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Impact and interaction of Samea multiplicalis (Lepidoptera: Pyralidae) and Cyrtobagous salviniae (Coleoptera: Curculionidae) on Salvinia minima in south Louisiana and the foraging behavior of Solenopsis invicta (Hymenoptera: Formicidae) on Salvinia minima

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IMPACT AND INTERACTION OF *SAMEA MULTIPLICALIS* (LEPIDOPTERA: PYRALIDAE) AND *CYRTOBAGOUS SALVINIAE* (COLEOPTERA: CURCULIONIDAE) ON *SALVINIA MINIMA* IN SOUTH LOUISIANA AND THE FORAGING BEHAVIOR OF *SOLENOPSIS INVICTA* (HYMENOPTERA: FORMICIDAE) ON *SALVINIA MINIMA*

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ABSTRACT

A field study was conducted in 2005 and 2006 to evaluate the impact and interaction of the herbivores *Cyrtobagous salviniae* Calder and Sands and *Samea multiplicalis* Guenee on common salvinia, *Salvinia minima* Baker in south Louisiana. It was a completely randomized experimental design in which treatments consisted of *C. salviniae* and *S. multiplicalis* feeding on common salvinia both independently and together along with a control.

Our study revealed that treatments consisting of *C. salviniae* and *S .multiplicalis* feeding both independently and together had a significant impact on the biomass of common salvinia. Sampling done in October of both 2005 and 2006 showed that the lowest biomass was recorded for the treatment with both *C. salviniae* and *S. multiplicalis*. There was also a significant treatment by month interaction with a linear decrease in biomass for the treatment consisting of feeding by both *C. salviniae* and *S. multiplicalis* in 2005. Also, biomass showed a quadratic trend for the treatment with only *S. multiplicalis* in 2005. Percentage terminal damage (PTD) and percentage mat green (PMG) showed a significant treatment effect and a significant treatment by month interaction in 2005. Also, PTD showed a significant treatment effect and a significant treatment by month interaction in 2006.

A field study was conducted in May of 2007 to document the foraging behavior of red imported fire ants (RIFA), *Solenopsis invicta* Buren, on common salvinia mats in flooded woodlands and dredged canals. RIFA mounds were found in flooded woodlands at the base of live trees and on dead tree stumps. The recruitment of RIFA to the bait stations was not uniform up to 100 m into the flooded woodlands and in most instances there was no linear relationship between distance from the levee and number of ants. Also, the recruitment at different times of the day was not the same. Results of this study provide evidence that RIFA forage extensively on

common salvinia in both flooded woodlands and dredged canals, and could possibly have an

adverse impact on the populations of native *S. multiplicalis* and also on the survival and

establishment of *C. salviniae*.

CHAPTER 1

INTRODUCTION

Classical biological control of weeds as a discipline has now been practiced for almost a century with numerous successful attempts worldwide (Waterhouse, 1998). Almost 70 countries have been involved in weed biological control efforts, with the United States and Australia at the forefront (Waterhouse, 1998). Despite the fact that these efforts involve the movement of organisms, including insects to exotic locations, very few cases have been reported of deleterious impacts on non target plants (Waterhouse, 1998). Stringent testing and quarantine protocols have been adopted in the last twenty five years to ensure the safety and reliability of biological control programs (Waterhouse, 1998).

The genus *Salvinia*, consisting of free-floating aquatic ferns, belongs to the family Salviniaceae. Two members of this family, *Salvinia molesta* D.S. Mitchell and *Salvinia minima* Baker, both originating in tropical Americas, are known to occur in the United States (Jacono et al., 2001). *Salvinia molesta*, also known as giant salvinia, is thought to be of recent introduction whereas *Salvinia minima*, also know as common salvinia, was probably introduced into the United States in the late 1920s or early 1930s (Jacono et al., 2001). Both of these species consist of a horizontal rhizome lying just below the surface of the water and a pair of floating leaves (Harley and Mitchell, 1981; USGS, 2005a). A highly dissected submerged third leaf is believed to function as a root (Room, 1988; Nauman, 1993).

The floating leaves of common salvinia are round to elliptical in shape and notched at the tip (Nauman, 1993). Leaf venation in common salvinia is obscure and areolate but the tips of veins are free, ending short of margins (Nauman, 1993). The surface of leaves in common salvinia plants have rows of branched hairs that remain free at the top whereas in the case of giant salvinia, these unite again at the apex to assume the shape of an "egg beater." Submerged leaves of common salvinia bear sporocarps which can either give rise to megasporangia with a

single megaspore or microsporangia with 64 microspores (Nauman, 1993). Both common and giant salvinia are considered to be sterile and reproduce vegetatively (Jacono et al., 2001). The plants of common and giant salvinia have three growth stages that are morphologically dissimilar and can be distinguished from each other. 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 the plants have been growing for some time and the edges of leaves start to curl upwards. 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 surface of water.

Common salvinia is believed to have been initially introduced into the United States as an ornamental plant, but later escaped into natural areas either accidentally or deliberately. Its presence has been recorded from over 690 locations in 89 freshwater drainage basins of Florida, Georgia, Louisiana, Alabama, Texas, South Carolina, Mississippi and Arkansas (USGS, 2005b) and in Louisiana and Texas, common salvinia has attained the status of a very problematic weed (Jacono et al., 2001). Common salvinia was first reported from Louisiana in 1980 at St. Mary Parish (Charles Dugas, personal communication, January 24, 2006) and is now estimated to infest more than 100,000 hectares (Johnson and Sanders, 2007). In Louisiana common salvinia is now described as one of the six most troublesome aquatic weeds (Johnson and Sanders, 2007).

Common salvinia thrives in freshwater wetlands, and Zedler and Kercher (2004) reported that wetlands are particularly prone to encroachment by invasive species. Freshwater wetlands occupy almost 27,328,826 hectares (inland and coastal) in the United States (Shaw and Fredine, 2006) and their susceptibility is corroborated by the fact that even though wetlands consist of only about 6% of the earth's land mass, nearly 24% (8 of 33) of the world's most invasive plants

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are wetland species (Zedler and Kercher, 2004). Zedler and Kercher (2004) used the term "landscape sinks" for wetlands as they end up amassing "debris, sediments, water, and nutrients," factors which create ideal conditions for invasive species to take over. Invasive plant species not only adversely impact the biodiversity and ecosystem of wetlands but also have a negative impact on their recreational use by humans (Zedler and Kercher, 2004). Lack of natural enemies, ability to overcome extreme environmental conditions and effective use of available resources are some of the reasons outlined by Zedler and Kercher (2004) as to why some of the wetland species become highly invasive.

Wetland habitats are currently under threat from non-native, floating aquatic plants like common salvinia, and Barataria Preserve of Jean Lafitte National Historic Park and Preserve (JELA) in Louisiana is one such example (USGS, 2005c). This unique habitat, consisting of freshwater floating marshes, native bottomland-hardwood swamps, and about 40 kilometers of natural bayous and waterways, is about 7,487 hectares in area. Common salvinia, the most abundant invasive plant species in the Preserve, occupies about 3,642 hectares and threatens to spread over the entire area (USGS, 2005c).

Common salvinia reproduces asexually through fragmentation at a very fast rate, covering the surface of water (USGS, 2005c). Thick mats of common salvinia prevent sunlight from reaching the submerged plants whereas floating plant species such as antler fern (*Ceratopteris pteridoides*) and duckweed (*Lemna spp.)* are also displaced (USGS, 2005c). Common salvinia mats lower the dissolved oxygen of infested water, thereby making it unsuitable for other life forms and can provide safe haven to pest species such as mosquitoes (USGS, 2005c).

Common salvinia infestations can also have a significant impact on a number of recreational and commercial activities. Duck hunting, for example, is estimated to have a total economic effect of $ 164,058,027 in Louisiana (Southwick Associates, 2005) and common salvinia threatens this important source of revenue by degrading the habitats suitable for ducks (Johnson and Sanders, 2007). Common salvinia infestations in Manchac Swamp, a prime duck hunting area in Louisiana, forced many hunters to relinquish their hunting leases (Charles Dugas, personal communication, January 24, 2006). Other recreational activities such as boating and fishing are also adversely affected by the thick floating mats of common salvinia, and these infestations may also hinder the ability of law enforcement agencies to effectively carry out their duties (USGS, 2005c). Commercial activities such as rice and crawfish farming, water drainage and electrical power generation can also be negatively impacted by common salvinia (Charles Dugas, personal communication, January 24, 2006).

Chemical herbicides are available, but asexual reproduction combined with the fast growth rate of common salvinia usually renders their application impractical and ineffective as the area to be treated is very large in most cases. Also, the cost of controlling common salvinia using herbicides by state and contract workers may range from $80 to $120 per acre whereas the cost to private land owners is much higher (Charles Dugas, personal communication, January 24, 2006). In some cases it is not physically possible to treat certain areas of common salvinia infestations with herbicides using boats and aircrafts due to inaccessibility. Chemical control also does not provide a long-term solution to the problem because common salvinia plants can easily spread to new areas with flowing water and quickly re-establish themselves because of a high rate of reproduction (USGS, 2005c).

Mechanical efforts to control this nuisance aquatic weed are often expensive, time consuming and generally not reliable (USGS, 2005c). Another problem with using a weed harvester to get rid of common salvinia infestations is the fact that it can operate only in navigable waterways and wooded swamps are thus left untreated (USGS, 2005c). Biological control, on the other hand, may offer a sustainable, long lasting and environmentally sound solution to the problem (McFadyen, 1998).

Cyrtobagous salviniae Calder and Sands (Coleoptera: Curculionidae), an aquatic weevil native to Brazil, Bolivia and Paraguay (Wibmer and O' Brien, 1986), 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, Zambia and Zimbabwe (Julien and Griffiths, 1998). The weevil can also survive and complete its life cycle on common salvinia (Tipping and Center, 2005).

This species was initially thought to be *Cyrtobagous singularis* Hustache but was later identified as *C. salviniae* (Sands, 1983). A population of *C. salviniae* was found to be established on common salvinia in Florida (Kissinger, 1966) and was initially thought to be *C. singularis* (Kissinger, 1966). Later it was shown that the Florida population was in fact *C. salviniae* (Calder and Sands, 1985) and it was also noticed that the Florida weevils were significantly smaller than those from Brazil (Calder and Sands, 1985). Molecular analysis indicated that the Florida population of the weevils was "significantly different" from the Brazilian population, which had been 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).

