the surface of water (Jacono 2005, USGS 2005b). Dry weight of giant salvinia (*Salvinia molesta* Mitchell), closely related to common salvinia, was reported to double in 2.5 d under optimum growing conditions (Room et al. 1981). The plants have 3 growth stages that are morphologically dissimilar and distinct. The initial growth stage, or primary stage, is characterized by isolated plants with leaves that lie flat on the water surface and is associated with initial colonization of a water body. The secondary stage is reached when plants have been growing for some time, and the edges of leaves start to curl upward. The tertiary or final stage is marked by crowding of plants, and the leaves curl to assume an almost vertical position. At this stage the infestation may resemble a "mat" covering the water surface. Thick mats of common salvinia prevent sunlight from reaching submerged plants, whereas floating plant species such as antler fern (*Ceratopteris pteridoides* [Hooker]) and duckweed (*Lemna* spp.) are also displaced (USGS 2005b). Common salvinia can lower the dissolved oxygen of infested water and provide safe haven to pest species such as mosquitoes (USGS 2005b). Motor crafts used for recreational activities such as boating and fishing get tangled in thick floating mats of common salvinia, making it extremely difficult to navigate, and these infestations may hinder the ability of law enforcement agencies to carry out their duties effectively (USGS 2005b). Commercial activities such as rice and crawfish farming, water drainage, and electrical power generation can also be negatively impacted by common salvinia (Charles Dugas, Louisiana Department of Wildlife and Fisheries, retired, pers. comm.).

Herbicides are available for control, but asexual reproduction combined with the fast growth rate of common salvinia usually renders their application impractical and ineffective because the area to be treated is very large in most cases. The cost of controlling common salvinia using herbicides by state and contract workers may range from $198 to $297/ha, depending on herbicide used, and the cost to private land owners is much higher (Charles Dugas, Louisiana Department of Wildlife and Fisheries, retired, pers. comm.). Other factors that limit use of herbicides are inaccessibility, spread of common salvinia plants to new areas with flowing water, and their ability to quickly re-establish because of high rate of reproduction (USGS 2005b). Mechanical efforts to control this nuisance aquatic weed are often expensive, time consuming, generally not reliable (USGS 2005b), and weed harvesters can operate only in navigable waterways, thus leaving wooded swamps untreated (USGS 2005b).

Cyrtobagous salviniae Calder and Sands (Coleoptera: Curculionidae) is an aquatic weevil native to Brazil, Bolivia, and Paraguay (Wibmer and O'Brien 1986) and has been used for the biological control of giant salvinia in a number of countries including Australia, Fiji, Ghana, India, Kenya, Malaysia, Namibia, Papua New Guinea, Republic of South Africa, Sri Lanka, Senegal, Zambia, Zimbabwe, and the Unites States (Julien and Griffiths 1998, Tipping and Center 2003, Diop

and Hill 2009). *Cyrtobagous salviniae* can survive and complete its life cycle on common salvinia (Tipping and Center 2005a). The adults are sub-aquatic in nature and can be spotted on or under leaves, within the leaf buds, or among the roots of giant salvinia plants (Forno et al. 1983). Eggs are laid singly and in the cavities formed from adults feeding on the leaves, rhizomes or "roots" (Forno et al. 1983). Adults of *C. salviniae* may feed on leaves, resulting in small irregular holes, or on terminal buds and consequently inhibit the growth of giant salvinia plants (Sands et al. 1983). Feeding by *C. salviniae* larvae causes the leaves to first darken to brown and then drop off (Forno et al. 1983).

Cyrtobagous salviniae was accidentally introduced to Florida sometime before 1960 (Jacono et al. 2001), and a population was subsequently discovered on common salvinia in Florida (Kissinger 1966). It was initially considered *Cyrtobagous singularis* Hustache (Kissinger 1966) but was later identified as *C. salviniae* (Calder and Sands 1985). These Florida weevils were significantly smaller than those from Brazil (Calder and Sands 1985). Molecular analysis indicated that this population was significantly different from the Brazilian *C. salviniae* population used for biological control in Australia (Goolsby et al. 2000). Recent molecular and morphological studies characterized the Florida and Brazilian populations of *C. salviniae* to be ecotypes (Madeira et al. 2006). *Cyrtobagous salviniae* adults of the Florida population lived an average of 96 d on common salvinia under laboratory conditions with a pre-oviposition period of about 45 d (Tipping and Center 2005b). Forno et al. (1983) reported an average larval development period of 23 d for the Brazilian population of *C. salviniae* reared on giant salvinia under laboratory conditions. *Cyrtobagous salviniae* adults were collected throughout the year from common salvinia in south Florida and from giant salvinia in south Brazil, although seasonal variation in the number of adults was reported in both studies (Forno et al. 1983, Tipping and Center 2005a). The Florida population is credited with keeping in check the spread of common salvinia in that state, and its absence in Louisiana and Texas has probably led to common salvinia becoming established there (Jacono et al. 2001).

