# AN EVALUATION OF TWO STRAINS OF *Cyrtobagous salviniae* CALDER AND SANDS AS NATURAL ENEMIES OF THE AQUATIC WEEDS *Salvinia molesta* MITCHELL AND *Salvinia minima* BAKER

A Thesis

by

JEREMIAH M. DYE

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

December 2005

Major Subject: Entomology

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Approved by:

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# ABSTRACT

An Evaluation of Two Strains of *Cyrtobagous salviniae* Calder and Sands as Natural Enemies of the Aquatic Weeds *Salvinia molesta* Mitchell and *Salvinia minima* Baker.

(December 2005)

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The floating aquatic weeds common salvinia (*Salvinia minima* Baker) and giant salvinia (*Salvinia molesta* Mitchell) degrade aquatic systems through fast, mat forming growth. The *Salvinia* specialist weevil *Cyrtobagous salviniae* Calder and Sands has been used to reduce the severity of giant salvinia infestations and associated with reduced severity of common salvinia infestations. Genetically, morphologically and biologically distinct strains of *C. salviniae* exist, but their relative potential for success as biological control agents of *Salvinia* species has not been evaluated. This thesis (1) describes a recirculating water system designed for conducting such studies and (2) reports the results of *C. salviniae* strain comparisons.

A recirculating water system with a high degree of replication and minimal variation in water flow, temperature and light intensity was used for laboratory experiments using sixty-day temperature profiles averaging 31.4, 26.5 and 8.0 ºC derived from surface water temperatures measured at lakes in expected range of *Salvinia* species in the North America. Larval and adult population numbers of two *C. salviniae*

strains (Australia and Florida) were determined for each temperature profile along with feeding induced plant necrosis on both *Salvinia* species. Australia *C. salviniae* had lower survivorship rates to adulthood on common salvinia than did Florida *C. salviniae* at the 31.4 and 26.5 ºC temperature profiles. Neither strain reproduced, and no significant between-strain differences in plant necrosis were detected at the 8.0 ºC temperature profile. At 31.4ºC there were no significant differences in adult counts, larval counts or plant damage between the two strains on giant salvinia. At 26.5ºC, however, significantly fewer larvae were collected from initially released adults and significantly less plant necrosis was associated with weevil feeding by Florida strain compared to Australia strain weevils. These results may have arisen from comparing Australia weevils from a growing colony to Florida weevils from a declining colony. Overall, the results indicate that only Florida *C. salviniae* should be released against common salvinia. Florida *C. salviniae* may be equally suitable to Australia *C. salviniae* for releases against giant salvinia, but further study is needed to fully assess the potential for using Florida *C. salviniae* against giant salvinia.

## ACKNOWLEDGEMENTS

I must thank my advisory committee members, Drs. Kevin Heinz, John Jackman and Stephen Davis III, for their encouragement, support and prodding. I am especially grateful to Dr. Heinz for my growth in critical thinking gained through his influence and example. I could not have persevered through my project without the camaraderie of my lab mates, Jason Mottern, Jarrad Prasifka, Jennifer Doles, Amy Bader and Jeremy Hudgeons. Above all, I must thank my dear wife, Wendy, for her company, help and encouragement when I needed it most.

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# CHAPTER I

## INTRODUCTION

Members of the genus *Salvinia* [Pterydophyta: Salviniecae] are aquatic, free floating ferns (Mitchell and Thomas 1972, Harley and Mitchell 1981, Oliver 1993). The plants grow as a series of nodes interconnected by horizontal rhizomes and can form thick mats covering the surface of slow moving water bodies. At each node, two photosynthetic leaves rest on top of the water surface, and a third leaf is submerged and finely divided. The plants lack true roots, but the submerged leaf functions in water and nutrient uptake. Heterosporous reproductive structures, when present, develop from the submerged leaves. Despite the presence of sexual reproductive structures, the primary mode of dispersal for *Salvinia* species is through budding. Rhizomes are easily broken, allowing small ramets to be carried to new habitats by wind and water currents. In uncrowded conditions, the two surface leaves are relatively smaller and lay flat on the surface, while rhizomes are longer and thinner. In crowded conditions the surface leaves are larger and folded along the midline, while the rhizomes are short and thick.

Unchecked salvinia growth can have dramatic effects on aquatic habitats. Thick mats, often colonized by other plants, can be nearly impassable to boats. When the plants cover the water surface they block the penetration of light into the water, reducing photosynthesis in the water column. Water beneath salvinia mats becomes stagnant due to an accumulation of organic matter coupled with reduced underwater photosynthesis

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This thesis follows the style and format of the Journal of Aquatic Plant Management.