The Florida population has been credited with keeping the spread of common salvinia in that state in check and its absence from Louisiana and Texas has probably led to common salvinia becoming a nuisance in these two states (Jacono et al., 2001). Tipping et al. (2004) conducted a study on the effects of *C. salviniae* (Brazilian population) on giant salvinia in eastern Texas and western Louisiana. After nearly four years of monitoring both release and control sites their conclusion was that the weevils had managed to establish themselves and suppress giant salvinia at the study sites.

The males of *C. salviniae* are slightly smaller than the females and newly emerged adults are brown, gradually darkening to shiny black (Forno et al., 1983). The adults are subaquatic in nature and can be spotted on or under leaves, within the leaf buds or among the roots of common salvinia plants (Forno et al., 1983). The adults are able to respire underwater with the help of an air film that sticks to their ventral surface (Forno et al., 1983). Eggs are laid singly and in the cavities that are formed during adult feeding on the leaves, rhizomes or "roots" (Forno et al., 1983). Temperature plays a crucial role in the different life history stages of this weevil. Females fail to oviposit at or below 21º C and eggs fail to hatch at or below 19 or at or above 37º C (Forno et al., 1983). Newly emerged larvae are white and may initially feed on young terminal buds but later tunnel into rhizomes to complete three instars (Forno et al., 1983). Larvae fail to complete development in the absence of rhizomes (Forno et al., 1983). Development of larvae within rhizome is dependent on temperature (Forno et al., 1983).

Time taken by larvae to complete development is related to nitrogen content of plant tissue with higher content resulting in reduced development time (Sands et al., 1983). Studies conducted on giant salvinia showed that the plant responded to feeding and tunneling damage by producing fresh growth with nitrogen content that is higher than that of intact plant or older

tissue of the same plant (Forno and Semple, 1987). Feeding on plant material with higher nitrogen content would be advantageous to both the larvae and adults of *C. salviniae* by positively influencing their development and reproductive capacity (Forno and Semple, 1987). Pupation takes place in a cocoon spun by the larva in close proximity to living plant tissues under the surface of water and it is usually found among the root mass and has the same brown color as the root mass (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 common salvinia plants (Sands et al., 1983).Feeding by weevil larvae causes the leaves to first darken to brown and then drop off (Forno et al., 1983). Browning is followed by root decay and its separation from the rhizome, eventually resulting in the death of nodes and internodes (Forno et al., 1983). Tunneling by larvae causes more severe damage to common salvinia plants as compared to feeding by adults (Sands et al., 1983).

Host specificity tests undertaken by Forno et al. (1983) showed that apart from giant salvinia, weevils did not feed on any other plant species except *Pistia stratiotes* (water lettuce) and *Ipomea batata* (sweet potato). Feeding on sweet potato took place when the leaves were in contact with water and adults feeding on water lettuce were unable to complete their life cycle. A no-choice test was done with sweet potato in a non-aquatic environment and there was no feeding recorded. Also, the adults failed to survive after seven days.

Samea multiplicalis Guenee (Lepidoptera: Pyralidae), a pyralid moth native to Brazil, is also found throughout the southeastern United States (Knopf and Habeck, 1976). This herbivore was released in Australia and showed potential as a biological control agent against giant salvinia (Sands and Kassulke, 1984). *Samea multiplicalis* was observed feeding on common salvinia plants in Florida but its impact was reported to be "negligible" (Tipping and Center, 2005).

Samea multiplicalis is a generalist herbivore and in Florida it was listed as the "most common natural control agent" feeding on *Pistia stratiotes* (water lettuce) (Newton and Sharkey, 2000). Semple and Forno (1987) reported five parasitoids and three pathogens of *S. multiplicalis* in Queensland, Australia with twenty-two percent of collected larvae being parasitized. Knopf and Habeck (1976) reported one dipterous and three hymenopterous parasitoids of *S. multiplicalis* from the United States. They estimated the parasitism level to be 52% at Port Charlotte, Florida. Natural populations of *S. multiplicalis* are present in Louisiana, and it was one of the three most common species captured using ultraviolet light traps from March 1995-October 1995 in the longleaf-pine savanna of Louisiana (Landau and Prowell, 1999).

The adults of *S. multiplicalis* are tan and have dark markings on both fore and hind wings with females being lighter than the males, particularly on the fore wings (DeLoach et al., 1979). The length of fore wings can range from 6.5mm to 10.5mm or more and with bands of cream and brown found on the apical half of the costa (Sands and Kassulke, 1984). Apart from being darker, males can also be differentiated from the females by the presence of black scales at the base of hind legs that are as long as the femur (Sands and Kassulke, 1984).

Eggs are laid singly among hairs on the upper surface of leaves in giant salvinia (Sands and Kassulke, 1984). After hatching, larvae feed on leaves by constructing a canopy composed of silk and epidermal hairs of the host plants (Knopf and Habeck, 1976). Pupation occurs in a silken cocoon that is made within old leaves (Sands and Kassulke, 1984). Knopf and Habeck (1976) studied the development time of different life stages (egg, larva and pupa) when *S. multiplicalis* was raised on water lettuce and giant salvinia. They reported the hatching of eggs to be synchronized and taking place on the 4th day after oviposition. The larval development time

ranged from 14-18 days whereas pupal development time ranged from 4-7 days at 28±1ºC and 14 h photoperiod.

Predation by natural enemies can have an adverse impact on the establishment of a biological control agent (Dray et al., 2001). Fire ants have been identified as important predators of lepidopteran larvae in both aquatic and terrestrial systems. Predation by fire ants was listed as one of the reasons responsible for the failure of *Spodoptera pectinicornis* Hampson (Lepidoptera: Noctuidae), a biological control agent released against *Pistia stratiotes* L. (waterlettuce), to establish and perpetuate in Florida (Dray Jr et al., 2001). Dray et al. (2001) documented that foraging by fire ants adversely influenced both adult and larval populations of *S. pectinicornis*. Freed and Neitman (1988) reported that red imported fire ants utilize aquatic vegetation to forage over the surface of a pond and Wilson (1986) noted that a number of *Solenopsis* species foraged in the vicinity of water. Red imported fire ants (*Solenopsis invicta* Buren) had a significant impact on the abundance of lepidopteran larvae and ranked second among control agents in cotton (Eubanks, 2001). Red imported fire ants were also reported to be an "active predator" of lepidopteran pests in soybean (Seagraves and McPherson, 2006).

The overall aim of this study was to determine the impact of the two herbivores, *C. salviniae* and *S. multiplicalis*, on common salvinia in south Louisiana and to examine the foraging behavior of red imported fire ants on common salvinia mats.

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CHAPTER 2

IMPACT AND INTERACTION OF *CYRTOBAGOUS SALVINAE* AND *SAMEA MULTIPLICALIS* ON COMMON SALVINIA (*SALVINIA MINIMA*) IN SOUTH LOUISIANA

INTRODUCTION

Non-indigenous weeds invade about 700,000 hectares of wildlife habitat per year (Babbitt, 1998) in the United States and the annual management costs for non-indigenous aquatic weed species is approximately $100 million (OTA, 1993). Common salvinia, *Salvinia minima* Baker is a free floating aquatic fern which occurs in nature as a sporophyte. 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 from over 690 locations in 89 freshwater drainage basins of Florida, Georgia, Louisiana, Alabama, Texas, South Carolina, Mississippi and Arkansas (USGS, 2005).

Common salvinia invades relatively calm and slow moving freshwater, multiplying rapidly and covering the water surface, with floating mats sometimes as thick as 20-25 cm (Jacono et al., 2001). It has attained the status of a "very troublesome weed" in Louisiana and Texas (Jacono et al., 2001). Due to its aggressive growth submerged plants are killed because sunlight does not reach them (Jacono et al., 2001). Common salvinia also displaces native surface plant species including duckweed (*Lemna* spp.), which is an important source of food for migrating waterfowl species (Jacono, 2003). Common salvinia degrades the quality of water and makes it unsuitable for other organisms, including fishery resources. Common salvinia mats also impede the movement of boats, making it almost impossible to navigate in heavily infested waters. The use of herbicides for controlling common salvinia infestations is often an expensive and impractical undertaking.

Cyrtobagous salviniae Calder and Sands is an aquatic weevil native to Brazil, Bolivia and Paraguay (Wibmer and O'Brien, 1986) which has been used for the biological control of giant salvinia, *Salvinia molesta* Mitchell, in a number of countries including Australia, Fiji, Ghana,

India, Kenya, Malaysia, Namibia, Papua New Guinea, Republic of South Africa, Sri Lanka, Zambia and Zimbabwe (Julien and Griffith, 1998). *Cyrtobagous salviniae* can also survive and complete its life cycle on common salvinia (Tipping and Center, 2005). A population of this weevil was found to be established on common salvinia in Florida (Kissinger, 1966) and was initially thought to be *Cyrtobagous singularis* Hustache (Kissinger, 1966). Later it was shown that the Florida population was in fact *C. salviniae* (Calder and Sands, 1985) and it was also reported that Florida weevils were significantly smaller that 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). 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 a nuisance in these two states (Jacono et al., 2001).

Samea multiplicalis Guenée is a pyralid moth that also feeds on common salvinia. Natural populations of this moth are present in Louisiana and were reported to be one of the three most common species captured using ultraviolet-light traps from March 1995-October 1995 in the longleaf pine savanna of Louisiana (Landau and Prowell, 1999). *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 *S. multiplicalis* and *C. salviniae* when feeding on common salvinia both independently and together in south Louisiana.

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MATERIALS AND METHODS

The study was conducted on a portion of a 4000 hectare tract of private property owned by Mr. James Boyce, located north of Gramercy, LA and adjacent to Highway 61 (30º10'46.77"N 90 º 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.

PVC pipes of diameter 0.0508 m (SCH.40) were used to construct frames of area 1m², the effective size of plots for the experiment. Sixteen frames were set up throughout the property and were at least 100 m apart. There were four treatments, each replicated four times, and applied randomly to the 16 plots (quadrates). The treatments were: (1) Common salvinia subjected to feeding by the weevil *C. salviniae* alone, (2) Common salvinia subjected to feeding by larvae of the moth *S. multiplicalis* alone, (3) Common salvinia subjected to feeding by both herbivores, (4) The control with no feeding. The frames were anchored using nylon ropes and bricks.