Samea multiplicalis (Guenée) (Lepidoptera: Crambidae), native to South America and the southeastern United States (Newton and Sharkey 2000), is a generalist herbivore that feeds on common salvinia in addition to other aquatic plants such as *Azolla caroliniana* Willd., *Azolla pinnata* R. Br., and *Psitia stratiotes* L. (Knopf and Habeck 1976, Sands and Kassulke 1984, Newton and Sharkey 2000, Tipping and Center 2005a). Natural populations of this moth are present in Louisiana and were reported to be one of the 3 most common species captured using ultraviolet-light traps from March to October 1995 in the longleaf pine savannas of Louisiana (Landau and Prowell 1999). The egg, larval, and pupal stages of *S. multiplicalis* lasted an average of 4, 29, and 8 d, respectively, when reared on giant salvinia under laboratory conditions (Sands and Kassulke 1984). Larvae construct and feed inside a refugium made of silk and plant hair, and growing apical buds are often damaged by larger larvae (Julien et al. 2002). *Samea multiplicalis* has been studied in Australia as a potential biological control agent against giant salvinia (Sands and Kassulke 1984).

The purpose of this study was to determine the impact of *C. salviniae* and *S. multiplicalis* on biomass of common salvinia when feeding both independently and together in south Louisiana.

MATERIALS AND METHODS

The study was conducted on portion of a 4000 ha tract of private property located north of Gramercy, Louisiana, and adjacent to Highway 61 (30°10'46.77"N 90°49'07.75"W). The site was flooded woodland, dominated by cypress and tupelo gum trees, with dredged canals that held water throughout the year, and was heavily infested with common salvinia. The depth of water in flooded woodlands and dredged canals fluctuated with rainfall but was 0.5 m on average in woodlands and 1.5 m or more in canals.

We used 5.08 cm dia (SCH. 40) PVC pipes to construct 1 m² frames, the size of the experiment plots. Sixteen frames were set up throughout the property with adjacent plots 100-500 m apart, and were anchored using nylon ropes and bricks. Four treatments, each replicated 4 times, were applied randomly to the 16 plots (quadrats). The treatments were (1) common salvinia subjected to feeding by the weevil *C. salviniae* only; (2) common salvinia subjected to feeding by *S. multiplicalis* larvae only; (3) common salvinia subjected to feeding by both *C. salviniae* and *S. multiplicalis*; and (4) the control with no feeding.

Weevils for the experiments were obtained from a Florida population and maintained in Louisiana State University campus greenhouses. The weevils used in 2005 were collected from Fort Lauderdale in September 2004 and 2005 by Dr. Phil Tipping (USDA-ARS, Invasive Plant Research Laboratory, Fort Lauderdale, FL). The weevils released in 2006 consisted of 2 different populations, one collected September 2005 by Dr. Phil Tipping at Fort Lauderdale and the other collected September 2005 by one of the authors (S. J. Johnson) at Coe's Landing on Lake Talquin, located near Tallahassee. The weevils were reared in 567.8 L tanks (Rubbermaid) stocked with common salvinia, which was replenished at regular intervals. Artificial grow lights (Bell Lighting Technologies Inc., Canada) maintaining a 14 h photoperiod and indoor heaters were used to provide optimum conditions (25-28 C) for the weevils to reproduce during winter months.

The study began in May of 2005 with the release of 40 weevils per plot in the 8 plots that received weevils (treatments 1 and 3). The sex ratio of weevils was not determined at release because there is no reliable external morphological or size difference between male and female Florida salvinia weevils. In August 2005, an additional 50 weevils per plot were released. The study was repeated in 2006 by releasing 100 weevils per plot in the 8 plots (treatments 1 and 3) in April and supplemented with another 50 weevils per plot in September. Treatments 2 and 3 resulted from natural infestation of *S. multiplicalis* at the study site. Treatments 1 and 4 were maintained free of *S. multiplicalis* by spraying with microbial insecticide (Thuricide concentrate, active ingredient: *Bacillus thuringiensis* subspecies *kurstaki*, equivalent to 4000 *Spodoptera* units or six million viable spores per milligram). This microbial formulation was used because it does not ad-

versely impact *C. salviniae* larvae and adults. In 2005, Thuricide was initially applied once a week, but in June we switched to twice a week for better control of *S. multiplicalis*, and this spraying schedule was followed throughout 2006. All plots were kept free of other aquatic vegetation by hand removal to maintain uniformity.

