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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 preoviposition 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 m^2 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 adversely 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 m^2, built with 2.5 cm dia PVC pipes, were haphazardly placed inside the 1 m^2 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 m^2 plot. In addition, 100 common salvinia plants were haphazardly selected at each sampling date from inside the 1 m^2 plot to check for damage to the terminal buds due to herbivore feeding (percent terminal-damage). The total number of *C. sal*viniae 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 m^2 plot after determination of percent terminal-damage. The area inside each 1 m^2 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 m^2 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 m^2 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 m^2 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

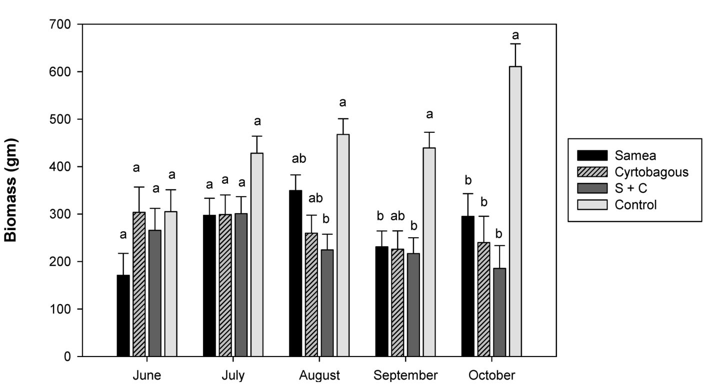

Figure 1. Least-squares mean biomass (with standard error) of common salvinia in different herbivore treatments at Gramercy, LA in 2005. For each month, treatments with the same letters were not statistically distinguishable (Tukey-Kramer, α = 0.05).

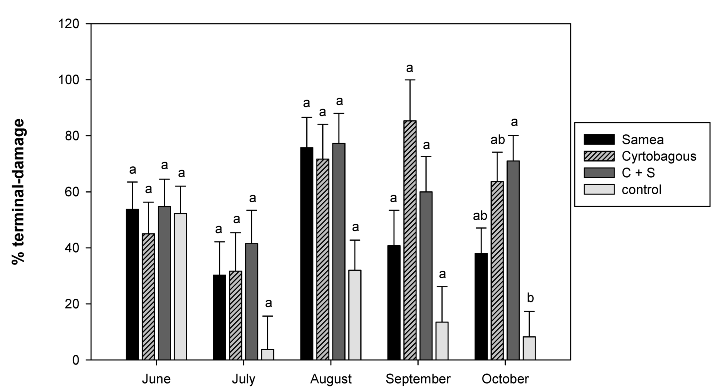

Figure 2. Least-squares mean percent terminal-damage (with standard error) on common salvinia in different herbivore treatments at Gramercy, LA in 2005. For each month, treatments with the same letters were not statistically distinguishable (Tukey-Kramer, α = 0.05).

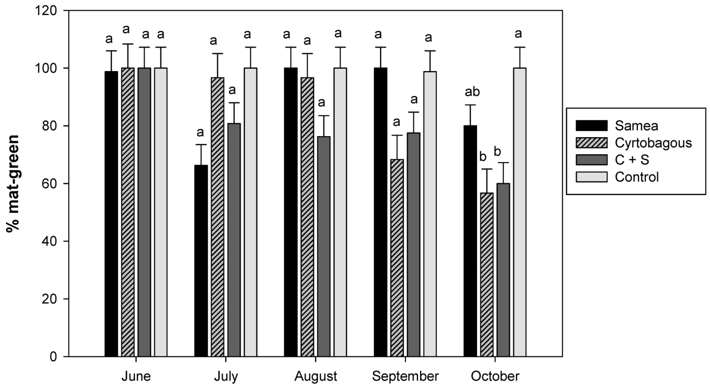

Figure 3. Least-squares mean percent mat-green (with standard error) of common salvinia in different herbivore treatments at Gramercy, LA in 2005. For each month, treatments with the same letters were not statistically distinguishable (Tukey-Kramer, $\alpha = 0.05$).

= 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.

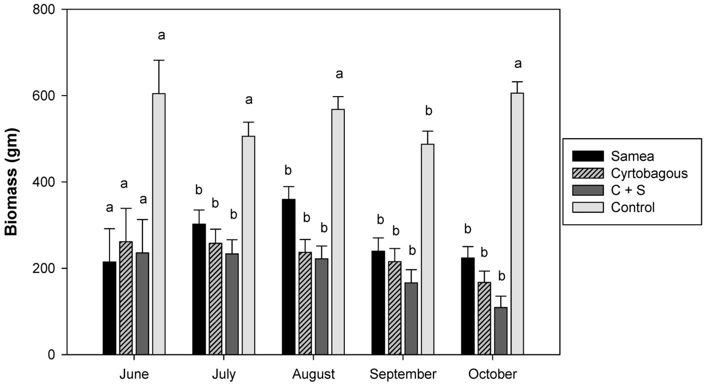

Figure 4. Least-squares mean biomass (with standard error) of common salvinia in different herbivore treatments at Gramercy, LA in 2006. For each month, treatments with the same letters were not statistically distinguishable (Tukey-Kramer, $\alpha = 0.05$).

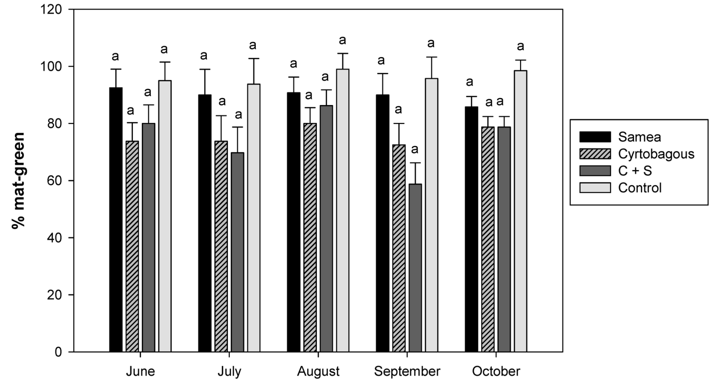

Figure 6. Least-squares mean percent mat-green (with standard error) of common salvinia in different herbivore treatments at Gramercy, LA in 2006. For each month, treatments with the same letters were not statistically distinguishable (Tukey-Kramer, $\alpha = 0.05$).

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).

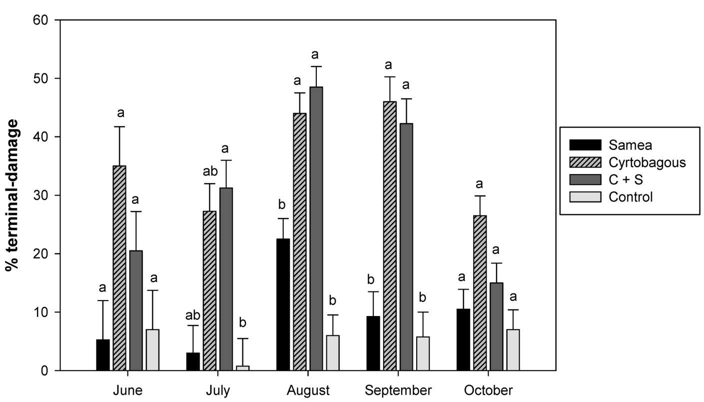

Figure 5. Least-squares mean percent terminal-damage (with standard error) on common salvinia in different herbivore treatments at Gramercy, LA in 2006. For each month, treatments with the same letters were not statistically distinguishable (Tukey-Kramer, $\alpha = 0.05$).

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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