A population of the weevils from Florida was maintained at Louisiana State University campus greenhouses and was the source of weevils used for the experiments. The weevils used in 2005 were collected from Fort Lauderdale, Florida in September 2004 by Dr. Phil Tippings (USDA-ARS, Aquatic Invasive Species Laboratory, Fort Lauderdale, Florida). The weevils released in 2006 consisted of two populations, one collected by Dr. Phil Tippings at Fort Lauderdale in September 2005 and the other collected by Dr. Seth Johnson (Louisiana State University, Baton Rouge, Louisiana) at Coe's Landing on Lake Talquin located near Tallahassee, Florida in September 2005. The weevils were reared in 567.8 liters 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 employed to provide optimum conditions (25 - 28ºC) for the weevils to reproduce during winter months.

The study began in May of 2005 with the release of forty weevils per plot in the eight plots that received weevils (treatments 1 and 3). In August of 2005, an additional fifty weevils were released in the plots. Since the weevils were not able to overwinter, the study was continued by releasing one hundred weevils per plot in the eight plots (treatments 1 and 3) that received weevils in April 2006 and supplemented with another fifty weevils in September. We depended upon natural infestation of *Samea multiplicalis* on common salvinia in plots of treatments 2 and 3. Treatments 1 and 4 were maintained free of *S. multiplicalis* by spraying twice a week with microbial insecticide (Thuricide® Concentrate, active ingredient: *Bacillus thuringiensis* subspecies *kurstaki,* equivalent to 4,000 *Spodoptera* units or six million viable spores per milligram). All plots were kept free of other aquatic vegetation by hand removal in order to avoid competition and any impact on growth of common salvinia.

Sampling was done monthly, starting in June of both 2005 and 2006 and continuing until October with five samples taken each year. Surface temperature, pH, growth stage, percentage area covered and percentage mat green of common salvinia inside the plot were recorded. Three quadrates of size 0.1m² built with PVC pipes of diameter 0.0254 m were randomly placed inside the 1 m² plot and the common salvinia enclosed within each plot was hand squeezed to remove excess water and weighed to determine the biomass. Fifteen such samples were randomly collected from the research site at the beginning of the study 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 the dry weights. Also, one hundred common salvinia plants were randomly selected from the plot and checked for damage to the terminal buds due to feeding by the herbivores. The data was analyzed using ANOVA and Tukey's Honest Significant Difference on SAS (2003) and JMP IN (2003). Linear regression analysis between wet and dry weights of common salvinia was done on SAS (2003). Trend analysis was also done on SAS (2003).

RESULTS

Linear Regression Analysis between Wet Weight and Dry Weight of Common Salvinia

The linear regression analysis between wet weight and dry weight of common salvinia was significant ($F=1079.87$; $df=1, 13$; $P<0.0001$; $r^2=0.9881$).

2005

Biomass of common salvinia was significantly lower in the treatment plots than in the control plots and for the treatments consisting of (1) only *C. salviniae* and (2)both *C. salviniae* and *S. multiplicalis*, and there was generally a decline in the biomass of common salvinia with each passing month. Biomass showed a significant treatment effect ($F=10.11$; $df=3, 11$; $P=0.0017$) and a significant interaction within treatments by month ($F=5.91$; $df=12, 44$; $P<0.0001$). Biomass also showed a linear trend ($F=7.20$; $df=1, 44$; $P=0.0102$) for the treatment consisting of both *C. salviniae* and *S. multiplicalis* and a quadratic trend ($F=7.52$; $df=1, 44$; $P=0.0088$) for the treatment consisting of only *S. multiplicalis* over time. In August of 2005 the biomass of common salvinia in the control plots was significantly higher than the biomass in treatments consisting of 1) only *C. salviniae* and 2) both *C. salviniae* and *S. multiplicalis* (Table 2.1). In September the biomass recorded in the control plots was significantly higher than the

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three treatments, 1) *C. salviniae* feeding alone, 2) *S. multiplicalis* feeding alone 3) both *C. salviniae* and *S. multiplicalis* feeding together. A similar trend continued in October.

Table 2.1: Biomass[1] of common salvinia (mean[2] ± S.E) exposed to different herbivore treatments at Gramercy, Louisiana in 2005.

Treatment	June	July	August	September	October
S[3]	170.6 ± 22.9 a[4]	297.3 ± 25.6 a	349.6 ± 17.7 ab	231.0 ± 37.5 b	295.8 ± 64.1 b
C	303.6 ± 44.3 a	299.4 ± 50.8 a	259.7 ± 48.1 b	226.0 ± 50.3 b	240.0 ± 77.5 b
S + C	265.8 ± 20.3 a	300.9 ± 50.9 a	224.5 ± 37.2 b	216.7 ± 29.6 b	185.6 ± 16.4 b
Control	304.9 ± 77.0 a	428.2 ± 13.6 a	467.7 ± 34.0 a	439.1 ± 22.6 a	610.3 ± 32.0 a

[1] Biomass refers to wet weight in grams
[2] Average of four replicates with each having single reading (average of three sub samples) **except** for treatment C, where mean is average of three replicates with each having one reading (average of three sub samples)
[3] S = *Samea multiplicalis*; C = *Cyrtobagous salviniae*
[4] Values with same letters are not significantly different (Tukey- Kramer HSD with alpha = 0.05)

Common salvinia in the treatment plots exposed to feeding by herbivores showed a significantly higher terminal bud feeding damage as compared to the control plots. Percentage terminal damage (PTD) showed a significant treatment effect (F=8.43; df=3, 11; P=0.0034) and a significant interaction within treatments by month (F=2.02; df=12, 44; P=0.0456). In September, PTD in the control plots was significantly less than the treatment consisting of only *C. salviniae*. In October the PTD in control plots was significantly less than treatments consisting of 1) *C. salviniae* alone and 2) both *C. salviniae* and *S. multiplicalis* (Table 2.2). The analysis of variance on PTD data was done on arcsine converted values.

The overall appearance of the common salvinia mats inside the control plots, with reference to the color, was significantly greener than the treatment plots exposed to feeding by the herbivores. Percentage mat green (PMG) showed a significant treatment effect (F=11.5; df=3, 11; P=0.0010) and a significant interaction within treatments by month (F=3.44; df=12, 44; P=0.0013).

Table 2.2: Percentage terminal damage (PTD) to common salvinia (mean[1] ± S.E) exposed to different herbivore treatments at Gramercy, Louisiana in 2005.

Treatment	June	July	August	September	October
S[2]	53.8 ± 13.7 a[3]	30.0 ± 16.2 a	75.8 ± 10.8 a	40.8 ± 12.2 ab	38.0 ± 12.5 ab
C	45.0 ± 3.2 a	31.7 ± 11.9 a	71.7 ± 5.4 a	85.3 ± 12.2 a	63.7 ± 0.3 a
S + C	54.8 ± 5.5 a	41.5 ± 13.3 a	77.3 ± 10.7 a	60.0 ± 18.7 ab	71.0 ± 11.9 a
Control	52.3 ± 11.3 a	3.8 ± 2.5 a	32.3 ± 13.5 a	13.5 ± 3.8 b	8.3 ± 1.9 b

[1] Average of four replicates with each having a single reading **except** for treatment C, where mean is average of three replicates
[2] S = *Samea multiplicalis*; C = *Cyrtobagous salviniae*
[3] Values followed by the same letters are not significantly different (Tukey-Kramer HSD with alpha = 0.05 using arcsine conversion)

In September, PMG in the treatment consisting of only *C. salviniae* was significantly less than the treatment consisting of only *S. multiplicalis* and the control (Table 2.3). In October PMG in the treatments consisting of 1) only *C. salviniae* and 2) both *C. salviniae* and *S. multiplicalis* was significantly less than the control plots. The analysis of variance on PMG data was done on arcsine converted values.

Table 2.3: Percentage mat green (PMG) of common salvinia inside treatment plots (mean[1] ± SE) at Gramercy, Louisiana in 2005.

Treatment	June	July	August	September	October
S[2]	98.7 ± 1.3 a[3]	66.3 ± 11.8 b	100.0 ± 0.0 a	100.0 ± 0.0 a	80.0 ± 12.2 ab
C	100.0 ± 0.0 a	96.7 ± 3.3 ab	96.7 ± 3.3 a	68.3 ± 11.7 b	56.7 ± 3.3 b
S + C	100.0 ± 0.0 a	80.8 ± 8.4 ab	76.3 ± 17.1 a	77.5 ± 10.3 ab	60 ± 10.8 b
Control	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	98.8 ± 2.5 a	100.0 ± 0.0 a

[1] Average of four replicates with each having a single reading **except** for treatment C, where mean is average of three replicates with each having single reading
[2] S = *Samea multiplicalis*; C = *Cyrtobagous salviniae*
[3] Values followed by the same letters are not significantly different (Tukey-Kramer HSD with alpha = 0.05 using arcsine conversion)

The data pertaining to the number of *C. salviniae* adults and *S. multiplicalis* larvae observed during sampling in 2005 has been summarized in Table 2.4. *Samea multiplicalis* was most abundant in the months of June and August whereas the highest numbers of weevil adults

were observed in the months of August and September in 2005. Growth stage and percentage

coverage of common salvinia inside the treatment plots for 2005 are summarized in Table 2.5.

The growth stage was tertiary for the remaining of the study period in the control plots, but in

October the growth stage was mostly primary/secondary for the treatment consisting of both the

herbivores (Table 2.5). Surface water temperature ($F=2.17$; df=3, 11; $P=0.1487$) and pH ($F=1.99$;

df=3, 11; $P=0.1740$) did not show significant treatment effects in 2005. Surface water

temperature and pH values in 2005 are listed in Table 2.6.

Table 2.4: The number of *C. salviniae* adults and *S. multiplicalis* larvae observed during
sampling at Gramercy, Louisiana in 2005.

Treatment	June		July		August		September		October		Total[1]	
	S.m[3]	C.s[4]									S.m	C.s
S[2]	34	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

[1] sum of a row
[2] S = *Samea multiplicalis*; C = *Cyrtobagous salviniae*
[3] no. of *S. multiplicalis* larvae belonging to all instars
[4] No. of C. salviniae adults

2006

Biomass of common salvinia was significantly lower in the treatment plots than in the control

plots and for the treatments consisting of (1) only *C. salviniae* and (2)both *C. salviniae* and *S.

multiplicalis*, there was a decline in the biomass of common salvinia with each passing month.

Biomass showed a significant treatment effect ($F=47.97$; df=3, 12; $P<0.0001$) and was

significantly higher in the control plots when compared to the other three treatments consisting

of 1) *C. salviniae* only 2) *S. multiplicalis* only 3) both *C. salviniae* and *S. multiplicalis* (Fig.

