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Authors: Parys, Katherine A., and Johnson, Seth J.

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BIOLOGICAL CONTROL OF COMMON SALVINIA (*SALVINIA MINIMA*) IN LOUISIANA USING *CYRTOBAGOUS SALVINIAE* (COLEOPTERA: CURCULIONIDAE)

Katherine A. Parys[1]* and Seth J. Johnson[2]
[1]USDA-ARS, Southern Insects Management Research, 141 Experiment Station Road, Stoneville, MS 38776, USA

[2]Department of Entomology, Louisiana State University Agricultural Center, 404 Life Sciences Building, Baton Rouge, LA 70803, USA

*Corresponding author; E-mail: katherine.parys@ars.usda.gov

Abstract

Common salvinia, *Salvinia minima* Baker, is an aquatic invasive fern that obstructs waterways and impacts water quality throughout the southeastern United States. In an effort to establish populations for classical biological control of this weed, the weevil, *Cyrtobagous salviniae* Calder and Sands, was released at multiple sites across Louisiana. Many of the release sites were lost due to a variety of ecological and anthropological disturbances. In 2008, *C. salviniae* was found to have successfully overwintered on *S. minima* in Gramercy, Louisiana. Attack by *Cyrtobagous salviniae* significantly increased the number of damaged terminal buds and decreased the fresh weight biomass of *S. minima*.

Key Words: classical biological control, aquatic weed, invasive species

Resumen

La salvinia común, *Salvinia mínima Baker*, es un helecho acuático invasor que obstruye vías fluviales y afecta la calidad de agua por el sureste de los Estados Unidos. Para fundar poblaciones de control biológico clásico el gorgojo *Cyrtobagous salviniae* Calder y Sands fue introducido en múltiples sitios por toda Louisiana. Fueron perdidos muchos sitios de introducción debido a varias alteraciones ecológicas y antropológicas. En 2008 fue notado que *C. salviniae* invernó exitosamente en *S. mínima* en Gramercy, Louisiana. El ataque de *Cyrtobagous salviniae* aumentó apreciablemente la cantidad de brotes terminales dañados y disminuyó la biomasa fresca de *S. minima*.

Palabras Clave: biológico clásico, maleza acuática, especie invasora

Common salvinia, *Salvinia minima* Baker (Salviniales: Salviniaceae), is a free floating aquatic invasive fern native to South America in the family Salviniaceae, and one of over ten species which occur worldwide in the genus *Salvinia* (Mitchell 1972). Infestation by *S. minima* has spread across the southeastern U.S. from an initial introduction in the St. Johns river in Florida during the late 1920's (Small 1931; Jacono et al. 2001). Established populations of *S. minima* are currently recorded from 14 states, and infestations are considered problematic in both Texas and Louisiana (Jacono et al. 2001; USDA & NRCS 2011). *Salvinia minima* has a history of being sold in the nursery trade, and is still widely available on the internet in spite of its invasive nature (Forno et al. 1983; Kay & Hoyle 2001).

Commonly misidentified in older literature, *S. minima* has been labeled as: *S. rotundifolia* Willdenow, *S. natans* Seguir, or *S. auriculata* Aublet (Jacono et al. 2001). *Salvinia minima* can be differentiated from its relative *Salvinia molesta* Michell by the presence of divided hairs on the abaxial leaf surface that are free and not joined at the tips (Mitchell 1972; Julien et al. 2002). Genetic analysis revealed that all the *S. minima* in the southern United States is closely related with the exception of a more recent introduction into Mississippi (Madeira et al. 2003).

Salvinia minima prefers lentic freshwater areas, especially marshes and low-lying forested woodlands. Louisiana has over 664,898 ha of freshwater marshes and 2,783,023 ha of forested wetlands, all of which could be susceptible to infestation by *Salvinia* spp.(Coreil 1993). Like other aquatic weeds, *S. minima* can easily spread between water bodies if boats and vehicles are not properly cleaned (Johnstone et al. 1985; Miller & Wilson 1989; Jacono 2003). Weather can also contribute to the spread of *Salvinia* spp., as mats fragment when flooding occurs (Harley & Mitchell 1981; Room 1983, 1990).