Sampling was done monthly, starting in June of both 2005 and 2006 and continuing until October, resulting in 5 samples taken each year. Three quadrats of 0.1 m², built with 2.5 cm dia PVC pipes, were haphazardly placed inside the 1 m² plot, and the common salvinia enclosed within each smaller quadrat was hand squeezed to remove excess water and weighed to determine the biomass. Plant material was replaced after weighing, and the 3 smaller quadrats were removed from the 1 m² plot. In addition, 100 common salvinia plants were haphazardly selected at each sampling date from inside the 1 m² plot to check for damage to the terminal buds due to herbivore feeding (percent terminal-damage). The total number of *C. salviniae* adults and *S. multiplicalis* larvae (all instars) observed during inspection of the 100 common salvinia plants for terminal damage was recorded. These plants were also replaced inside the 1 m² plot after determination of percent terminal-damage. The area inside each 1 m² plot covered with common salvinia (percentage coverage) and the area inside each plot appearing green (percentage mat-green) was estimated by visual inspection. Values for pH and surface-water temperature inside the 1 m² plots were recorded at each sampling date. The relationship between wet and dry weight of common salvinia was determined at the beginning of study; destructive sampling of common salvinia was not possible due to the presence of herbivores in the samples and the experimental design that required collection of data over time. Fifteen samples of common salvinia were collected from different locations at the study site using a 0.1 m² quadrat, and their wet-weight was recorded. These samples were brought to the laboratory in coolers and dried in an oven (Precision Scientific, Model 144) for 72 h at 100 C to determine dry weights.

Additional samples of common salvinia were collected from both inside and approximately 1 m outside the 8 weevil treatment plots using 0.1 m² quadrats in April 2006 to check for the presence of *C. salviniae* adults. Three samples were collected from inside the plot and 4 samples from the outside, for a total of 7 samples per site. The same number of samples were also removed from the remaining 8 treatment plots to maintain uniformity. Samples from *C. salviniae* release plots were brought back to the lab in coolers and put in Berlese funnel for 72 h under 60 w light bulbs. One or 2 common salvinia plant were placed in a clear 118 mL Whirl-Pak bag containing tap water to attract weevil adults. These bags were attached to the base of the Berlese funnel and checked every 24 h for presence of weevil adults and replaced with a new bag containing fresh common salvinia plants.

Regression analysis (SAS 2003) was used to determine the relationship between wet and dry weights of common salvinia. Repeated- measures analysis of variance (ANOVA) with an unstructured variance-covariance matrix was used to determine whether herbivore treatments had differential effects on biomass of common salvinia over time. Proc mixed (SAS

2003) was used to analyze the data with plots as repeated units. Similar analyses were performed on data pertaining to pH, surface-water temperature, percentage terminal-damage, and percentage mat-green. Tukey-Kramer was used to separate the treatment least square means on each sampling date for biomass, percent terminal-damage, and percent mat-green data. For the treatment consisting of only *S. multiplicalis*, we compared the number of larvae observed during sampling in 2005 and 2006. Within each year, we also compared the number of *S. multiplicalis* larvae observed during sampling in the treatments consisting of (1) only *S. multiplicalis* and (2) both *C. salviniae* and *S. multiplicalis*; and the number of *C. salviniae* adults observed during sampling in the treatments consisting of (1) only *C. salviniae* and (2) both *C. salviniae* and *S. multiplicalis*.

RESULTS AND DISCUSSION

The wet weight of common salvinia in the 15 samples ranged from 61 to 478 gm with a mean of 224 gm, and the dry weight of samples ranged from 49 to 70 gm with a mean of 57 gm. The regression analysis of dry weight on wet-weight of common salvinia was significant (F = 1079.87, df = 1, 13; P < 0.0001, r^2 = 0.9881) and suggests that wet weight of common salvinia can be a reliable way of comparing plant material among the different treatments.