2.1).

Table 2.5: Growth stage and percentage coverage of common salvinia inside treatment plots at Gramercy, Louisiana in 2005.

Treatment/ Replicate		June		July		August		September		October	
		gs^2	$\%c^3$								
S^1	1	p/s	100	s	100	s	100	p/s	85	p/s	95
	2	s/t	100	s/t	100	t	100	s/t	80	p/s	85
	3	t	100	t	100	t	100	t	100	t	100
	4	p/s	100	t	100	t	100	t	100	t	100
C	1	s	100	s/t	100	s	100	p	85	p	50
	2	t	100	t	100	t	100	t	100	t	100
	3	t	100	t	100	s	100	p/s	100	p/s	90
S + C	1	t	100	s	100	s	70	t	95	p/s	70
	2	t	100	t	100	s/t	95	t	100	p/s	80
	3	s/t	100	t	100	t	100	s/t	95	t	100
	4	p/s	100	s	100	s	95	p/s	95	p	100
Control	1	s	100	t	100	t	100	t	100	t	100
	2	p	100	t	100	t	100	t	100	t	100
	3	t	100	t	100	t	100	t	100	t	100
	4	t	100	t	100	t	100	t	100	t	100

[1] S = *Samea multiplicalis*; C = *Cyrtobagous salviniae*
[2] growth stage of common salvinia inside plot; (p = primary, s = secondary, t = tertiary)
[3] percentage coverage of common salvinia inside plot

Table 2.6: Surface water temperature and pH inside the treatment plots at Gramercy, Louisiana in 2005.

Treatment/ Replicate		June		July		August		September		October	
		pH	swt^1								
S^2	1	6.6	33.0	7.0	27.2	6.6	27.2	6.8	28.2	7.0	17.7
	2	6.8	32.7	7.1	28.3	6.9	28.5	6.5	27.5	7.1	19.5
	3	6.7	28.1	6.2	25.8	6.8	24.7	6.5	26.9	7.4	18.8
	4	6.6	26.4	6.7	26.5	6.6	24.7	6.8	26.5	6.9	17.2
C	1	6.5	25.7	6.6	26.1	6.3	25.5	6.4	25.7	6.5	15.5
	2	6.5	28.3	6.5	27.3	6.9	24.3	6.4	29.5	6.6	18.7
	3	6.8	26.5	7.0	26.1	6.7	27.8	6.3	25.7	6.6	17.0
S + C	1	6.8	33.3	7.1	27.7	6.8	29.7	6.6	28.1	6.8	17.6
	2	6.5	27.8	6.9	26.7	6.4	24.7	6.3	29.2	6.7	17.1
	3	6.5	26.4	6.5	26.2	6.6	24.3	6.6	25.7	6.7	18.3
	4	6.7	25.6	7.0	25.5	6.6	25.1	6.5	25.2	6.7	17.1
Control	1	7.6	36.7	7.2	27.2	7.1	27.1	6.9	27.1	7.0	16.5
	2	6.6	35.0	7.0	27.2	6.7	28.2	6.5	25.8	6.9	17.5
	3	7.0	33.2	6.3	26.6	6.6	28.2	6.6	27.5	6.9	20.2
	4	6.6	31.6	7.1	28.3	6.6	24.7	6.3	29.1	6.9	17.8

[1] surface water temperature inside the plot in ºC
[2] S = *Samea multiplicalis*; C = *Cyrtobagous salviniae*

Unlike 2005, the interaction term for treatments by month was non significant in 2006 and thus we focused only on the treatment effect and discounted the significant linear trend (F=6.12; df=1, 48; P=0.0169) of biomass in the treatment with both *C. salviniae* and *S. multiplicalis* and a significant quadratic trend (F=6.30; df=1, 48; P=0.0155) in the treatment with only *S. multiplicalis*.
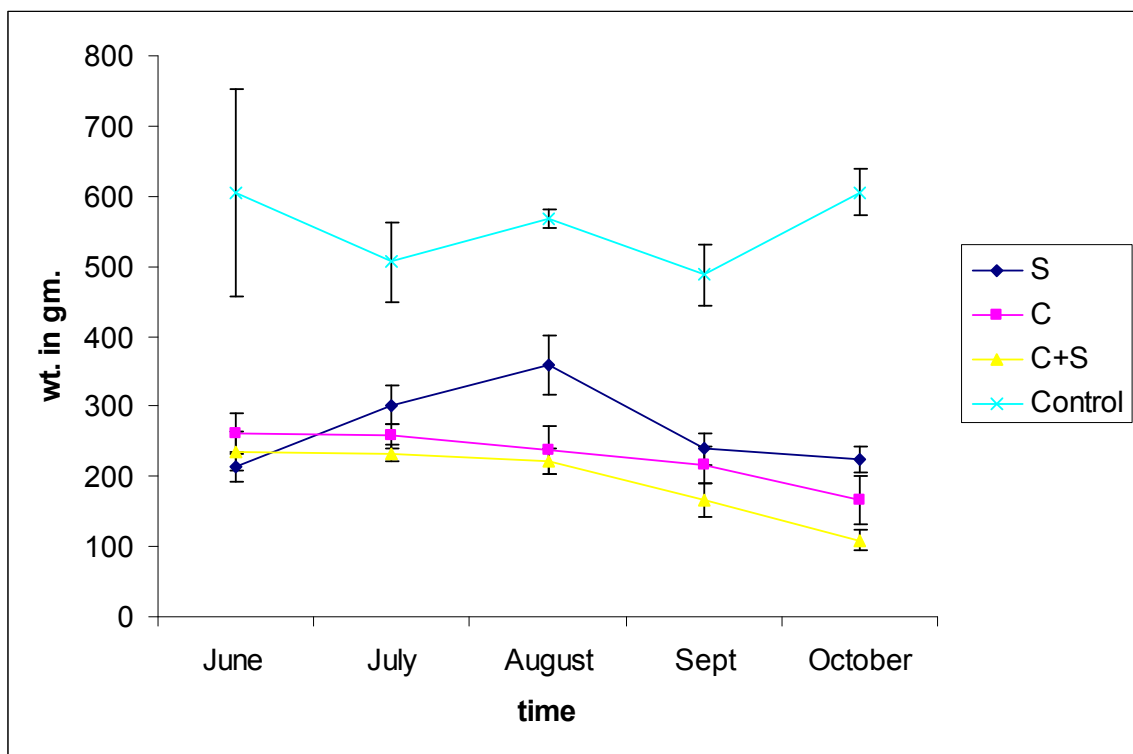
Fig. 2.1: Biomass of common salvinia in different herbivore treatments at Gramercy, Louisiana in 2006.
 S = *Samea multiplicalis*; C = *Cyrtobagous salviniae*

Common salvinia in the treatment plots exposed to feeding by herbivores showed a significantly higher terminal bud feeding damage as compared to the control plots. Percentage terminal damage (PTD) showed a significant treatment effect (F=30.55; df=3, 12; P<0.0001) and a significant interaction within treatments by month (F=2.96; df=12, 48; P=0.0037). In June PTD

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in the treatment only with only *C. salviniae* was significantly higher than the treatment with *S.*
multiplicalis. In July PTD in the treatments consisting of *C. salviniae* only and both *C. salviniae*
and *S. multiplicalis* was significantly higher than the treatment consisting of *S. multiplicalis* only
and the control plots (Table 2.7). In August, PTD in *C. salviniae* only and both *C. salviniae* and
S. multiplicalis treatments was significantly higher than the control and *S. multiplicalis* only
treatment and PTD for the *S. multiplicalis* treatment was significantly higher than in the control
plots. In September, the PTD trend was similar to July of 2006 and in October PTD for the *C.*
salviniae only treatment was significantly higher than the *S. multiplicalis* only treatment and the
control.

Table 2.7: Percentage terminal damage (PTD) to common salvinia (mean[1] ± S.E) exposed to
different herbivore treatments at Gramercy, Louisiana in 2006.

Treatment	June	July	August	September	October
S[2]	5.3 ± 2.3 b[3]	3.0 ± 0.7 b	22.5 ± 4.5 b	9.3 ± 2.8 b	10.5 ± 3.2 b
C	35.0 ± 10.6 a	27.3 ± 6.7 a	44.0 ± 4.2 a	46.0 ± 4.3 a	26.5 ± 4.8 a
S + C	20.5 ± 7.6 ab	31.3 ± 6.6 a	48.5 ± 3.2 a	42.3 ± 6.5 a	15.0 ± 2.8 ab
Control	7.0 ± 2.2 ab	0.8 ± .3 b	6.0 ± 1.4 c	5.8 ± 1.9 b	7.0 ± 2.3 b

[1] Average of four replicates with each having a single reading
[2] S = *Samea multiplicalis*; C = *Cyrtobagous salviniae*
[3] Values followed by the same letters are not significantly different (Tukey-Kramer HSD with
alpha = 0.05 using arcsine conversion)

The numbers of *C. salviniae* adults and *S. multiplicalis* larvae observed during sampling
in 2006 are summarized in Table 2.8. *Samea multiplicalis* was most abundant in the months of
June and August, but the highest numbers of weevil adults were observed in the months of July
and September in 2005. Percentage mat green (PMG) showed a significant treatment effect
(F=8.28; df=3, 12; P= 0.0030) with *C. salviniae* only and both *C. salviniae* and *S. multiplicalis*
treatments significantly different from the control. Also, for PMG the interaction within
treatment by month was non significant (F=0.61; df=3, 12; P=0.8248) in 2006. Growth stage,
percentage coverage and percentage mat green values recorded for different treatments in 2006

are presented in Table 2.9. Growth stage remained tertiary in the control plots for the entire study period whereas for the treatment consisting of both the herbivores, growth stage was mostly secondary/tertiary in the month of September and primary/secondary in the month of October. Also, the growth stage was mostly secondary/tertiary in the month of October for the treatment consisting of only *C. salviniae*. Surface water temperature ($F=2.64$; $df=3, 12$; $P=0.0974$) and pH ($F=0.89$; $df=3, 12$; $P=0.4759$) did not show significant treatment effect in 2006. Table 2.10 displays the values recorded for surface water temperature and pH in 2006.

Table 2.8: The number of *C. salviniae* adults and *S. multiplicalis* larvae observed during sampling at Gramercy, Louisiana in 2006.