(Harley and Mitchell 1981).

Escape from intentional cultivation is the means by which salvinia species become naturalized outside of their native ranges. For example, *Salvinia molesta* Mitchell is native to South America (Forno and Harley 1979) and was first carried outside of its native range to Sri Lanka in 1939 by researchers at the University of Colombo (Room and Fernando 1992). Within four years it had escaped confinement in an outdoor pond, and by 1954 had infested thousands of hectares of Sri Lankan rice paddies. Subsequently, infestations have been reported around the world including areas of Africa, Australia, Papua New Guinea and India (Oliver 1993). The first infestation in North America was detected in 1995 in a water garden at an elementary school in Houston (Jacono 1999), and giant salvinia is now reported in 12 US states (Jacono et al. 2001).

The invasion and subsequent range expansion of *Salvinia minima* Baker to the US follow a similar pattern of spread following human cultivation, and have been reported by Jacono et al. (2001) as follows. The native range of common salvinia extends from southern Mexico to northern Argentina, and some have also considered it to be native to Florida. However, the authors showed that the species became naturalized in Florida in the 1930's after being was cultivated in US greenhouses and gardens as early as the 1880's. Common salvinia is now also naturalized in South Carolina, Georgia, Mississippi, Missouri, Louisiana and Texas.

The rapid growth and ready dispersal of giant salvinia make chemical and

mechanical control methods ineffective because they require indefinitely repeated treatments to maintain an acceptable level of control, while biological control agents have the potential for successful and long-term control. The weevil *Cyrtobagous salviniae* Calder and Sands has been used to successfully control giant salvinia in many countries (Oliver 1993). The species was first used in Australia, where individuals collected from giant salvinia located near Joinville, Brazille and tested for host specificity were released to control giant salvinia infestations (Thomas and Room 1986). The releases were deemed successful, and the weevils destroyed several thousand tons of giant salvinia within a few years after release (Room et al. 1981). Weevils collected from Australia have been used to successfully control giant salvinia in many other areas including Papua New Guinea (Thomas and Room 1986), India (Joy et al. 1985) and Africa (Cilliers 1991, Giliomee 1986).

The time required to bring infestations under control using *C. salviniae* in temperate regions is generally longer than is required in tropical regions. Cilliers (1991) reported that control was achieved four years after initial releases in the more temperate Southeastern Cape of South Africa, while the salvinia mats in the more tropical regions of the country were controlled in only one or two years. Similarly, when *C. salviniae* was released in Australia, control was achieved within two years of the initial release except at two temperate sites in the southern part of the country where significant reductions in giant salvinia biomass were not observed until four years after the initial releases. (Room et al. 1984).

Differences in time to achieve control in different climates can be explained by the

influences of temperature on *C. salviniae.* Sands et al. (1986) found in a laboratory study that adult survivorship increased from 13 weeks to 20 weeks as rearing temperature decreased from 31°C to 23°C. These authors found that average lifetime fecundity was 374 eggs per female over 15 weeks at 27°C and decreased to 274 eggs produced over 13 weeks at 31°C. However, with this increase in temperature, developmental time to adulthood decreased from 5.15 weeks to 4.14 weeks, and a population was thus expected to increase 1000 fold in 22 weeks at either temperature. At 23°C the average lifetime fecundity was 320 eggs over 20 weeks, but the population would take 50 weeks to increase 1000 fold due to an 8.21 week developmental time to adulthood. Another explanation of the longer time to control giant salvinia with *C. salviniae* in temperate regions may be that females stop laying eggs below 21°C, and eggs must be above 19°C to hatch (Cilliers 1991). In contrast, though giant salvinia grows fastest at 30°C, it can continue to grow even at 12°C (Room 1986).

In addition to temperature, biological control efforts against giant salvinia in the US using *C. salviniae* are further complicated by potential variability among strains. In their 1985 description of *C. salviniae*, Calder and Sands reclassified *C. singularis* from Brazil, Paraguay, Argentina and Florida as *C. salviniae*. In South America the species was recorded feeding on *Salvinia auriculata* Aublet, *Salvinia herzogii* de la Sota, *Salvinia biloba* Raddi, and giant salvinia. The reclassified specimens from Florida were collected from common salvinia.