Cyrtobagous salviniae failed to establish in one of the plots in 2005 and was not included in data analysis. In 2005, the repeated-measures ANOVA value for biomass was significant (F = 10.11; df = 3, 11; P = 0.0017), showing an overall impact on biomass due to feeding by herbivores as compared to control. The treatment * date term was also significant (F = 5.91; df = 12, 11; P < 0.0001) for 2005, reflecting gradually increasing biomass in the control plots over time and decreasing biomass in treatments consisting of (1) only *C. salviniae* and (2) both *C. salviniae* and *S. multiplicalis* (Figure 1). For the treatment consisting of both *C. salviniae* and *S. multiplicalis*, there was a significant linear trend in the biomass of common salvinia (F = 6.87; df = 1, 11; P = 0.0238). For the treatment consisting of only *S. multiplicalis*, there was an increase in biomass of common salvinia from June to August and a decline thereafter, a significant quadratic trend (F = 4.58; df = 1, 11; P = 0.0557), and may have contributed to significant treatment * date interaction (Figure 1). Herbivore feeding also had a significant impact on percentage terminal-damage (F = 7.64; df = 3, 11; P = 0.0049) as compared to the control plots. For the treatment consisting of only *C. salviniae*, percentage terminal-damage increased from 45% in June to 85% in September, while for the treatment consisting of both *C. salviniae* and *S. multiplicalis*, percentage terminal-damage increased from 55% in June to 71% in October (Figure 2). Percentage terminal-damage for the treatment consisting of only *C. salviniae* decreased to 64% in October (Figure 2). This trend was reflected in the significant treatment * date interaction (F = 4.15; df = 12, 11; P = 0.0125). For percentage mat-green analysis, we dropped plot as the repeated unit because there was insufficient variability in data. Herbivore feeding had a significant impact on percentage-mat green inside the treatment plots (F = 47.97; df = 3, 55; P = 0.0003). For the treatment consisting of only *C. salviniae*, percentage mat-green decreased from 100% in June to 57% in October, while for treatment consisting of both *C. salviniae* and *S. multiplicalis*, percentage mat-green decreased from 100 to 60% during the same period (Figure 3). Percentage coverage, surface-water temperature and pH did not show a significant treatment effect in 2005.

In 2005, the number of *S. multiplicalis* larvae observed during sampling for the treatments consisting of (1) only *S. multiplicalis* and (2) both *C. salviniae* and *S. multiplicalis* varied significantly over time (F = 5.33; df = 4, 6; P = 0.0355), and the highest number of larvae were recorded in June and August (Table 1). Because there was insufficient variability, we dropped plot as the repeated unit from the analysis when the number of *C. salviniae* adults were compared between treatments consisting of (1) only *C. salviniae* and (2) both *C. salviniae* and S. *multiplicalis*. A significantly higher number of weevil adults were observed in the treatment consisting of only *C. salviniae* as compared to the treatment with both the herbivores (F = 6.27; df = 1, 25; P = 0.0191; Table 1).

Cyrtobagous salviniae adults were not recovered from any of the 8 weevil treatment plots in April 2006. As in 2005, the repeated-measures ANOVA value for biomass was significant (F
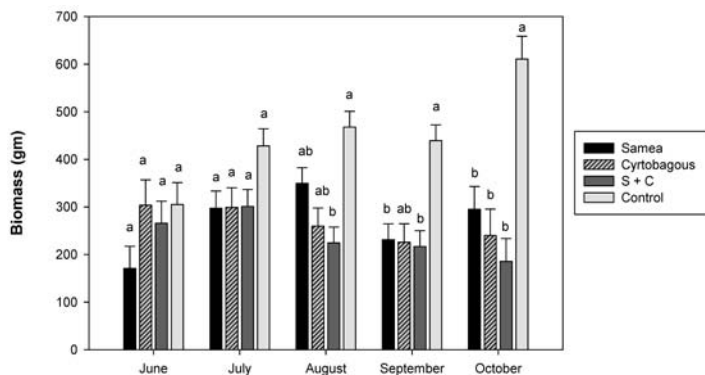

Figure 1. Least-squares mean biomass (with standard error) of common salvinia in different herbivore treatments at Gramercy, LA in 2005. For each month, treatments with the same letters were not statistically distinguishable (Tukey-Kramer, α = 0.05).
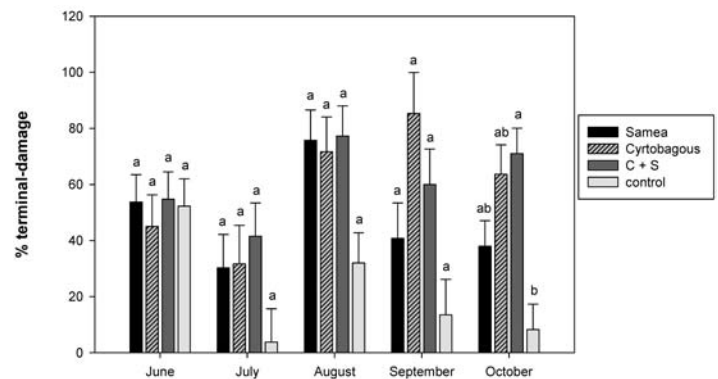