Treatment	June		July		August		September		October		Total[1]	
	S.m[3]	C.s[4]									S.m	C.s
S[2]	10	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

[1] sum of a row
[2] S = *Samea multiplicalis*; C = *Cyrtobagous salviniae*
[3] no. of *S. multiplicalis* larvae belonging to all instars
[4] no. of *C. salviniae* adults

DISCUSSION

Biomass of common salvinia was measured in terms of wet weight and not dry weight as destructive sampling was not feasible because of the presence of herbivores in plant material, and also the experimental design, which required collection of data over time. However, linear regression analysis of wet and dry weights was highly significant with almost 98% of the variability explained by the model and justified measuring and analyzing biomass data as wet weights.

Table 2.9: Growth stage, percentage coverage and percentage mat green of common salvinia inside treatment plots at Gramercy, Louisiana in 2006.

Treatment/ Replicate		June gs[2]	%c[3]	%mg[4]	July gs	%c	%mg	August gs	%c	%mg	September gs	%c	%mg	October gs	%c	%mg
S[1]	1	s	90	95	s	100	100	t	100	95	s/t	100	95	s	100	98
	2	s	100	90	t	100	80	t	90	90	t	95	90	t	95	90
	3	t	95	95	t	100	85	t	100	98	t	80	75	t	80	80
	4	s	100	90	s/t	100	95	t	95	80	t	95	95	s	100	75
C	1	s/t	110	80	s	100	50	s/t	80	50	p/s	90	70	s/t	80	70
	2	s/t	100	95	s	100	95	s/t	100	90	p/s	80	60	p	80	80
	3	t	100	40	s	100	50	t	100	90	s/t	90	80	s/t	90	80
	4	s	95	80	s/t	100	100	t	100	90	t	100	80	s/t	100	85
S + C	1	s/t	100	70	s/t	90	50	s/t	90	90	s/t	50	20	p	80	80
	2	s/t	100	80	s/t	100	60	t	100	85	s/t	99	70	p/s	85	70
	3	s	100	80	s	100	70	s/t	95	80	s/t	100	70	s	100	75
	4	s	100	90	s	100	99	s/t	95	90	p/s	90	75	p/s	90	90
Control	1	t	100	95	t	100	95	t	100	98	t	100	95	t	100	98
	2	t	100	85	t	100	95	t	100	99	t	100	99	t	100	98
	3	t	100	100	t	100	95	t	100	100	t	100	90	t	100	98
	4	t	100	100	t	100	90	t	100	99	t	100	100	t	100	99

[1] S = *Samea multiplicalis*; C = *Cyrtobagous salviniae*
[2] growth stage of common salvinia inside plot (p = primary, s = secondary, t = tertiary)
[3] percentage coverage of common salvinia inside plot
[4] percentage mat green of common salvinia inside plot

Table 2.10: Surface water temperature and pH inside the treatment plots at Gramercy, Louisiana in 2006.

Treatment/ Replicate		June pH	swt[1]	July pH	swt	August pH	swt	September pH	swt	October pH	swt
S[2]	1	6.7	24.3	6.5	24.6	7.1	24.4	7.3	21.8	6.5	16.5
	2	6.7	27.5	6.4	25.8	6.8	24.3	6.8	21.5	6.9	14.0
	3	6.8	27.8	6.7	25.8	6.9	24.0	6.9	23.3	7.1	14.0
	4	6.7	27.8	6.4	26.0	6.6	24.0	7.3	21.0	7.5	16.0
C	1	6.9	25.7	6.6	26.0	7.0	26.7	7.3	24.0	6.9	17.0
	2	6.6	24.8	6.8	25.3	6.6	23.9	7.0	20.7	7.2	18.0
	3	6.8	27.8	6.5	26.2	6.5	24.8	6.7	21.0	7.2	17.0
	4	6.4	24.6	6.7	24.9	7.5	24.4	7.2	22.2	6.9	17.0
S + C	1	6.8	27.9	6.5	25.2	6.7	24.2	6.8	22.0	7.1	19.8
	2	6.8	28.4	6.6	25.6	6.5	24.6	6.7	21.0	6.9	17.2
	3	6.7	27.6	6.5	25.8	6.6	23.9	6.9	22.0	7.0	16.4
	4	6.5	25.1	6.6	26.4	6.8	24.2	7.1	22.0	7.0	18.0
Control	1	6.9	25.6	6.7	26.0	7.0	26.8	7.2	24.6	7.1	17.0
	2	7.0	28.4	6.6	26.1	6.7	22.4	7.1	23.0	6.8	17.4
	3	6.9	25.7	6.9	26.3	7.2	26.6	7.6	25.0	7.3	17.4
	4	6.7	27.1	6.4	25.9	6.6	24.6	6.7	22.0	6.7	16.0

[1] surface water temperature inside the plot in ºC
[2] S = *Samea multiplicalis*; C = *Cyrtobagous salvinia*

A

B

C

Fig. 2.2: Treatments showing feeding damage. (A) Feeding by both *S. multiplicalis* and *C. salviniae*. (B) Feeding by *C. salviniae* alone. (C) Feeding by *S. multiplicalis* alone. (D) Control. (fig. continued)
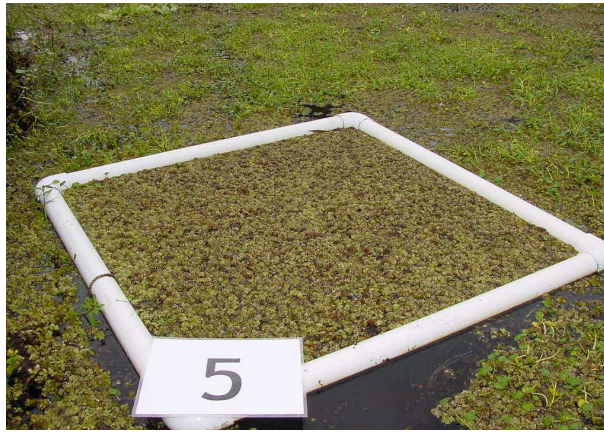
D

Fig. 2.3: Satellite image of the study site at Gramercy, Louisiana (DOQQ, 2005).

This study showed for the first time that *S. multiplicalis* had a significant impact on biomass of common salvinia, and contradicted earlier reports that it had a "negligible impact" on common salvinia in Texas and Louisiana (Tippings and Center, 2005). The quadratic trend in biomass of common salvinia over time in the treatment consisting of only *S. multiplicalis* in 2005 gives an insight into its population dynamics. Beginning in June, biomass showed an upward trend until August which was indicative of a decline in *S. multiplicalis* population. In September, there was a sharp decline in biomass brought about by a rebound in the *S. multiplicalis* population. These fluctuations may have been caused by natural population cycles of the

herbivore which seems to do better in spring and fall. Parasitism of *S. multiplicalis* larvae may be one of the reasons responsible for this trend. Knopf and Habeck (1976) reared four parasitoids (three ichneumonids and one tachinid) from *S. multiplicalis* larvae in Florida. During the course of this project, some *S. multiplicalis* larvae collected from the field and reared in the lab were parasitized by a braconid wasp. Also, Semple and Forno (1987) mentioned the recovery of five parasitoids and three pathogens from *S. multiplicalis* larvae in Queensland, Australia. Although not experimentally established, red imported fire ants, *Solenopsis invicta* Buren, could also have negatively impacted *S. multiplicalis* populations as workers were frequently observed foraging on common salvinia mats infested with *S. multiplicalis*.

The numbers of *S. multiplicalis* larvae observed in 2005 were higher than those observed in 2006, but the trend for *C. salviniae* was just the reverse with more adults observed in 2006 than 2005 (Tables 2.4 and 2.8). Although it is not clear what factors lead to the decline of *S. multiplicalis* populations in 2006, this situation may have favored the initial establishment of *C. salviniae* at release sites, given the higher number of adult *C. salviniae* observed in 2006. Alternatively in 2005, when the populations of native *S. multiplicalis* were high, they could have had a negative impact on the survival and establishment of *C. salviniae* since both the species were competing for a common resource. The greater number of *C. salviniae* adults released per plot in 2006 may also have contributed to the higher population of *C. salviniae* observed. The non significant interaction within treatment by months in 2006 could also have been a result of lower *S. multiplicalis* populations.

Also, our study was unique in the sense that *C. salviniae* adults released in 2005 failed to over-winter and consequently had to be released again in 2006. As a result, we could not document their impact from one year to the next. If the *C. salviniae* had overwintered a more

comprehensive understanding of the efficiency and interaction of these two agents in bringing about biological control of common salvinia would have been possible. Had *C. salviniae* successfully over- wintered, we would have had the opportunity in 2006 to study and compare the impact of two established herbivores on common salvinia.

The mean percentage terminal damage recorded was higher for all treatments in 2005 when compared to 2006 probably because the population of *S. multiplicalis* was higher in 2005 which would have resulted in more feeding on terminal buds. Also, percentage terminal damage data were collected by two different individuals in 2005 and 2006 and this could have played some role in the above mentioned difference. The effect of feeding by both *C. salviniae* and *S. multiplicalis* on the growth stages of common salvinia was discernible towards the end of study period (October) in both 2005 and 2006 with most treatment plots showing primary, secondary and primary/secondary growth stages as compared to the tertiary growth stage of the control plots.

Environmental variables such as pH and surface water temperature did not show a treatment effect in this study. The reason could be the small size of our plots (1 m²), which in some cases were surrounded by other aquatic vegetation. Any treatment effects on water quality, if they occurred, were probably obscured by the impacts of other surrounding vegetation on the water quality of the plots. Ideally, the impact of common salvinia on water quality variables should be measured when large and pure stands of common salvinia are exposed to the different treatments.

This study was thus 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 combination of *C. salviniae* and *S. multiplicalis* feeding together

appears to have the greatest impact on common salvinia, at least in the initial year when non native *C. salviniae* was introduced. We base this conclusion on the fact that the biomass of common salvinia in the treatment consisting of both *C. salviniae* and *S. multiplicalis* feeding together was the least (although not significantly less than the treatments consisting of either *C. salviniae* or *S. multiplicalis* alone) in both 2005 and 2006 (Table 2.1 and Figure 2.1).

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 its larvae and adults feeding on common salvinia may ultimately turn out to be a better control agent than *S. multiplicalis* since common salvinia can multiply at exceedingly fast rates and a constant feeding pressure must be maintained to have any kind of long term impact on its growth and spread. The gap between succeeding larval generations of *S. multiplicalis* gives common salvinia an opportunity to rebound from feeding damage and thus 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. It is also recommended based on our observations to keep the populations of *S. multiplicalis* low at the sites where *C. salviniae* adults have been released, preferably by using microbial insecticides, during the initial couple of months so that the weevils get a chance to establish without competing for resources.