Uncontrolled *S. minima* forms dense mats of plant material that decrease aesthetic value and limit use of aquatic areas (Montz 1989). Infestations obstruct waterways, decrease light availability, reduce available dissolved oxygen, and alter pH levels (Hatch 1995; Flores & Carlson 2006). Persistence of these mats also raises human health issues, as *Salvinia* spp. provides ideal habitat for *Mansonia* spp. (Diptera: Culicidae) which have been identified as vectors in the spread of West Nile Virus, St. Louis Encephalitis and Venezuelan Equine Encephalitis (Chow et al. 1955; Ramachandran 1960; Lounibos et al. 1990). Several species of biting midges (Diptera: Ceratopogonidae) have also associated with *Salvinia* infestations (Buckingham & Balciunas 1994; Borkent & Craig 2001). Utilization of freshwater resources for activities like fishing, migratory bird hunting, and alligator harvests contributed a total positive economic effect of over US$ 1.2 billion to the state of Louisiana in 2006, making them an important asset to protect (Southwick Associates 2008).

Control options for *S. minima* include chemical control, mechanical control, and biological control. Chemical control is non-selective and the price for herbicide application can range from $198 to $297/ha (Tewari & Johnson 2011). Mechanical control is not feasible as fragmentation of plants results in additional vegetative growth, and removal by hand is impossible in most areas. Classical biological control programs using *Cyrtobagous salviniae* Calder and Sands (Coleoptera: Curculionidae) have been successful against *S. molesta* and were significantly less expensive than other available methods of control (Chikwenhere & Keswani 1997).

Cyrtobagous salviniae is a semi-aquatic weevil native to Brazil, Paraguay, and Bolivia, which has been introduced into 16 countries worldwide for control of *S. molesta* (Wibmer & O'Brien 1986; Julien & Griffiths 1998; Julien et al. 2002). While widely released to control *S. molesta*, *C. salviniae* also feeds on *S. minima* (Tipping & Center 2005). *Cyrtobagous salviniae* was originally misidentified as *Cyrtobagous singularis* Hustache, and a difference in size was noted between *C. salviniae* populations from North and South America (Kissinger 1966; Calder & Sands 1985). Recent studies by Madeira et al. (2006) suggested that the size difference between populations is more likely a case of 2 different ecotypes. The smaller Florida ecotype damages both *S. minima* and *S. molesta* more extensively than the Brazilian ecotype (Tipping et al. 2010).

Cyrtobagous salviniae is credited with keeping *S. minima* under control in Florida while explosive growth in Texas and Louisiana has been attributed to the absence of the weevil (Jacono et al. 2001; Tipping et al. 2012). The Florida ecotype of *C. salviniae* was initially introduced into Texas and Louisiana in 1999 to control infestations of *S. molesta,* while the Brazilian ecotype was introduced beginning in 2001 (Goolsby et al. 2000; Tipping & Center 2003; Tipping et al. 2008). Prior releases of *C. salviniae* into infestations of *S. minima* from between 2002-2005 were considered to have failed and overwintering had not been previously observed. The goal of this project was to successfully introduce and establish a population of *C. salviniae* that would overwinter in Louisiana and control infestations of *S. minima*.

Materials and Methods

Origins of *Cyrtobagous salviniae* Populations

A colony of *C. salviniae* was established at Louisiana State University (LSU) using a founding group of 300 individuals, and reared in a greenhouse on the main campus in Baton Rouge beginning in 2002. Initial populations of the Florida ecotype of *C. salviniae* were collected in 2002 by Dr. Phil Tipping (United States Department of Agriculture-Agricultural Research Service) from the Ft. Lauderdale area in southern Florida. Additional shipments of weevils collected from the same area were mailed in 2004, 2005, and 2006 to bolster the number of individuals in the colony at LSU.

A second colony using *C. salviniae* from northern Florida was established in a separate greenhouse during the fall of 2005. Founding individuals were collected during a trip made in September 2005 to Lake Talquin near the town of Quincy in northern Florida. Approximately 200 *C. salviniae* were collected by hand while an additional 600 individuals were recovered by Berlese funnel, and returned to LSU. Field collections to augment the second colony were again made in northern Florida from Lake Talquin (2005-2006) and Lake Miccosukee (2007-2008). Approximately 1,400 weevils were collected from Lake Talquin in 2006, while an additional 2,000 weevils were collected by a combination of hand picking and Berlese funnels from Lake Miccosukee in both 2007 and 2008.