Figure 2. Least-squares mean percent terminal-damage (with standard error) on common salvinia in different herbivore treatments at Gramercy, LA in 2005. For each month, treatments with the same letters were not statistically distinguishable (Tukey-Kramer, α = 0.05).
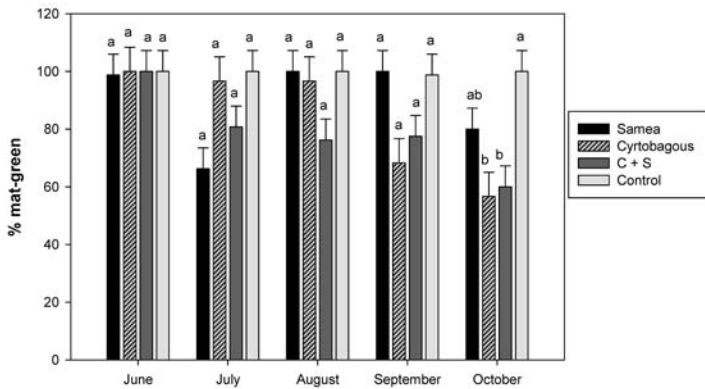
Figure 3. Least-squares mean percent mat-green (with standard error) of common salvinia in different herbivore treatments at Gramercy, LA in 2005. For each month, treatments with the same letters were not statistically distinguishable (Tukey-Kramer, α = 0.05).

= 47.97; df = 3, 12; $P < 0.0001$) in 2006, representing an overall reduction of biomass due to feeding by herbivores as compared to control. The treatment * date term was also significant ($F = 8.48$; df = 12, 12; $P = 0.0004$) for 2006, and for the treatments consisting of (1) only *C. salviniae* and (2) both *C. salviniae* and *S. multiplicalis,* there was a gradual decrease in biomass of common salvinia from June to October (Figure 4), although not a significant linear trend as observed in 2005. For the treatment consisting of only *S. multiplicalis,* there was an increase in biomass of common salvinia from June to August and a decline thereafter, a significant quadratic trend ($F = 9.52$; df = 1, 12; $P = 0.0094$), which may have also contributed to significant treatment * date interaction (Figure 4). However, unlike 2005, the biomass in control plots remained high throughout the sampling period and did not show an increasing trend over time (Figure 4). We attribute this to increased control of *S. multiplicalis* larvae in 2006 as a result of twice a week application of Thuricide throughout the study period. Herbivore feeding also had a significant impact on percentage terminal-damage ($F = 31.91$; df = 3, 12; $P < 0.0001$) as compared to the control plots. For the treatment consisting of only *C. salviniae,* percentage terminal-damage increased from 35% in June to 46% in September, whereas for the treatment consisting of both *C. salviniae* and *S. multiplicalis,* percentage terminal-damage increased from 21% in June to 48% in August (Figure 5). Percentage terminal-damage for the treatment consisting of only *C. salviniae* decreased to 27% in October and to 15% for the treatment consisting of both *C. salviniae* and *S. multiplicalis,* reflected in significant quadratic trend for both the treatments ($F = 11.22$; df = 1, 12; $P = 0.0058$; and $F = 75.92$; df = 1, 12; $P < 0.0001$, respectively) and a significant treatment * date interaction ($F = 14.26$; df = 12, 12; $P < 0.0001$; Figure 5). Percentage mat green showed significant effect ($F = 6.50$; df = 3, 12; $P = 0.0073$), and for the treatment consisting of both *C. salviniae* and *S. multiplicalis,* the area inside the plot appearing green decreased from 80% in June to 59% in September (Figure 6). Percentage coverage, surface-water temperature and pH did not show a significant treatment effect in 2006.

For the treatment consisting of only *S. multiplicalis,* a significantly higher number of larvae were observed during sampling in 2005 as compared to 2006 ($F = 4.59$; df = 1, 30; $P = 0.0405$; Tables 1 and 2).

In contrast to an earlier report that *S. multiplicalis* had "negligible impact" on common salvinia in Florida (Tipping and Center 2005a), our results indicate that the native herbivore may suppress common salvinia in south Louisiana. However, the fact that biomass of common salvinia in *S. multiplicalis* plots increased during the first 3 months (Jun-Aug) of sampling in both 2005 and 2006 indicates its inability to maintain constant feeding pressure throughout the growing season, an attribute essential to control rapidly multiplying aquatic plant species like common salvinia. Percent terminal-damage for the treatment consisting of just *S. multiplicalis* was highest in August and corresponded with one of the highest number of larvae observed during sampling in both 2005 and 2006. For the same treatment, we observed a decline in the biomass of common salvinia in September of both years, which may have been a result of injury to the terminal buds caused by larval feeding in August. Although feeding by *S. multiplicalis* larvae may damage terminal buds and slow growth of common salvinia, the impact is not as severe as that caused by the internal feeding of *C. salviniae* larvae, which cause the rhizomes to break apart, thus preventing further spread by fragmentation. As a result, common salvinia can rebound even after heavy infestation by *S. multiplicalis* once larval feeding has declined.