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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CHAPTER 3

FORAGING BEHAVIOR OF *SOLENOPSIS INVICTA* (HYMENOPTERA: FORMICIDAE) ON COMMON SALVINIA

INTRODUCTION

Red imported fire ants (RIFA), *Solenopsis invicta* Buren, were introduced into the United States in the 1930s (Tschinkel, 2006) and have spread throughout the southeastern parts of the country (Gotelli and Arnett, 2000). The diet of RIFA is very broad and consists of numerous arthropods and invertebrates in addition to vegetative matter (Tschinkel, 2006). Since the feeding behavior of RIFA is indiscriminate, they usually have an impact on the populations of other animal species around them (Tschinkel, 2006) and in some cases may adversely affect beneficial insects and arthropods (Eubanks, 2001). RIFA are able to colonize disturbed habitats (Taber, 2000) and have been documented to occur in proximity to ponds (Tschinkel, 1988).

RIFA impact the populations of a number of lepidopteran insect spp. (eggs, larvae and adults) in different ecological niches and agroecosystems. Reagan et al. (1972) discussed the increase in *Diatraea saccharalis* (F.) (sugarcane borer) infestations when insecticides were used to eliminate or reduce RIFA populations in Louisiana sugarcane fields. McDaniel and Sterling (1979) reported RIFA was the major predator of *Heliothis virescens* F. (tobacco budworm) eggs in cotton. Eger et al. (1983) studied the winter survival of *H. virescens* and *Heliothis zea* Boddie (cotton bollworm) in Texas and suspected RIFA had a negative impact on the survival of the two species. Elvin et al. (1983) suggested that RIFA dominated the predation of small and medium *Anticarsia gemmatalis* Hübner (velvetbean caterpillar) larvae in soybeans. RIFA was the second ranked control agent of lepidopteran larvae in cotton (Eubanks, 2001) and Seagraves and McPherson (2006) described RIFA predation on the eggs of lepidopteran pests in soybean.

A number of studies have documented the foraging behavior of RIFA in aquatic ecosystems. Dray et al. (2001) documented the foraging of fire ants on mats of waterlettuce plants (*Pistia stratiotes* L.) in Florida. Predation by fire ants was implicated as being one of the

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reasons for the failure of *Spodoptera pectinicornis* Hampson (Lepidoptera: Noctuidae), a biological control agent of waterlettuce, to establish in Florida despite numerous attempts. Both adults and larvae of *S. pectinicornis* were targeted by fire ants which were found in high numbers at the release cages. Freed and Neitman (1988) reported RIFA utilizing aquatic vegetation to forage over the surface of water and Patrock (in press) recorded RIFA worker trails on long leaf pondweed (*Potamogeton nodosus*). RIFA were observed using long leaf pondweed as a "platform" to forage over the surface of water about 8 m from the shore of a man made lake (Patrock, in press).

Samea multiplicalis Guenee, a generalist herbivore which occurs in the southeastern United States, was studied as a potential biological control agent of *Salvinia molesta* D. S. Mitchell (giant salvinia) in Australia (Sands and Kassulke, 1984). Tippings and Center (2005) reported *S. multiplicalis* feeding on *Salvinia minima* Baker (common salvinia) in Florida. RIFA were observed on common salvinia mats during the course of the study described in chapter 2 and are possibly an important natural enemy of *S. multiplicalis*.

Documenting the foraging behavior of RIFA on common salvinia mats is the first step in determining the importance of RIFA as a natural enemy of *S. multiplicalis* on common salvinia.

MATERIALS AND METHODS

This study to determine the foraging behavior of RIFA on common salvinia mats was conducted on the private property of Mr. James Boyce, located north of Gramercy, Louisiana and adjacent to Highway 61 ((30º10'46.77"N 90 º49'07.75"W). Crawfish ponds, flooded woodlands dominated by cypress and tupelo gum trees, and dredged canals running parallel to levees were the main topographical features of the location. The woodlands and canals were heavily infested with common salvinia when the study was undertaken.

Four locations with about 70% shade were selected in the flooded woodlands and designated study sites A, B, C and D (Fig. 3.1). Sites A and B were located on the northern part of the property and were about 50 m apart. Both of these sites were accessible by a levee which ran between the flooded woodland and a dredged canal. Sites C and D were located on the southern part of the property and separated by a levee that ran across the flooded woodland. Five additional sites exposed to full sunlight at least six hours a day were also randomly selected throughout the property and designated as sites 1, 2, 3, 4 and 5 (Fig. 3.1). These sites were in dredged canals that held water throughout the year and heavily infested with common salvinia when the study was conducted.

Bait stations consisted of a floating platform made of 0.06 m² Styrofoam squares (0.01 m thick) and 0.012×0.075 m disposable culture tubes (Kimble Glass Inc.) held in position with utility duct tape (Mainstays™) (Fig. 3.2). The Styrofoam base stabilized the glass tube with bait on the surface of common salvinia. Surface temperature readings were taken with a handheld infrared thermometer (Mini Temp Testr IR, Oakton®). Vienna sausage (Libby's®) was used as bait in all the stations. All photographs were taken using FinePix S5200 digital camera (Fujifilm®). The study was conducted in May of 2007.

Bait stations were placed at distances of 1, 2, 3, 4, 5, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90, and 100 m from the levee at sites A and B (Fig. 3.3). The same interval was followed for sites C and D but it was not physically possible to place the bait stations beyond 50 m at site C and beyond 70 m at site D. In canals the bait stations were placed at 1 to 3 m distance from the levee depending upon depth and accessibility. Bait stations were also placed on the levee between sites A and B. Bait stations were placed up to 15 m from the levee at sites A, B, C and D on 7 and 11 May of 2007. On 17 and 18 May of 2007 the bait stations were placed at 20, 30, 40, 50, 60, 70,

80, 90, and 100 m from the levee at sites A and B. Bait stations were also placed at 20, 30, 40 and 50 m from the levee at site C and at 20, 30, 40, 50, 60 and 70 m from the levee at site D on May 17 and 18. Two bait stations were placed on the levee between sites A and B and a single bait station per site was placed at the five sites in the canals on May 17 and 18, 2007.

Bait stations were set up at 0830 hours CST and checked at 1130 hours and 1430 hours CST at sites A and B. At sites C and D the stations were set up at 0900 hours CST and checked at 1200 hours and 1500 hours CST. Bait stations on the levee and in canals were set up at 0930 hours CST and checked at 1230 hours and 1530 hours CST. Stations that recruited RIFA were collected and replaced with new ones. Surface temperature was also recorded at each of the individual bait stations. The ants collected at the bait stations were brought back to the laboratory in a cooler and held in the refrigerator for identification and counting.

Data on the number of RIFA collected at different distances from the levee and at different times of the day for sites A, B, C and D were analyzed with the chi square goodness of fit test and linear regression on JMP IN (2003) and SAS (2003). Data on the number of RIFA collected at different times of the day for sites 1, 2, 3, 4 and 5 were analyzed for difference in mean with the t test in SAS (2003).

RESULTS

Site A

RIFA were observed foraging ca. 50 m from the levee and recruited to bait stations placed at 1, 2, 3, 4, 5, 10, 15, 20, 40 and 50 m (Table 3.1). The number of RIFA collected at individual bait stations placed along the transect ranged from 13 to 156 and no other ant species were collected. The surface temperature at bait stations ranged from 21.8ºC in the shaded part of the flooded woodland to 26.5ºC in patches exposed to sunlight.
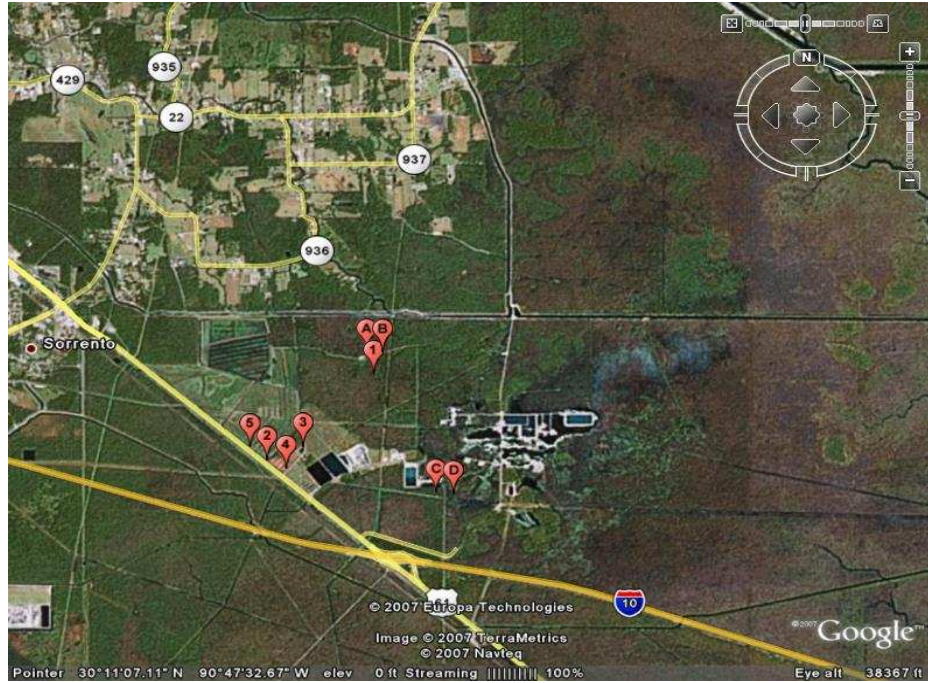
Fig. 3.1 Satellite image of the study site with locations in the flooded woodland (A, B, C and D) and in canals (1, 2, 3, 4 and 5) at Gramercy, Louisiana. (© Google Earth)

Fig. 3.2 Fire ant bait station on the surface of common salvinia.

Fig. 3.3 Transect of bait stations in flooded woodland near Gramercy, Louisiana at site A in May 2007.

The foraging trail of RIFA at the 1 m bait station led to the levee. RIFA observed at 20 m recruited from a mound located at the base of a tree in the vicinity of the bait station (Fig. 3.4). Another RIFA mound, in a dead tree stump, was found located ca. 60 m from the levee (Figs. 3.4 and 3.5) but no trails were observed leading to any of the bait stations from this mound.

