IMPACT OF THE RED IMPORTED FIRE ANT, *SOLENOPSIS INVICTA* (HYMENOPTERA: FORMICIDAE), ON BIOLOGICAL CONTROL OF *SALVINIA MINIMA* (HYDROPTERIDALES: SALVINIACEAE) BY *CYRTOBAGOUS SALVINIAE* (COLEOPTERA: CURCULIONIDAE)

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Abstract

A 2-yr study of the impacts of the red imported fire ant, *Solenopsis invicta* Buren, on the success of *Cyrtobagous salviniae* Calder & Sands as a biological control agent of common salvinia, *Salvinia minima* Baker, was undertaken in southern Louisiana. Floating moats were constructed around 8 release sites where *C. salviniae* was introduced as a biological control agent. These moats were used to restrict access to the release site by red imported fire ants in half the quadrats. Moating was successful in limiting the number of red imported fire ants in the release sites over the course of the study ($P < 0.0001$). In 2010, locations where access by red imported fire ants was limited by the presence of a moat had lower biomass ($P = 0.04$) and higher populations of *C. salviniae* ($P < 0.0001$). Our study suggests that the presence of red imported fire ants negatively impacts the success of *C. salviniae* as a biological control agent.

Key Words: Common salvinia, predation, natural regulation

Resumen

Fue realizado en el sur de Louisiana un estudio de dos años de los impactos de la hormiga roja importada, *Solenopsis invicta* Buren, sobre el éxito del *Cyrtobagous salviniae* Calder y Sands como agente de control biológico de *Salvinia minima* Baker. Fosos flotantes fueron construidos alrededor de ocho sitios de introducción de *C. salviniae* como agente de contro biológico. Estos fosos fueron empleados para restringir el accesso de *S. invicta* al sitio de introducción en la mitad de los cuadrantes. El empleo de fosos logró limitar el número de hormigas rojas importadas en los sitios de introducción durante el estudio (p < 0.0001). En el 2010, los locales en donde el accesso de la hormiga roja importada fue limitado por la presencia de un foso tenían menos biomasa (p = 0.04) y poblaciones mayores de *C. salviniae* (p < 0.0001). Nuestro estudio sugiere que la presencia de la hormiga roja importada afecta negativamente el éxito de *C. salviniae* como agente de control biológico.

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Common salvinia, *Salvinia minima* Baker, is an aquatic invasive freshwater fern from South America that is currently established in waterways across the Gulf Coast of the United States (Jacono et al. 2001). Common salvinia is considered problematic in both Texas and Louisiana where infestations form dense mats that impede access, outcompete native vegetation, and degrade water quality (Montz 1989; Hatch 1995; Richards 2003; Flores & Carlson 2006). These infestations also provide habitat for *Mansonia* spp. (Diptera: Culicidae), known to vector several infectious diseases to humans (Howitt et al. 1949; Chow et al. 1955; Chamberlain et al. 1956; Lounibos et al. 1990; CDC 2009).

Common salvinia reproduces asexually from small plant fragments, and like many aquatic invasive plants spreads easily through boat movement (Johnstone et al. 1985; Miller & Wilson 1989; Jacono 2003). Weather also contributes to the spread of salvinia*,* as mats can fragment with flooding and water movement (Harley & Mitchell 1981; Room 1983, 1990). Current chemical control options for salvinia infestations are non-selective and expensive, ranging in price from US$ 198 to US$ 297/ha (Tewari & Johnson 2011). Biological control of giant salvinia, *Salvinia molesta* Mitchell, has been successful at a much lower cost than other available methods of control (Chikwenhere & Keswani 1997).

Cyrtobagous salviniae Calder and Sands (Coleoptera: Curculionidae) is a semi-aquatic weevil native to South America that has been used to successfully control *S. molesta* in 16 countries (Thomas & Room 1986; Wibmer & O'Brien 1986; Cilliers 1991; Julien et al. 2002). Although *C. salviniae* has been widely used to control giant salvinia, it also has been credited with keeping Flor-

ida populations of common salvinia under control (Jacono et al. 2001). *Cyrtobagous salviniae*, which is adventive in Florida, was first introduced into both Texas and Louisiana in 2000 by the US Department of Agriculture to help control the spread of giant salvinia (Goolsby et al. 2000).