TABLE 1. THE NUMBER OF *C. SALVINIAE* ADULTS AND *S. MULTIPLICALIS* LARVAE OBSERVED DURING SAMPLING AT GRAMERCY, LA IN 2005.

Treatment	Jun		Jul		Aug		Sep		Oct		Total[a]	
	S.m[c]	C.s[d]									S.m	C.s
S[b]	34[e]	0	8	0	22	0	3	0	7	0	74	0
C	2	0	4	5	1	7	1	6	0	4	8	22
S+C	9	0	1	0	7	1	2	3	8	2	27	6
Control	8	0	0	0	7	0	0	0	0	0	15	0

[a]Sum of a row.
[b]S = *Samea multiplicalis*; C = *Cyrtobagous salviniae.*
[c]Number of *S. multiplicalis* larvae belonging to all instars.
[d]Number of *C. salviniae* adults.
[e]Each value in the table represents the total number of *C. salviniae* adults and/or *S. multiplicalis* larvae (all instars) observed during inspecting the haphazardly picked 100 common salvinia plants for terminal damage from the four replicate plots of each treatment.
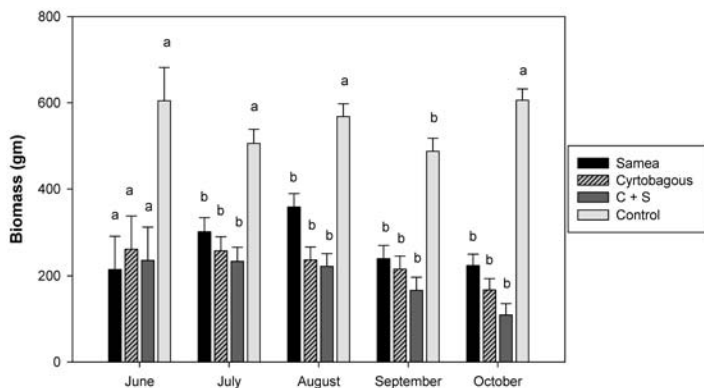
Figure 4. Least-squares mean biomass (with standard error) of common salvinia in different herbivore treatments at Gramercy, LA in 2006. For each month, treatments with the same letters were not statistically distinguishable (Tukey-Kramer, α = 0.05).

Figure 6. Least-squares mean percent mat-green (with standard error) of common salvinia in different herbivore treatments at Gramercy, LA in 2006. For each month, treatments with the same letters were not statistically distinguishable (Tukey-Kramer, α = 0.05).

The number of *S. multiplicalis* larvae observed in the treatment plots varied over time in 2005, and a similar but nonsignificant trend was also recorded in 2006. Common salvinia was available at all the treatment plots for larval feeding throughout the sampling period and does not seem to be a factor in observed population fluctuations of the herbivore. We believe this may be a result of natural population cycles of the *S. multiplicalis*, which seems to do better in spring and fall (S. Johnson, pers. observ.). Parasitism of *S. multiplicalis* larvae may also be responsible for the observed trend. During the course of this study, some *S. multiplicalis* larvae collected from the field and reared in the lab were found to be parasitized by a braconid wasp. Knopf and Habeck (1976) reared 4 parasitoids (3 ichneumonids and 1 tachinid) from *S. multiplicalis* larvae in Florida. Semple and Forno (1987) mentioned the recovery of 5 parasitoids and 3 pathogens from *S. multiplicalis* larvae in Queensland, Australia. Taylor and Forno (1987) reported that *S. multiplicalis* females avoided ovipositing on plants damaged from earlier feeding, and the resulting dispersal was another reason for the failure of this herbivore as a biological control agent of giant salvinia in Australia (Briese 2004).
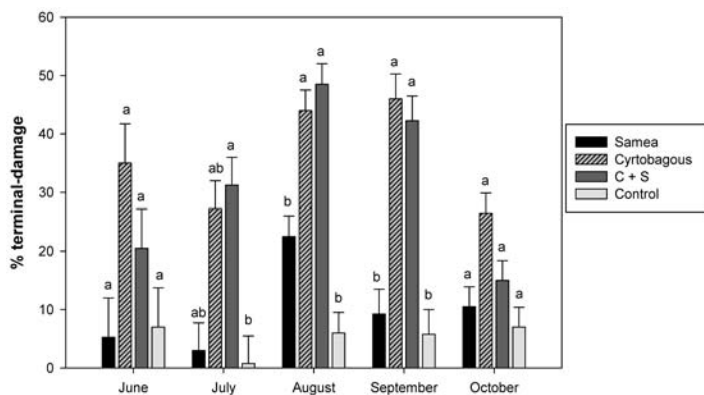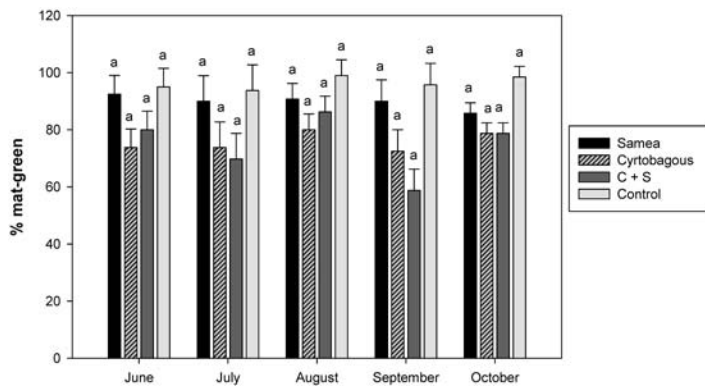