The presence of *C. salviniae* in already the US resulted in a different approach to dealing with giant salvinia when it was first detected in the US in 1995 (Jacono 1999). Instead

of importing *C. salviniae* weevils from Australia, the first biological control attempts were made in 1999 using weevils collected from common salvinia in Florida (Tipping and Center 2005). However, the dramatic reductions in giant salvinia commonly resulting from *C. salviniae* releases with were not observed, though environmental instability at the release sites hindered a full evaluation of the *C. salviniae* strain from Florida. At the same time, Goolsby et al. (2000) compared DNA for 28S ribosomal RNA from both strains and found base pair differences between the two strains that were as frequent as have been observed between described species in the same genus in other studies. Goolsby et al. (2000) used these differences to suggest the possibility that the two strains might actually be different species. Similarly, in the original description of the species, Calder and Sands (1985) noted that the weevils collected from Florida were significantly smaller than those on *S. molesta* from Brazil. Subsequent releases in the US have included only the use of *C. salviniae* from Australia because uncertainty relating to the species status of Florida strain *C. salviniae* gave cause to question whether it would be able to control giant salvinia as well as the better studied Australia strain (Tipping and Center 2005).

The potential for use of the Florida strain of *C. salviniae* for biological control of both giant salvinia and common salvinia is currently unknown, though the presence of this strain in Florida may explain why common salvinia is much less aggressive there than in other states (Jacono et al. 2001). In addition, the potential for use of Australia strain *C. salviniae* against common salvinia has not been evaluated. The goal of my research, which is described in chapter III, was to compare the Florida and Australia

strains of *C. salviniae* relative to their ability to reproduce and damage giant salvinia and common salvinia at the temperatures the weevils can be expected to experience in the US. In order to be able to conduct this comparison, I first designed and built a replicated, temperature-controlled experimental system capable of simulating daily temperature fluctuations and seasonal temperature and photoperiod changes. This system is described in detail in Chapter II.

# CHAPTER II

# A RECIRCULATING WATER SYSTEM FOR BIOLOGICAL AND ECOLOGICAL STUDIES OF AQUATIC ORGANISMS

## Introduction

Manipulative experimental studies of the floating aquatic ferns in the genus *Salvinia* (Pteridophyta: Salviniaceae) have generally been conducted at three different scales: containers up to two liters in size in growth chambers, containers ranging is size from two liters to 4400 liters in greenhouses or outside, and floating cages or quadrats on existing outdoor water bodies. Many, such as in Forno and Bourne (1985, 1986, 1988), Gardner and Al-Hamdani (1997) and Sands et al. (1983, 1986), are conducted in ≤ 2 liter containers, placed in temperature controlled chambers and often hold individual plants as units of study. Small containers in growth chambers allow full control of temperature and photoperiod. Effects of water quality and nutrient availability can also be studied in this way, but can be limited by the need to frequently replenish the nutrient solution. Generally such studies are limited in duration to days or weeks. Others have used 2 liter to 4400 liter containers in greenhouses or outside (Chrisholm 1979, George 1976, Jayanth and Nagarkatti 1987). Larger outside containers allow the scale, duration and conditions of the experiment to be more realistic. Others have used floating cages or quadrats on outdoor water bodies to increase the scale of the experiment and to gain confidence in the relevance of results to field conditions (Julien et al. 1987, Room and

Thomas 1985). However, reproducibility of field studies becomes a challenge due to daily variation and stochasticity in outdoor conditions. Unrestricted outdoor studies are also not possible in cases where quarantine procedures are mandated. In addition, temperate zone seasonality may limit outdoor experiments to a small window each year when conditions are suitable for the organism to be tested.

To gain many of the advantages of both outdoor systems and laboratory systems, while avoiding many of the disadvantages of each, we designed and built a recirculating water system capable of regulating temperature and photoperiod of a large number of homogeneous experimental units to match daily and seasonal changes from any site for which such data exists. The system was designed for investigation of the influences of host plant and temperature regimes on the usefulness of different strains of the weevil *Cyrtobagous salviniae* Calder and Sands, a natural enemy of the aquatic weeds giant salvinia (*Salvinia molesta* Mitchell) and common salvinia (*Salvinia minima* Baker) for biological control of these species. Here we delineate the features and utility of this system in conducting replicated, manipulative experiments.