The red imported fire ant, *Solenopsis invicta* Buren, is an adventive exotic pest discovered in the port of Mobile, Alabama in the 1930s (Buren 1972). The red imported fire ant is native to South America where populations are limited by competition and parasitoid pressure (Buren et al. 1974; Jouvenaz 1983). It has a broad omnivorous diet, eating both plants and animals with other invertebrates making up a large part of their diet (Vinson 1997). The voracious appetite and indiscriminate diet of the red imported fire ant has earned a reputation as beneficial for eating pests in some agricultural systems, though diet preference is hard to predict and can include other beneficial insects (Sterling 1978; Eubanks 2001).

Red imported fire ant colonies are known to use water to disperse and display unique rafting behavior when flooded (Morrill 1974; Mlot et al. 2011). Freed & Neitman (1988) first noted red imported fire ants using aquatic vegetation to forage over water. They were recorded crossing long leaf pondweed (*Potamotegon nodosus* Poir.*),* and foraging up to 15 m from the shore in a Texas pond (Patrock 2007). Tewari (2007) found populations of red imported fire ants foraging extensively on common salvinia mats in both forested wetlands and canals in southern Louisiana up to 80 m from the levee. They have been known to venture into other wet habitats to prey on intertidal polychaetes, young sea turtles, neonate alligators, and a variety of other wildlife (Allen et al. 1997; Allen et al. 2001;Palomo et al. 2003; Allen et al. 2004).

Biological control programs for other aquatic plants have been impacted by the presence of fire ants. Red imported fire ants have been observed preying on *Spodoptera pectinicornis* Hampson (Lepidoptera: Noctuidae)*,* an introduced biological control agent of waterlettuce, *Pistia stratiotes* L. (Arales: Araceae) (Dray et al. 2001). *Samea multiplicalis* (Guneé) (Lepidoptera: Crambidae) and *Stenopelmus rufinasus* Gyllenhal (Coleoptera: Curculionidae), native control agents of the floating fern *Azolla* (Hydropteridales: Salviniaceae) also are negatively impacted by red imported fire ants (Cuda et al. 2004). The objective of this study was to determine whether the presence of red imported fire ants is negatively affecting the success of the biological control of common salvinia in southern Louisiana.

Materials and Methods

The experimental site was an artificial pond heavily infested with *S. minima* on privately owned property located near the town of Tunica, Louisiana (30.951656 N, -91.480719 W). The levee adjacent to the pond had known populations of red imported fire ants. To allow red imported fire ants to forage freely, no insecticide treatment was used to during the course of our study. No biological control agents had previously been released at this site. Eight exclusion quadrats (Fig. 1) were constructed and placed in the experimental pond parallel to the levee. Each quadrat consisted of 2 nested square quadrats made from 5.08 cm diameter SCH40 PVC pipe. The inner quadrat measured 1.00 m^2 whereas the outer was 1.50 m^2, leaving a 0.25 m moat between the inner and outer quadrats. The 2 quadrats were rigidly connected to each other in 4 locations 0.13 m underwater by 12.70 mm SCH40 PVC to prevent the moat from being compromised. Assembled quadrats were placed in the water and anchored to the levee on the bank of the pond, leaving 1 m both between quadrats and between quadrats and the levee. The quadrats were cleared of all plant material prior to the beginning of the study.

In both 2009 and 2010, each of the inner quadrats was filled with 3 kg of common salvinia and seeded with 150 *C. salviniae* individuals (in 2010, plant material was transported from another location to prevent previously established populations of *C. salviniae* from confounding results). Every other quadrat's moat was filled with an ad-
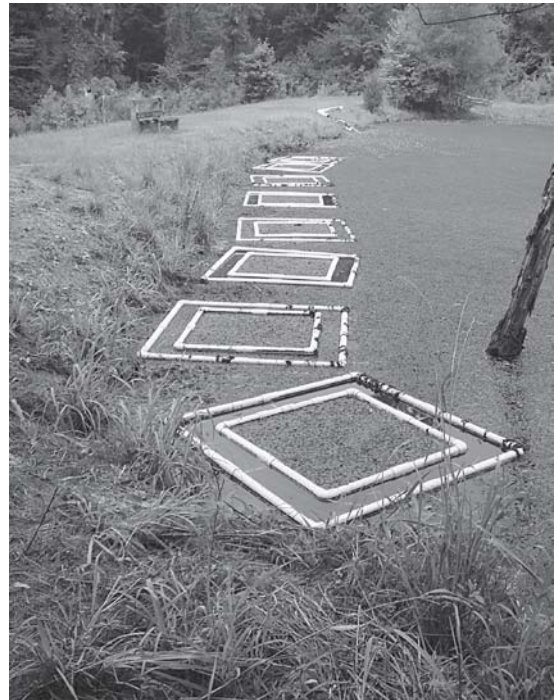

Fig. 1. Exclusion quadrats in the field at the site in Tunica, Louisiana.

ditional 3 kg of plant material to cover the open water and allow red imported fire ants access to the inner quadrat (Fig. 1). Moated release quadrats were cleared of any introduced plant material and plants growing in the quadrat other than common salvinia were removed every other week. Inner quadrats also received a floating pitfall trap to monitor for activity of red imported fire ants (Parys & Johnson 2011).