Figure 5. Least-squares mean percent terminal-damage (with standard error) on common salvinia in different herbivore treatments at Gramercy, LA in 2006. For each month, treatments with the same letters were not statistically distinguishable (Tukey-Kramer, α = 0.05).

Water-lettuce (*Pistia stratiotes*) is another aquatic plant utilized by *S. multiplicalis* larvae, and its presence at our research site may have influenced the number of larvae observed inside the treatment plots in both 2005 and 2006. However, neither the oviposition preference of *S. multiplicalis* females nor the feeding behavior of different instar larvae when multiple host plants occur together has been studied in Louisiana. Although not experimentally established in our study, red imported fire-ants (RIFA; *Solenopsis invicta* Buren) could have negatively impacted *S. multiplicalis* populations. RIFA workers were frequently observed foraging on common salvinia mats infested with *S. multiplicalis*, and RIFA mounds were noticed at the base of trees in flooded woodlands. RIFA impact the populations of a number of lepidopteran insect species (eggs, larvae, and adults) in different aquatic and terrestrial habitats (Reagan et al. 1972, McDaniel and Sterling 1979, Eger et al. 1983, Elvin et al. 1983, Dray et al. 2001, Eubanks 2001, Seagraves and McPherson 2006).

The fewer *S. multiplicalis* larvae observed inside the treatment plots in 2006 as compared to 2005 may have been a result of environmental factors like rainfall. The average rainfall recorded for May and June in 2006 (2.45 and 1.34 in, respectively) was low compared to the same months in 2004 (9.48 and 10.46 in) and 2005 (7.70 and 6.59 in) at Lutcher, Louisiana (SRCC 2009), about 16 km from the research site. Common salvinia is a floating plant that is totally dependent on water levels (Tipping and Center 2005a), especially in shallow flooded woodlands. Low rainfall in 2006 (May and Jun) may have impacted common salvinia infestations at our research site, and possibly *S. multiplicalis* populations, the source of larvae for our plots.

In our study, *C. salviniae* adults released in 2005 were not recovered at the 8 weevil treatment plots in 2006 and consequently had to be replaced. The minimum air temperature recorded at Reserve, Louisiana (SRCC 2009), about 30 km from the research site, was below freezing point (0 C) for one day in January 2006 and 2 consecutive days in February 2006. Exposure to these extreme conditions may have negatively impacted the survival of *C. salviniae* at our research site. However, Tipping and Center (2003) reported that *C. salviniae* adults of Brazilian population (imported from Australia)

TABLE 2. THE NUMBER OF *C. SALVINIAE* ADULTS AND *S. MULTIPLICALIS* LARVAE OBSERVED DURING SAMPLING AT GRAMERCY, LA IN 2006.