Cyrtobagous salviniae Release Locations

In Louisiana, releases of *C. salviniae* onto infestations of *S. minima* have been made by 3 groups: Louisiana State University (LSU), the United State Department of Agriculture Agricultural Research Service's Invasive Plant Research Laboratory (USDA-ARS), and the Louisiana Department of Wildlife and Fisheries (LaDWF). LSU released 11,426 *C. salviniae* at 11 locations between 2003 and 2010 across southern Louisiana (Table 1; Fig. 1). USDA-ARS made several releases at the Barataria Preserve of Jean Lafitte National Historical Park and Preserve (N 29°48'17" W 90°07'09") between 2002 and 2005. LaDWF made releases at 16 additional locations in 2007 and 2008 (Fig. 1) (A. Perret, LaDWF, personal communication).

TABLE 1. LOCATIONS IN LOUISIANA WHERE *CYRTOBAGOUS SALVINIAE* HAS BEEN RELEASED BY LOUISIANA STATE UNIVERSITY. THE NUMBER OF RELEASE PLOTS AT EACH LOCATION IS LISTED IN PARENTHESES NEXT TO THE NAME OF THE LOCATION. THE NUMBER OF *C. SALVINIAE* PRESENTED IS PER PLOT (WITH THE TOTAL NUMBER OF *C. SALVINIAE* RELEASED AT EACH LOCATION OVER THE YEAR PRESENTED ON THE LINE BELOW IN PARENTHESES). RELEASES IN 2003 AND 2004 WERE MADE BY JOHNSON, RELEASES IN 2005 WERE MADE BY TEWARI AND JOHNSON, THOSE IN 2006 BY TEWARI, JOHNSON, AND PARYS, AND THOSE IN 2007-2008 BY PARYS AND JOHNSON.

Release Location (# of plots)	2003	2004	2005	2006	2007	2008
Henderson Swamp (1) 30°28'02"N, 91°40'01"W	150 (150)	—	—	—	—	—
St. James (1) 29°58'23"N, 90°53'31"W	150 (150)	100 (100)	—	275 (275)	—	—
Joyce Wildlife Management Area (1) 30°28'14"N, 90°25'30"W	150 (150)	275 (275)	—	275 (275)	—	—
Maurepas Wildlife Management Area (1) 30°07'27"N, 90°46'15"W	150 (150)	309 (309)	—	275 (275)	—	—
McElroy Swamp (1) 30°19'90"N, 90°45'36"W	—	315 (315)	—	—	—	—
Cypress Lake (1) 30°19'00"N, 93°17'08"W	—	307 (307)	—	50 (50)	—	—
Gramercy (multiple plots) 30°11'04"N, 90°49'08"W	—	—	90 (8) (720)	150 (10) (1500)	500 (3) (1500)	300 (3) (900)
Alligator Bayou (1) 30°18'36"N, 91°00'57"W	—	—	—	275 (275)	—	—

The primary study site for this study was a 4,000 ha tract of private property located just north of Gramercy, Louisiana. Of the sites listed in Table 1, Gramercy was used from 2005-2010, which had the longest continuous usage out of the 11 release sites used by LSU. The Gramercy site was initially used as a release site for a previous study on the combined effectiveness of *C. salviniae* and *Samea multiplicalis* (Guenée) (Lepidoptera: Crambidae) for the control of *S. minima* from 2005-2006 (Tewari & Johnson 2011).