7 May 2007 (1130 hours): The recruitment of RIFA at the bait stations placed 1, 2, 3, 4, 5, 10 and 15 m from the levee was not uniform (X^2=660.0; df=6; P<0.0001) and the linear relationship between the no. of RIFA at a bait station and the distance from the levee was non significant (F=1.15; df=1, 5; P=0.3317).

7 May 2007 (1430 hours): The recruitment of RIFA at the bait stations was not uniform (X^2=446.2; df=6; P<0.0001) and the linear relationship between the no. of RIFA at a bait station and the distance from the levee was non significant (F=0.66; df=1, 5; P=0.4522). Also, the

spatial recruitments of RIFA at 1130 hours and 1430 hours were significantly different from each other (X^2=214.1; df=3; P<0.0001).

11 May 2007 (1130 hours): The recruitment of RIFA at the bait stations placed 1, 2, 3, 4, 5, 10 and 15 m from the levee was not uniform (X^2=615.5; df=6; P<0.0001) and the linear relationship between the no. of RIFA at a bait station and the distance from the levee was non significant (F=0.53; df=1, 5; P=0.5006).

11 May 2007 (1430 hours): The recruitment of RIFA at the bait stations was not uniform (X^2=141.6; df=6; P<0.0001) and the linear relationship between the no. of RIFA at a bait station and the distance from the levee was non significant (F=0.24; df=1, 5; P=0.6425). Also, the spatial recruitments of RIFA at 1130 hours and 1430 hours were significantly different from each other (X^2=322.1; df=5; P<0.0001).

17 May 2007 (1130 hours): The recruitment of RIFA at the bait stations placed 20, 30, 40, 50, 60, 70, 80, 90 and 100 m from the levee was not uniform (X^2=783.2; df=8; P<0.0001) and the linear relationship between the no. of RIFA at a bait station and the distance from the levee was non significant (F=3.16; df=1, 7; P=0.1188).

17 May 2007 (1430 hours): The recruitment of RIFA at the bait stations was not uniform (X^2=654.5; df=8; P<0.0001) and the linear relationship between the no. of RIFA at a bait station and the distance from the levee was non significant (F=2.13; df=1, 7; P=0.1878). Also, the spatial recruitments of RIFA at 1130 hours and 1430 were significantly different from each other (X^2=8.3; df=2; P=0.0160).

18 May 2007 (1130 hours): The recruitment of RIFA at the bait stations placed 20, 30, 40, 50, 60, 70, 80, 90 and 100 m from the levee was not uniform (X^2=715.4; df=8; P<0.0001)

and the linear relationship between the no. of RIFA at a bait station and the distance from the levee was non significant (F=1.10; df=1, 7; P=0.3287).

18 May 2007 (1430 hours): The recruitment of RIFA at the bait stations was not uniform (X^2=820.6; df=8; P<0.0001) and the linear relationship between the no. of RIFA at a bait station and the distance from the levee was non significant (F=0.47; df=1, 7; P=0.5131). Also, the spatial recruitments of RIFA at 1130 hours and 1430 hours were significantly different from each other (X^2=8.5; df=1; P=0.0036).

Fig. 3.4 RIFA mound at the base of a tree ca. 20 m from the levee (Site A) in flooded woodland at Gramercy, Louisiana in May 2007.

Site B

RIFA were observed foraging ca. 80 m from the levee and recruited to bait stations placed at 1, 2, 3, 4, 5, 10, 20, 30, 40, 50 and 80 m (Table 3.2).

A

B

Fig. 3.5 RIFA mound in a dead tree stump at site A in flooded woodland at Gramercy, Louisiana in May 2007 ca. 60 m from the levee. (A) RIFA mound (B) close up of the mound with RIFA visible in the top right corner.

Table 3.1: Number of RIFA collected at hot dog baits from site A in flooded woodland at Gramercy, Louisiana.

Distance[1]	7 May 2007 1130[2]	1430	11 May 2007 1130	1430	17 May 2007 1130	1430	18 May 2007 1130	1430
1	150	130	132	32	-----	-----	-----	-----
2	0	0	0	46	-----	-----	-----	-----
3	0	0	13	52	-----	-----	-----	-----
4	0	0	0	25	-----	-----	-----	-----
5	60	0	0	0	-----	-----	-----	-----
10	0	60	0	100	-----	-----	-----	-----
15	0	100	135	30	-----	-----	-----	-----
20	-----	-----	-----	-----	96	48	54	26
30	-----	-----	-----	-----	0	0	0	0
40	-----	-----	-----	-----	117	110	0	0
50	-----	-----	-----	-----	156	120	119	125
60	-----	-----	-----	-----	0	0	0	0
70	-----	-----	-----	-----	0	0	0	0
80	-----	-----	-----	-----	0	0	0	0
90	-----	-----	-----	-----	0	0	0	0
100	-----	-----	-----	-----	0	0	0	0

[1] Distance from the levee in m
[2] Time of the day (CST)

The number of RIFA collected at individual bait stations placed along the transect ranged from 5 to 250 (Table 3.2) and no other ant species were collected. The surface temperature at bait stations ranged from 21.8ºC in the shaded part of the flooded woodland to 27.2ºC in patches exposed to sunlight. The foraging trail of RIFA at the 1 m bait station led to the levee. RIFA were also observed moving between bait stations placed at 1, 2, 3, 4, and 5 m and appeared to be coming from the levee. RIFA collected at 50 m recruited from a mound located on a dead tree stump in the vicinity of the bait station (Fig. 3.6).

7 May 2007 (1130 hours): The recruitment of RIFA at the bait stations placed 1, 2, 3, 4, 5, 10 and 15 m from the levee was not uniform ($X^2=506.3$; df=6; $P<0.0001$) but the linear relationship between the no. of RIFA at a bait station and the distance from the levee was

significant (F=7.06; df=1, 5; P=0.0450). The number of RIFA decreased with distance from the levee.

7 May 2007 (1430 hours): The recruitment of RIFA at the bait stations was not uniform (X^2=725.9; df=6; P<0.0001) and the linear relationship between the no. of RIFA at a bait station and the distance from the levee was non significant (F=3.29; df=1, 5; P=0.1296). Also, the spatial recruitments of RIFA at 1130 hours and 1430 hours were significantly different from each other (X^2=98.3; df=4; P<0.0001).

11 May 2007 (1130 hours): The recruitment of RIFA at the bait stations placed 1, 2, 3, 4, 5, 10 and 15 m from the levee was not uniform (X^2=473.8; df=6; P<0.0001) and the linear relationship between the no. of RIFA at a bait station and the distance from the levee was non significant (F=1.86; df=1, 5; P=0.2304).

11 May 2007 (1430 hours): The recruitment of RIFA at the bait stations was not uniform (X^2=292.1; df=6; P<0.0001) and the linear relationship between the no. of RIFA at a bait station and the distance from the levee was non significant (F=1.26; df=1, 5; P=0.3199). Also, the spatial recruitments of RIFA at 1130 hours and 1430 hours were significantly different from each other (X^2=136.2; df=5; P<0.0001).

17 May 2007 (1130 hours): The recruitment of RIFA at the bait stations placed 20, 30, 40, 50, 60, 70, 80, 90 and 100 m from the levee was not uniform (X^2=552.9; df=8; P<0.0001) and the linear relationship between the no. of RIFA at a bait station and the distance from the levee was non significant (F=3.23; df=1, 7; P=0.1155).

17 May 2007 (1430 hours): The recruitment of RIFA at the bait stations was not uniform (X^2=479.5; df=8; P<0.0001) but the linear relationship between the no. of RIFA at a bait station and the distance from the levee was significant (F=9.23; df=1, 7; P=0.0189). Also, the spatial

recruitments of RIFA at 1130 hours and 1430 hours were significantly different from each other (X^2=85.9; df=4; P<0.0001).

18 May 2007 (1130 hours): The recruitment of RIFA at the bait stations placed 20, 30, 40, 50, 60, 70, 80, 90 and 100 m from the levee was not uniform (X^2=418.8; df=8; P<0.0001) and the linear relationship between the no. of RIFA at a bait station and the distance from the levee was non significant (F=3.37; df=1, 7; P=0.1089).

18 May 2007 (1430 hours): The recruitment of RIFA at the bait stations was not uniform (X^2=318.0; df=8; P<0.0001) but the linear relationship between the no. of RIFA at a bait station and the distance from the levee was significant (F=6.15; df=1, 7; P=0.0422). Also, the spatial recruitments of RIFA at 1130 hours and 1430 hours were significantly different from each other (X^2=5.5; df=1; P=0.0189).

Table 3.2: Number of RIFA collected at hot dog baits from site B in flooded woodland at Gramercy, Louisiana.

Distance[1]	7 May 2007 1130[2]	1430	11 May 2007 1130	1430	17 May 2007 1130	1430	18 May 2007 1130	1430
1	200	110	0	10	-----	-----	-----	-----
2	140	250	178	51	-----	-----	-----	-----
3	60	81	98	154	-----	-----	-----	-----
4	105	32	40	74	-----	-----	-----	-----
5	6	5	85	62	-----	-----	-----	-----
10	0	0	0	30	-----	-----	-----	-----
15	0	0	0	0	-----	-----	-----	-----
20	-----	-----	-----	-----	0	72	20	34
30	-----	-----	-----	-----	110	90	67	52
40	-----	-----	-----	-----	70	130	0	0
50	-----	-----	-----	-----	82	110	0	0
60	-----	-----	-----	-----	0	0	0	0
70	-----	-----	-----	-----	0	0	0	0
80	-----	-----	-----	-----	0	24	0	0
90	-----	-----	-----	-----	0	0	0	0
100	-----	-----	-----	-----	0	0	0	0

[1] Distance from the levee in m
[2] Time of the day (CST)

Fig. 3.6 RIFA mound covering a dead tree stump ca. 50 m from the levee at site B in Gramercy, Louisiana.

Site C

RIFA were observed foraging ca. 30 m from the levee and recruited to bait stations placed at 1, 2, 3, 4, 5, 15 and 30 m (Table 3.3). The number of RIFA collected at individual bait stations placed along the transect ranged from 9 to 157 (Table 3.3). The surface temperature at bait stations ranged from 24.4ºC in the shaded part of the flooded woodland to 31.8ºC in patches exposed to sunlight.

The foraging trail of RIFA at the 1 m bait station led to the levee. Carpenter ants (*Camponotus* spp.) were collected from the bait station placed 40 m from the levee. This was the only other genus of ants observed at site C.