Treatment	Jun		Jul		Aug		Sep		Oct		Total[a]	
	S.m[b]	C.s[c]									S.m	C.s
S[d]	10[e]	0	1	0	10	0	6	0	0	0	27	0
C	1	15	1	16	0	11	0	21	0	10	2	73
S+C	2	8	3	18	3	11	2	13	0	10	10	60
Control	0	0	0	0	1	0	1	0	0	0	2	0

[a]Sum of a row.
[b]S = *Samea multiplicalis*; C = *Cyrtobagous salviniae*.
[c]Number of *S. multiplicalis* larvae belonging to all instars.
[d]Number of *C. salviniae* adults.
[e]Each value in the table represents the total no of *C. salviniae* adults and/or *S. multiplicalis* larvae (all instars) observed during inspecting the haphazardly picked 100 common salvinia plants for terminal damage from the four replicate plots of each treatment.

were able to over-winter on giant salvinia in Texas and Louisiana under adverse conditions with temperatures falling below 0 C on multiple days. The Toledo Bend Reservoir release site in the aforementioned study (Tipping and Center 2003) is approximately 275 mi north of our study location and raises the possibility of establishing the Brazilian population of *C. salviniae* for controlling common salvinia in Louisiana. Although no study to date has documented the impact of Brazilian *C. salviniae* on common salvinia in Louisiana, Tipping and Center (2005b) cautioned that the larger size of Brazilian weevils (both adult and larvae) may limit their ability to utilize relatively smaller common salvinia plants with narrow rhizomes.

As a result, we could not document the impact of *C. salviniae* from one year to the next at our research site. For the majority of the sampling period in both 2005 and 2006, however, the treatment with both *S. multiplicalis* and *C. salviniae* had the least biomass of common salvinia, and unlike the treatment with only *S. multiplicalis*, we observed a progressive decline in biomass when both the herbivores were present. The impact of internal feeding on the rhizomes of common salvinia by *C. salviniae* larvae was evident in the browning of individual plants, reflected in lower values of percentage mat-green recorded on most sampling dates. Percent terminal-damage for the treatment consisting of only *C. salviniae* in both 2005 and 2006 increased from July to September before declining in October. *Cyrtobagous salviniae* adults are capable of walking and flight dispersal (Tipping and Center 2005a), and this behavior may have resulted in reduced feeding on common salvinia inside the treatment plots in October and thus a decline in percent terminal-damage. Weevil adults were also observed outside the treatment plots toward the end of sampling period in both 2005 and 2006. Dispersal of weevils from the treatments plots may have resulted in a higher number of adults being recorded in the treatment consisting of only *C. salviniae* when compared to the treatment with both the herbivores in 2005. We released fewer weevil adults at the beginning of study in 2005, and this too may have contributed to the aforementioned result (more adults recorded in *C. salviniae* treatment only) because in 2006 we did not detect any difference in the number of *C. salviniae* adults between the 2 treatments. As a result of our experimental design, destructive sampling was not possible, and we were unable to determine the number of *C. salviniae* adults per unit weight of common salvinia or per unit area of our treatment plots. Tipping and Center (2005a) projected *C. salviniae* to exceed more than 100 adults per square meter, a number they suggested was sufficient to control common salvinia in south Florida. In closely related giant salvinia, Room (1988) estimated that 300 adults and 900 larvae of *C. salviniae* per square meter could effectively control most infestations.

Although feeding by the herbivores had an impact on the biomass of common salvinia, we did not detect any difference among the treatments in terms of area inside the plot that was covered with common salvinia, a result we attribute to its aggressive vegetative reproduction. Environmental variables such as pH and surface water temperature also did not show treatment effect in our study. The size of our plot was relatively small (1 m^2) in comparison to the common salvinia infestation at the research site, and in some cases these plots were surrounded by other aquatic vegetation (in addition to common salvinia). Any treatment effects, if they occurred, were probably obscured by the impacts of surrounding vegetation on the water quality of plots.

This study was able to show that although *S. multiplicalis* exhibits seasonal variations in its population dynamics, it still had a significant impact on the biomass of common salvinia in south Louisiana. The findings thus indicate that *C. salviniae* would be an ideal biological control agent to complement the native herbivore *S. multiplicalis*. *Cyrtobagous salviniae*, with both larvae and adults feeding on common salvinia, may ultimately turn out to be a better control agent than *S. multiplicalis* because common salvinia can multiply at exceedingly fast rates, and constant feeding pressure must be maintained to have any kind of long term impact on its growth and spread. The gap between successive larval generations of *S. multiplicalis* most likely gives common salvinia an opportunity to rebound from feeding injury, and even high populations of the herbivore at certain times of the year (spring and fall) seem to have only an occasional impact on its growth and spread. The feeding characteristics of *C. salviniae* are thus better suited to our objective of controlling common salvinia.

Biological control agents can provide a sustainable, economical and environmentally sound alternative to chemical control of common salvinia. In the absence of biological control efforts, common salvinia will continue to remain a nuisance aquatic weed and spread unchecked in the numerous fresh waterways throughout Louisiana and neighboring states of Arkansas, Mississippi, and Texas.

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