The 10 additional sites where LSU released *C. salviniae* to control infestations of *S. minima* faced a variety of challenges (Table 1). During August 2003, LSU released *C. salviniae* from southern Florida at 4 sites: Henderson Swamp, St. James, Joyce Wildlife Management Area (WMA), and Maurepas WMA. In early 2004, 3 of the 4 sites showed no signs of *C. salviniae* and Henderson Swamp was not sampled due to inaccessibility. The use of Henderson Swamp as a field site was discontinued in 2004, and 2 new locations were established at McElroy Swamp and Cypress Lake with southern Florida weevils. Additional releases of *C. salviniae* were made in 2006 with southern and northern Florida weevils at Joyce WMA, Maurepas WMA, St. James, Cypress Lake, and Alligator Bayou. Two of these sites were discarded after the 2006 field season: Alligator Bayou became infested with water hyacinth (*Eichornia crassipes* (Mart.) Solm.) (Commenales: Pontederiaceae) by the fall and the site at Joyce WMA was destroyed by vandalism. Releases after 2006 were made only with northern Florida weevils. Releases made in Vacherie and Hammond (2008-2010) were lost to decreased water levels, while the releases made in Tunica are detailed further in Parys & Johnson (2012).

Sampling

Research plots at sites used by LSU for releasing *C. salviniae* were established using 1 m^2 experimental quadrats constructed from 5.08 cm diam SCH 40 PVC (polyvinyl chloride). These floating quadrats were sealed to float and served as experimental plots. Two of these plots were placed at each field location, at least 500 m apart in similar habitat areas, and anchored with a nylon rope tied to 2 bricks. This allowed repeated sampling of the same location. Of the paired plots, one was designated as a *C. salviniae* release while the other was maintained as a control. Any vegetation other than *S. minima* found growing in the plots was removed monthly by hand weeding. Plots were sampled monthly with a few exceptions. A total of 36 sampling dates were used over 5 years: Jul-Sep 2006, Jun 2007-Aug 2008, Mar-Oct 2009, and May-Dec