During 2009, *C. salviniae* were introduced in early July and allowed to establish for 2 mo before sampling commenced in Sep. Quadrats were sampled once a month for 2 mo before heavy rain caused the pond to overtop the levee, compromising the mat and exclosures. The 2009/2010 winter was unusually cold, with the pond's water surface reaching a low of 1.18 °C. In 2010, the experiment was not re-established until the month of July when the mat of common salvinia had reformed across the water's surface. *Cyrtobagous salviniae* were again allowed to establish for 2 mo before sampling was initiated in Sep. Sampling the mat of common salvinia was accomplished by placing three 0.1 m² mini-quadrats constructed from 2.5 cm dia SCH40 PVC pipe. Plant material was removed from within each of the mini-quadrats, samples were hand squeezed to remove excess water and returned to the lab. During 2010, biomass was recorded from these samples as well. Tewari & Johnson (2011) established that wet weights of common salvinia samples were significantly correlated with dry weights, suggesting that wet weights were an efficient and reliable way of comparing treatments. Plant samples were returned to the laboratory and submerged for 24 h to count *C. salviniae* present. Both the plant material and weevils were returned to the original quadrat following counts of *C. salviniae*. In addition to the samples of plant material, each floating pitfall trap was emptied and refilled, and catches returned to the lab for processing and identification.

The presence of *S. multiplicalis*, a native herbivore documented to negatively impact the biomass of common salvinia in Louisiana also was noted at the field site (Tewari & Johnson 2011). To reduce confounding results, quadrats were sprayed with a microbial insecticide (Thuricide® concentrate, active ingredient: *Bacillus thuringiensis kurstaki,* approximately 4000 *Spodoptera* units/6 million viable spores per milligram) when larvae and adults of *S. multiplicalis* were observed. A HOBO® Pro v2 Water Temperature Data Logger (#U22-001, Onset Computer Corporation, Bourne, Massachusetts) set to record water temperature hourly, was set in place at the site during the summer of 2009, and allowed to run throughout the length of the study.

Data collected from experiments in 2009 and 2010 were analyzed separately by years and as a pooled group where possible. Analysis of Variance (ANOVAs) was performed with Proc MIXED (SAS Institute 2008). Data from *S. invicta* populations, *C. salviniae* populations, and *S. minima* biomass were compared in two-way ANOVAs with treatment (moating of release sites) and sampling date as factors. When differences were detected by ANOVAs ($P < 0.05$) least square means were separated by the least significant differences (LSD, $\alpha = 0.05$).

RESULTS

Differences in the number of individual *S. invicta* collected were not detected in 2009 between moated and non-moated quadrats ($F = 3.21$; df = 1,12; $P = 0.0984$), but were different during 2010 ($F = 64.14$; df = 1,36; $P = 0.0005$) (Figs. 2 and 3). Sampling date also was significant in 2010 ($F = 4.58$; df = 5,36; $P = 0.0025$). Differences between treatments were significant when data from 2009 and 2010 were pooled, indicating that the moat was successful in keeping most individuals from accessing the inner area throughout the study ($F = 17.88$; df = 1,48; $P < 0.0001$). The sampling date ($F = 6.20$; df = 7,48; $P < 0.0001$) and treatment × date ($F = 8.95$; df = 7,48; $P < 0.0001$) interaction also were significant in the pooled model. No other species of ants were collected from the traps during either yr.

Population data of *C. salviniae* sampled during the study was subdivided into 2 sets by year. During 2009, numbers of *C. salviniae* per sample were not different between treatments ($F = 0.20$; df= 1,12; 6; $P = 0.6665$) (Fig. 4). For 2010, one data point (one quadrat from Oct 2010) stood out from the others as over 6 standard deviations from the mean, possibly indicating a severe outlier. Data for 2010 were analyzed using Dixon's Q, which
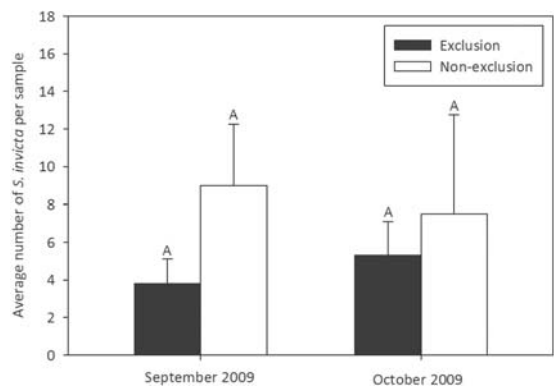