7 May 2007 (1200 hours and 15000 hours): RIFA were not collected at any of the bait stations placed 1, 2, 3, 4, 5, 10 and 15 m from the levee at site C.

11 May 2007 (1200 hours): The recruitment of RIFA at the bait stations placed 1, 2, 3, 4, 5, 10 and 15 m from the levee was not uniform (X^2=535.6; df=6; P<0.0001) and the linear

relationship between the no. of RIFA at a bait station and the distance from the levee was non significant ($F=0.65$; df=1, 5; $P=0.4577$).

11 May 2007 (1500 hours): The recruitment of RIFA at the bait stations was not uniform ($X^2=270.3$; df=6; $P<0.0001$) and the linear relationship between the no. of RIFA at a bait station and the distance from the levee was non significant ($F=1.15$; df=1, 5; $P=0.3329$). Also, the spatial recruitments of RIFA at 1200 hours and 1500 hours were significantly different from each other ($X^2=84.7$; df=5; $P<0.0001$).

17 May 2007 (1200 hours): RIFA were not collected from any of the bait stations placed at 20, 30, 40 and 50 m from the levee.

17 May 2007 (1500 hours): The recruitment of RIFA at the bait stations was not uniform ($X^2=378.0$; df=3; $P<0.0001$) and linear relationship between the no. of RIFA at a bait station and the distance from the levee was non significant ($F=0.14$; df=1, 2; $P=0.7418$).

18 May 2007 (1200 hours and 1500 hours): RIFA were not collected at any of the bait stations placed at 20, 30, 40 and 50 m from the levee at site C.

Table 3.3: Number of RIFA collected at hot dog baits from site C in flooded woodland at Gramercy, Louisiana.

| Distance[1] | 7 May 2007 | | 11 May 2007 | | 17 May 2007 | | 18 May 2007 | |
	1200[2]	1500	1200	1500	1200	1500	1200	1500
1	0	0	58	70	-----	-----	-----	-----
2	0	0	34	110	-----	-----	-----	-----
3	0	0	0	32	-----	-----	-----	-----
4	0	0	157	155	-----	-----	-----	-----
5	0	0	0	30	-----	-----	-----	-----
10	0	0	0	0	-----	-----	-----	-----
15	0	0	9	45	-----	-----	-----	-----
20	-----	-----	-----	-----	0	0	0	0
30	-----	-----	-----	-----	0	126	0	0
40	-----	-----	-----	-----	0	0	0	0
50	-----	-----	-----	-----	0	0	0	0

[1] Distance from the levee in m
[2] Time of the day (CST)

Site D

RIFA were observed foraging only ca. 10 m from the levee and recruited to bait stations placed at 2, 3, 4, 5 and 10 m (Table 3.4). The number of RIFA collected at individual bait stations placed along the transect ranged from 6 to 168. The surface temperature at bait stations ranged from 23.3ºC in the shaded part of the flooded woodland to 28.8ºC in patches exposed to sunlight.

Carpenter ant (*Camponotus* spp.) adults were collected from the bait station placed 30 m from the levee. This was the only other genus of ants observed at site D. No RIFA mounds were observed in proximity of the transect at site D.

7 May 2007 (1200 hours): The recruitment of RIFA at the bait stations placed 1, 2, 3, 4, 5, 10 and 15 m from the levee was not uniform (X^2=600.0; df=6; P<0.0001) and the linear relationship between the no. of RIFA at a bait station and the distance from the levee was non significant (F=0.02; df=1,5; P=0.8938).

7 May 2007 (1500 hours): The recruitment of RIFA at the bait stations was not uniform (X^2=195.0; df=6; P<0.0001) and the linear relationship between the no. of RIFA at a bait station and the distance from the levee was non significant (F=0.10; df=1, 5; P=0.7679). Also, the spatial recruitments of RIFA at 1200 hours and 1500 hours were significantly different from each other (X^2=41.9; df=1; P<0.0001).

11 May 2007 (1200 hours): The recruitment of RIFA at the bait stations placed 1, 2, 3, 4, 5, 10 and 15 m from the levee was not uniform (X^2=650.7; df=6; P<0.0001) and the linear relationship between the no. of RIFA at a bait station and the distance from the levee was non significant (F=0.37; df=1, 5; P=0.5672).

11 May 2007 (1500 hours): The recruitment of RIFA at the bait stations was not uniform (X^2=294.8; df=6; P<0.0001) and the linear relationship between the no. of RIFA at a bait station and the distance from the levee was non significant (F=1.08; df=1, 5; P=0.3467). Also, the spatial recruitments of RIFA at 1200 hours and 1500 hours were significantly different from each other (X^2=269.0; df=4; P<0.0001).

17 May 2007 (1200 hours and 1500 hours): RIFA were not collected at any of the bait stations placed 20, 30, 40, 50, 60 and 70 m from the levee.

18 May 2007 (1200 hours and 1500 hours): RIFA were not collected at any of the bait stations placed 20, 30, 40, 50, 60 and 70 m from the levee.

Table 3.4: Number of RIFA collected at hot dog baits from site D in flooded woodland at Gramercy, Louisiana.

| Distance[1] | 7 May 2007 | | 11 May 2007 | | 17 May 2007 | | 18 May 2007 | |
	1200[2]	1500	1200	1500	1200	1500	1200	1500
1	0	0	0	0	-----	-----	-----	-----
2	0	0	0	168	-----	-----	-----	-----
3	0	0	139	133	-----	-----	-----	-----
4	0	25	95	76	-----	-----	-----	-----
5	100	45	201	97	-----	-----	-----	-----
10	0	0	6	90	-----	-----	-----	-----
15	0	0	0	0	-----	-----	-----	-----
20	-----	-----	-----	-----	0	0	0	0
30	-----	-----	-----	-----	0	0	0	0
40	-----	-----	-----	-----	0	0	0	0
50	-----	-----	-----	-----	0	0	0	0
60	-----	-----	-----	-----	0	0	0	0
70	-----	-----	-----	-----	0	0	0	0

[1] Distance from the levee in m
[2] Time of the day (CST)

Canals

RIFA recruited to bait stations placed at all five sites in the canals (Table 3.5). Surface temperature at sites 1, 2, 3, 4 and 5 ranged from 32ºC to 34.6ºC.

17 May 2007

There was no significant difference (t =0.77; df=8; P=0.4655) between the number of ants collected at 1230 hours and 1530 hours at sites 1, 2, 3, 4, and 5.

18 May 2007:

There was no significant difference (t =0.25; df=8; P=0.8053) between the number of ants collected at 1230 hours and 1530 hours at sites 1, 2, 3, 4, and 5.

Table 3.5: Number of RIFA collected at hot dog baits from sites located in dredged canals.

| | 17 May 2007 | | 18 May 2007 | |
Site	1230[1]	1530	1230	1530
1	73	225	176	84
2	47	52	0	0
3	24	15	64	86
4	0	32	0	108
5	53	30	23	34

[1] Time of the day (CST)

Levee

Bait stations were placed on the levee between sites A and B. RIFA recruited to all the stations and a considerable number were found under the Styrofoam base when the surface temperature reached 40.8ºC, while few were observed on top of the bait stations. Bait was then placed on the surface of levee without the station (Fig. 3.7) and 41 RIFA were collected at a single location when the surface temperature was 40ºC.

DISCUSSION

This study was able to document extensive foraging by RIFA over common salvinia mats in both flooded woodlands and canals. The mounds of RIFA were not limited to the levees but also found to be established within the flooded woodlands at different locations and on varied substrates such as the base of a live tree or dead tree stumps. RIFA are known to form a floating

ball during floods (Taber, 2000) and this adaptation can help them to survive in the flooded environment of the woodlands where water level fluctuates often and can rise considerably, especially after heavy rainfall.

Fig. 3.7 RIFA recruiting to hot dog bait placed on the levee at 40ºC (surface temperature) in Gramercy, Louisiana.

Analysis of the number of RIFA collected at bait stations placed along transects at different distances from the levee indicated that they were not uniformly distributed. Also, this distribution was not linear at sites A, C and D while at site B, a linear trend was observed at 1130 hours on 7 May 2007, 1430 hours on 17 May 20007 and 1430 hours on 18 May 2007. These findings are supported by the discovery of randomly distributed RIFA mounds present in the flooded woodlands. RIFA recruiting to any particular bait station appears to depend upon the proximity of the bait station to a mound. Also, there does not seem to be a clear relationship between the number of RIFA recruiting to a bait station and the time of day.

One shortcoming of this study was that the bait stations were not placed along the entire length of the transect (1 to 100 m for sites A and B, 1 to 50 m for site C and 1 to 70 m for site D) on the same day and during the same sampling period. These data would have provided a better understanding of the temporal and spatial foraging behavior of RIFA in flooded woodlands. Such a study is planned for the summer of 2007 and it would also provide information regarding the foraging behavior of RIFA in a different climatic regime as compared to spring.

Analysis of data for sites 1, 2, 3, 4 and 5 in the canals illustrates that RIFA were foraging in late morning as well as early afternoon. However, there was no clear temporal effect on the number of RIFA collected in late morning or early afternoon. The highest temperature recorded on the surface of common salvinia in the canals during this study was ca. 35ºC and it did not inhibit the foraging activity of RIFA. The average surface temperature in flooded woodlands was much lower than the canals and the highest recorded was 31.8ºC at site C. Thus in spring, temperature thus does not appear to be a limiting factor in the foraging activity of RIFA on common salvinia, especially in flooded woodlands which are about 70% shaded.

Temperature was found to be one of the most important factors influencing foraging by RIFA (Porter and Tschinkel, 1987). RIFA foraged "regularly" on land when surface temperatures were in excess of 40ºC and continued to do so even at temperatures greater than 50ºC (Porter and Tschinkel, 1987). These results are consistent with our study as RIFA were observed foraging on the levee at 40ºC, the highest temperature recorded on the surface. However, Porter and Tschinke (1987) found soil temperature at 2 cm to be the "best predictor" of "foraging rates" by RIFA and the maximum foraging took place between 22 and 36ºC.

The extensive foraging of RIFA on the mats of common salvinia could thus have a negative impact on the populations of native *S. multiplicalis* and also possibly on the survival

and establishment of *Cyrtobagous salviniae* Calder and Sands (Coleoptera: Curculionidae), a

biological control agent being released in Louisiana for the control of common salvinia.

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