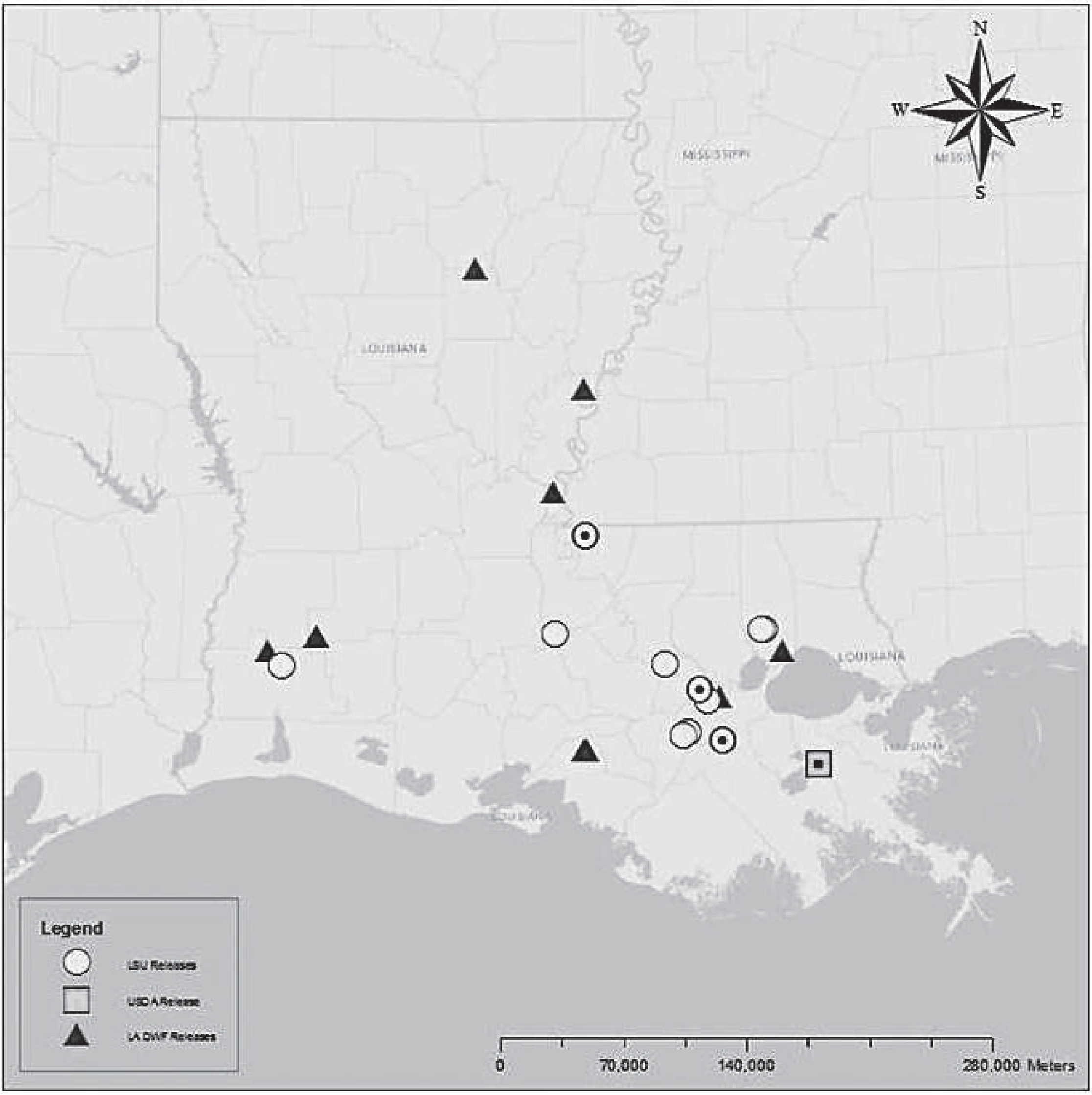

Fig. 1. Map showing *Cyrtobagous salviniae* release locations across Louisiana. Locations where overwintering was confirmed contain a mark in the center of the symbol. Several releases made by Louisiana Department of Wildlife and Fisheries were so close that they do not appear as separate symbols.

2010. Access to field sites was limited during fall 2008 after Hurricanes Gustav and Ike backed up several feet (about 1 m) of storm surge onto the property in Gramercy where release plots were located. The PVC frames survived both hurricanes.

To sample each plot, three 0.1 m^2 smaller floating quadrats were constructed from 2.5 cm diam SCH 40 PVC. These were haphazardly placed into the larger 1 m^2 plot. All *S. minima* plant material was removed from inside each smaller quadrat, placed in a plastic bag and hand squeezed to remove excess water prior to recording biomass. All plant material was returned to the plot after sampling. The relationship between wet and dry biomass of *S. minima* was determined by Tewari & Johnson (2011) to eliminate the need for destructive sampling within the plots.

To evaluate and quantify damage from herbivores, 100 stems of *S. minima* were haphazardly selected from each plot and terminal buds were examined for signs of feeding. *Cyrtobagous salviniae* was considered established at field locations when adults and/or characteristic feeding damage were observed more than 2 months following the release. Weevils were considered overwintered and established when adults were observed in a plot in the spring, after the winter.

Presence and number of herbivores including *C. salviniae, S. multiplicalis*, and *Synclita obliteralis* (Walker) (Lepidoptera: Crambidae) were noted and counted when observed on terminal buds. All plant material and insects were returned to the plot after monthly sampling.

Each plot was also evaluated by visual estimation for percent surface coverage by *S. minima*. Discoloration of *S. minima* mats have been previously associated with herbivore damage and stressed plants, similar to *S. molesta* (Room et al. 1981). Percentage of area inside the plot that appeared green was visually estimated to the nearest 10%. Measurements for surface water temperature, dissolved oxygen, pH, and conductivity were taken using a handheld meter with the probes located just under the surface of the *S. minima* mat at each sampling date (YSI 556 Multi Probe System; PCSTestr 35 Multi Parameter Meter). To eliminate confounding effects of feeding by native lepidopteran herbivores *S. multiplicalis* and *S. obliteralis,* all plots were sprayed with Thuricide weekly (*Bacillus thuringiensis kurstaki,* equivalent to 4,000 *Spodoptera* units or 6×10^6 viable spores per mg). *Bacillus thuringiensis kurstaki* is specific to Lepidoptera and has no reported effects on Coleoptera (MacIntosh et al. 1990).

Statistical Analysis

The data analysis for this paper was generated using SAS software, Version 9.2 of the SAS System for Windows. A mixed linear model was used to evaluate and compare data from release and control plots collected between 2006-2010. Treatment effect of the weevil introduction was evaluated through Analysis of Variance (ANOVA) using the REPEATED statement with autoregressive heterogeneous variances in PROC MIXED. When a treatment was significant, means were separated into letter groups by Fisher's Protected LSD Test at alpha = 0.05 using the PDMIX800 Macro (Saxton 1998). Data was analyzed both as a single large dataset (2006-2010), as well as individually by year (2006, 2007-2008, 2009, 2010), with data from 2007-2008 presented as one "year," since sampling was continuous throughout the time frame.