Fig. 2. Average number of individual *Solenopsis invicta* recovered from a floating pitfall trap placed within the inner-quadrat for 2 sampling dates from Sep-Oct of 2009. When significant differences were detected between treatments, bars capped with same letter are not significantly different (LSD, $\alpha = 0.05$).
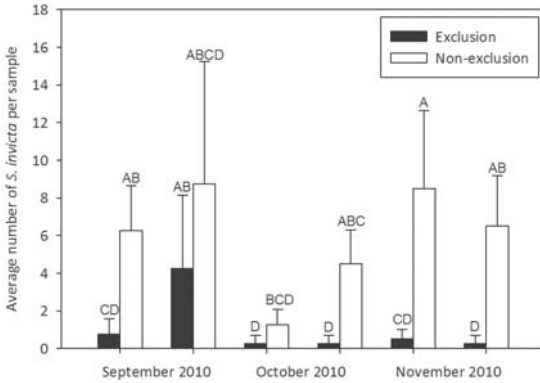
Fig. 3. Average number of individual *Solenopsis invicta* recovered from a floating pitfall trap placed within the inner-quadrat over 6 sampling dates (2 per mo) from Sep-Nov of 2010. Bars capped with the same letters are not statistically different (LSD, $\alpha = 0.05$).
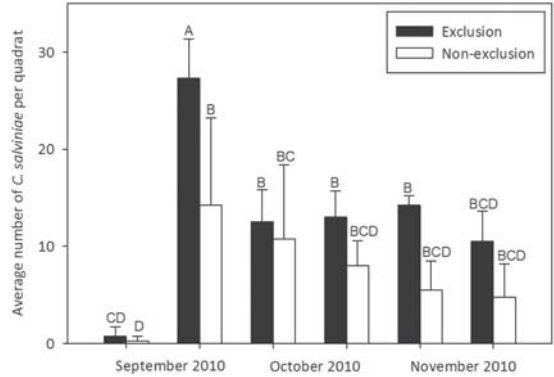

Fig. 5. Number of individuals of *Cyrtobagous salviniae* removed from $0.1\,m^2$ area of *Salvinia minima* over 6 sampling dates (2 per month) from Sep-Nov of 2010 (Adjusted using Dixon's Q). Bars capped with the same letters are not statistically different (LSD, $\alpha = 0.05$).

suggested rejecting the outlier from analysis with a 95% confidence level ($Q = 0.86$) (Dean & Dixon 1951; Rorabacher 1991). After removing the outlier from the 2010 data, the number of individuals of *C. salviniae* per sample of *S. minima* was significantly higher in quadrats with a moat ($F = 22.0$; df = 1,35; $P < 0.0001$) (Fig. 5), indicating that the treatment affected *C. salviniae*. Sampling date in 2010 for *C. salviniae* population was also significant ($F = 17.81$; df = 5,35; $P < 0.0001$). Data for *C. salviniae* populations were not pooled between years for analysis because normality assumptions were violated.

Biomass of the of *S. minima* samples was only analyzed for 2010 and was normally distributed. The biomass of *S. minima* samples was lower in moated quadrats than those that were unmoated ($F = 4.42$; df = 1,36; $P = 0.0425$). Biomass declined

during the course of the study causing estimates to differ among sampling dates ($F = 43.46$; df = 5,36; $P < 0.0001$) (Fig. 6).

Discussion

During this 2 yr study, *C. salviniae* was introduced as a biological control agent for common salvinia at 8 sites, and half of those sites were moated to prohibit red imported fire ants from reaching release sites. Moats successfully excluded red imported fire ants during 2010, positively affected *C. salviniae* populations, and negatively affected common salvinia biomass.

Differences observed among dates in red imported fire ant populations are likely a response to changes in foraging behavior as a response to abiotic factors such as rainfall and seasonal tem-
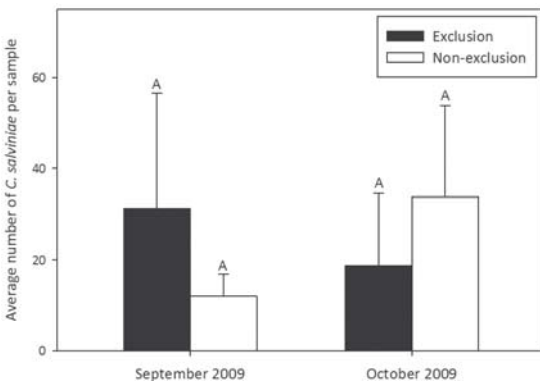

Fig. 4. Average number of individuals of *Cyrtobagous salviniae* removed from $0.1\ m^2$ area of *Salvinia minima* from 2 sampling dates, Sep-Oct of 2009. Bars capped with the same letters are not statistically different (LSD, $\alpha = 0.05$).
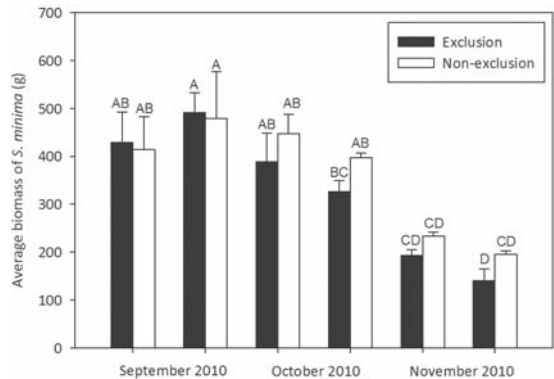

Fig. 6. Average biomass of $0.1\ m^2$ of *Salvinia minima* over 6 sampling dates (2 per mo) from Sep-Nov of 2010. Bars capped with the same letters are not statistically different (LSD, $\alpha = 0.05$).

perature changes. Other studies have found that optimum foraging occurs between 22 and 36 °C (Porter & Tschinkel 1987). Whereas moats were an effective method to significantly decrease the number of red imported fire ant individuals within a quadrat, several individuals managed to cross the moat. Vegetation growing along the bank could have provided temporary access, although plant material was diligently cleared to eliminate any possible bridge substrates from the area. Red imported fire ants are well documented to survive water and flooding so it is not surprising that some individuals might cross 0.25 m of open water (Morrill 1974; Mlot et al. 2011).

Many species of ants are documented to interfere with biological control programs, both by defending food sources and through intra-guild predation (Cudjoe et al. 1993; Stechmann et al. 1996; González-Hernández et al. 1999; Eubanks et al. 2002; Kaplan & Eubanks 2002; Wyckhuys et al. 2007). Discussion of predation on weevils from other arthropods is scattered in the literature across a wide variety of ecological systems (Barney et al. 1979; Alfaro & Borden 1980; Barney & Armbrust 1980; Richman et al. 1983; Barker et al. 1989). The best documented impact of arthropod predation on a weevil is the use of red imported fire ants as a control agent for the boll weevil (Sterling 1978; Jones & Sterling 1979; Fillman & Sterling 1983). Red imported fire ants also have been documented impacting the *Azolla* weevil, *S. rufinasus* (Cuda et al. 2004), which is of a similar adult size (2 mm) to *C. salviniae* (Hill 1998; Tipping et al. 2010).

Although not addressed in this study, red imported fire ants that were actively foraging on the mat of common salvinia were likely also preying on both larvae and eggs of *S. multiplicalis*. Red imported fire ants interfered with the introduction and establishment of a similar lepidopteran, *S. pecticornis,* released for the biological control of waterlettuce (Dray et al. 2001) and have been observed preying on *S. multiplicalis* in Florida (Cuda et al. 2004). Lepidopteran eggs and larvae in a variety of terrestrial systems also are prey for red imported fire ants (Reagan et al. 1972; McDaniel & Sterling 1979; Eger et al. 1983; Elvin et al. 1983; Eubanks 2001; Seagraves & McPherson 2006). Thuricide® was sprayed to control *S. multiplicalis* within our study area, but the majority of the pond was untreated and supported large populations of larvae as a food source throughout the study. Although *S. multiplicalis* had a negligible effect on common salvinia in Florida (Tipping & Center 2005), Tewari & Johnson (2011) documented that *S. multiplicalis* had a significant negative effect on the biomass of common salvinia in Louisiana.

This study suggests red imported fire ants are actively impacting the success of the biological control of common salviniae by decreasing the population of *C. salviniae*. In combination with probable predation on the only other herbivore, *S. multiplicalis*, populations of red imported fire ants should be controlled where possible to increase the success of biological control for either salvinia species. Although not cost effective or possible for large wetland areas or water bodies, controlling red imported fire ants around smaller infestations of common salvinia when infested with *C. salviniae* will increase chances of successful biological control.

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