The loss of study sites due to environmental change and human interference reduced our planned statistical power within the study, so sites were pooled by treatment for analysis. Data presented from 2006 was collected from Joyce WMA, St. James, and Alligator Bayou, data from 2007 was collected from St. James and Gramercy, and data from 2008-2010 was from Gramercy. Differences in the number of terminal buds damaged between treatment and control, surface water temperature, dissolved oxygen, pH, and conductivity were analyzed by t-test.

Results

Impact of *Cyrtobagous salviniae* Releases

Throughout the study, the mean fresh weight biomass of *S. minima* in Gramercy varied greatly, ranging from a minimum average weight of 80.22 g/0.1 m^2 (release plots, May 2010) to a maximum average of 679 g/0.1 m^2 (control plots, Jul 2009). Analysis indicated that introducing *C. salviniae* into our field sites significantly decreased the fresh weight biomass of *S. minima* over the entire course of our study ($F = 58.36$, df = 1,4, $P = 0.0016$) (Fig. 2A). When broken up by year, 2006 ($F = 10.58$, df = 1,4, $P = 0.0313$), 2007-2008 ($F = 55.58$, df = 1,4, $P = 0.0017$), and 2009 ($F = 58.36$, df = 1,4, $P < 0.0001$) all showed a significant treatment effect (Fig. 2B-D). Biomass between release and control plots during 2010 was not significantly different ($F = 6.54$, df = 1,4, $P = 0.0628$) (Fig. 2E).

Plots that had *C. salviniae* introduced had a significantly higher number of *S. minima* terminal buds damaged than control sites ($t = 5.572$, df = 70, $P < 0.0001$) (Fig. 3). The mean number of terminal buds damaged ranged from a high of 66/100 (release plots, Sep 2007) to a low of 1.5/100 (control plots, Jan-Mar 2008). None of the other variables monitored as part of the study were significantly different between release and control plots: percentage of the mat covering the quadrat ($t = -0.92$, df = 70, $P = 0.358$), percentage of the mat that was green ($t = -1.164$, df = 70, $P = 0.112$), pH ($t = 1.161$, df = 70, $P = 0.25$), dissolved oxygen ($t = 0.564$, df = 70, $P = 0.588$), conductivity ($t = 1.172$, df = 70, $P = 0.254$), or temperature at the water's surface ($t = -0.487$, df = 70, $P = 0.28$).

Overwintering *Cyrtobagous salviniae*

The first *C. salviniae* known to have successfully established and overwintered on *S. minima* in Louisiana were observed at a single plot in Gramercy on 14 Apr 2008. Additional individuals were seen at the first plot and a second plot in Gramercy on 17 Jun 2008 along with visible damage to the *S. minima* mat, leaving patches of open water. Plant material from the plots where individual *C. salviniae* were observed was brought back to the laboratory and processed through a Berlese funnel for a more thorough population evaluation. The 2 release plots where *C. salviniae* were observed had 12 and 136 adult weevils per kg of *S. minima* in Jun 2008.

Cyrtobagous salviniae successfully overwintered at all 3 plots in Gramercy that were used in 2008-09. In the spring of 2009, we recovered 18 *C. salvinae*/kg of *S. minima* from one of the

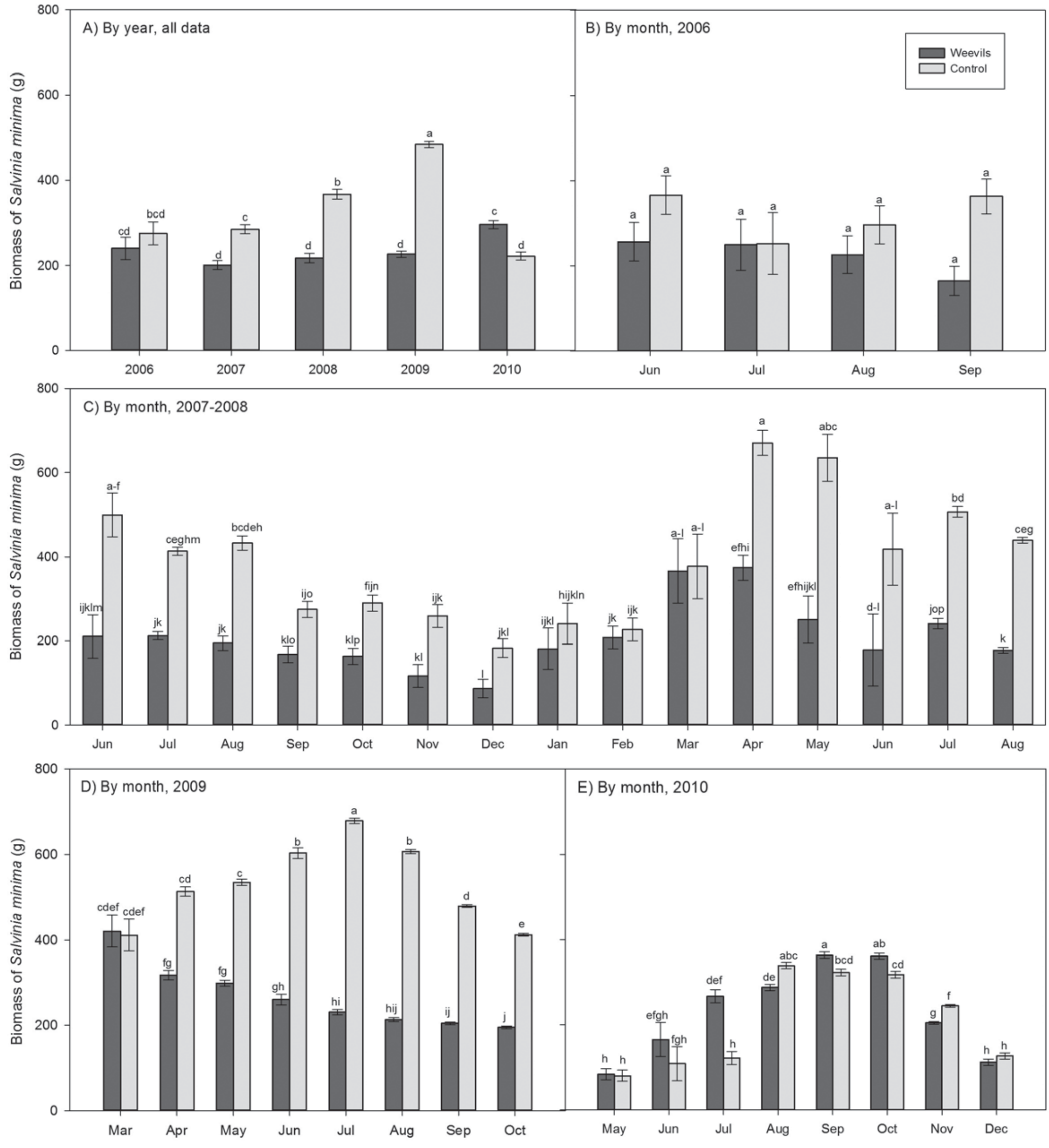

Fig. 2. Average *Salvinia minima* biomass grams per 0.1 m^2 area removed from both treatment and control quadrats in Gramercy, Louisiana. Data presented are from Jul 2006-Dec 2010.

release sites. Sampling at Gramercy was continued throughout 2009 until freezing temperatures sank the majority of the mat of *S. minima*. Throughout Nov and Dec 2009 low air temperatures with frosts occurred, culminating in a low of -5 °C on 11 Jan 2010. Our water surface temperature data recorder for Gramercy was lost during the winter for 2009/2010. The closest USGS data available lists water surface temperatures of 2.8 °C for 13, 14, 15, and 18 Jan 2010 (54 km from research site) (USGS 2010). The mat did not rebound from the freezing temperatures to cover the water's surface until Jun 2010.

During the summer of 2008, additional releases had been planned in the Jean Lafitte National Park's Barataria Preserve at the request of the National Park Service. Prior to the scheduled release on 18 Jul 2008, adult *C. salviniae* were unexpectedly observed during preliminary sampling on *S. minima* in the Twin Canals area of

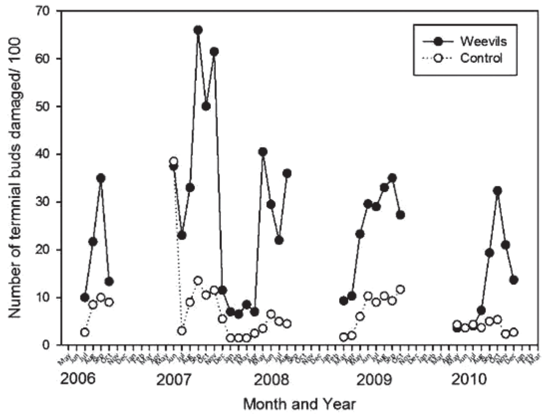

Fig. 3. Average number of terminal buds of *Salvinia minima* damaged per 100 randomly checked from both treatment and control sites in Gramercy, Louisiana. Data presented are from Jul 2006- Dec 2010.

the park. These *C. salviniae* are assumed to be a residual population from the USDA's original releases made in the area from 2002-2005, though *C. salviniae* had also been released in other areas of the park to control *S. molesta*. Adult *C. salviniae* were again observed 19 May 2009 at another location within the park, Bayou Coquille, with 4 *C. salviniae*/kg of *S. minima*.

Discussion

We suspected original introductions of *C. salviniae* in Louisiana made between 2003 and 2005 from southern Florida near Ft. Lauderdale may not have been temperately adapted to the cooler climate in Louisiana, or initial release numbers were too low to adequately establish populations. Introductions using *C. salviniae* from northern Florida were begun in 2006, assuming that they would be better adapted to the local climate. *Cyrtobagous salviniae* from northern Florida were found to have successfully established on *S. minima* in Louisiana beginning in 2007 and were found through 2010. Additionally, *C. salviniae* were discovered in low numbers at Jean Lafitte National Park beginning in 2008, suggesting that original releases by the USDA established in low numbers but had not been successful in controlling *S. minima*.

While *C. salviniae* adults were not recovered in Gramercy during the spring of 2010, the mat of *S. minima* was sparse and never recolonized to the pre-freeze extent present in previous years. Most of the former mat's area was lost to waterhyacinth invasion (*E. crassipes*). *Salvinia* infestations are warmer than the surrounding air, suggesting that regardless of the freeze *C. salviniae* could still be present in low numbers (Room & Kerr 1983). *Cyrtobagous salviniae* has also successfully established on *S. molesta* here in Louisiana and in areas of temperate Australia that experience similar winter temperatures and conditions to those recorded here in Louisiana (Tipping & Center 2003; Sullivan & Postle 2010; Sullivan et al. 2011).

While *C. salviniae* was successfully introduced and established in southern Louisiana and biomass of *S. minima* was significantly lower in release areas, the infestations at most of the field sites were not completely controlled. Other factors may be influencing the effectiveness of *C. salviniae* as a herbivore, possibly including nutrient limiting and interspecies interactions with other arthropods (Sands et al. 1983). The red imported fire ant (*Solenopsis invicta* Buren) (Hymenoptera: Formicidae) has been known to impact populations of *C. salviniae* and prey on other biological control agents for aquatic plants (Dray et al. 2001; Cuda et al. 2004; Parys & Johnson 2012). Other reports of predation on *C. salviniae* include *Hydrochus* sp. (Coleoptera: Hydrophilidae) as well by an unidentified spider (Sands et al. 1986; Triplet et al. 2000).

This study illustrates that while *C. salviniae* did not completely control *S. minima* at our main research site, it significantly decreased the biomass and significantly increased the number of terminal buds damaged in southern Louisiana. No significant differences were observed in pH, dissolved oxygen, percentage of the mat covered, percentage of the plant material green, and conductivity between release plots where *C. salviniae* was introduced and control plots. Working in concert, *C. salviniae* along with native herbivores *S. multiplicalis* and *S. obliteralis* may provide an ecologically sound and economically practical alternative to chemical or mechanical control of *S. minima* in southern Louisiana where the red imported fire ant can be controlled.

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