## Materials and Methods

### Experimental units

The system permits a high degree of replication through the inclusion of 48 experimental units. Each experimental unit (Figure 1-A) was a 70 liter plastic storage bin (Model 1730 Tote Box, Sterilite Corporation, Townsend, MA) measuring 60.5 cm long x 43 cm wide x 39 cm high. Each unit had a water depth of 25 cm, resulting in 45.5 l of water and a water surface (Figure 1-B) area of 51 cm by 38 cm. Twelve utility

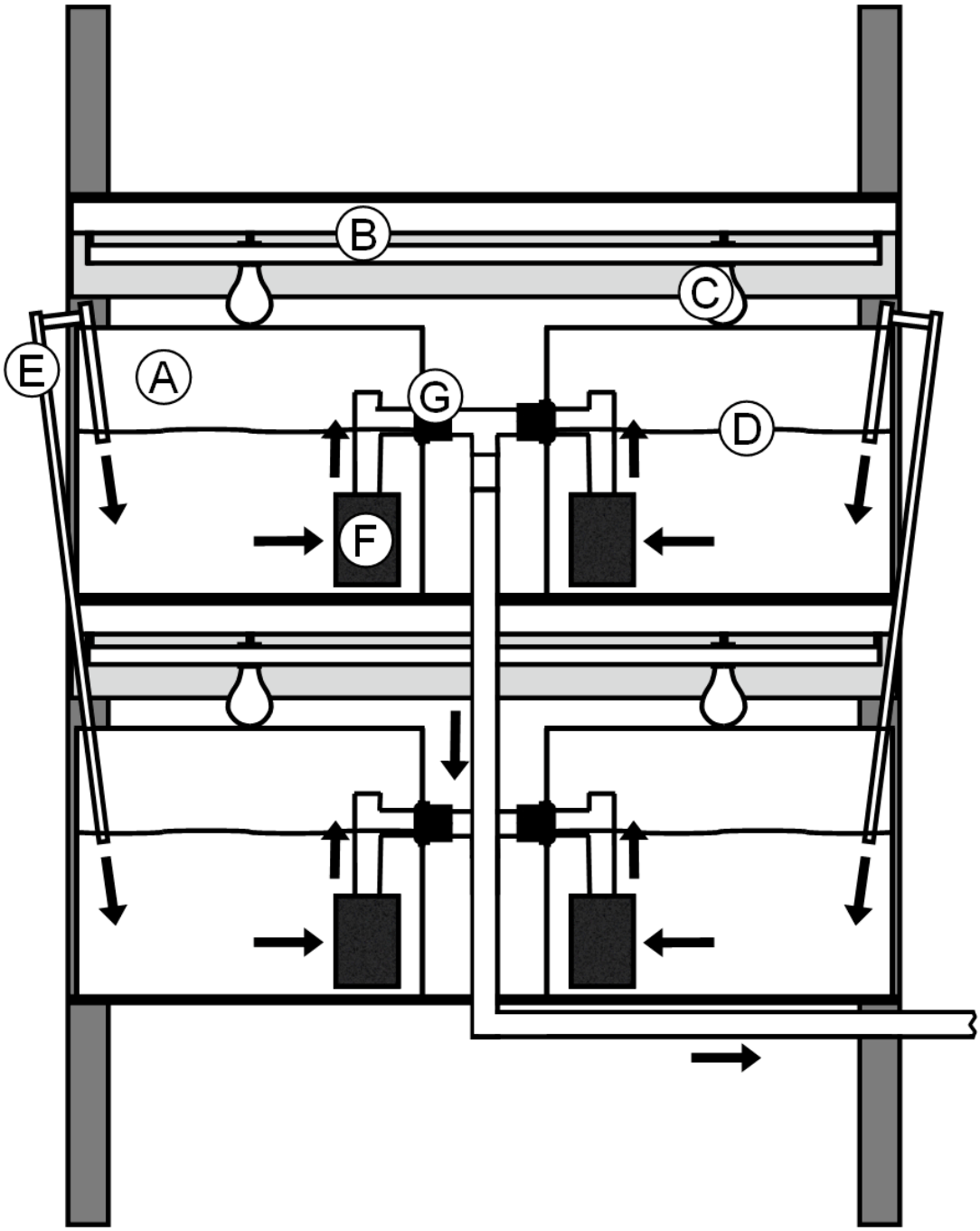


Figure 1. Diagram of one shelving unit and associated experimental units (A), fluorescent light fixtures (B), incandescent light bulbs (C) water surface (D), water delivery pipes (E), submerged filters (F), bulkhead fittings (G). Arrows indicate direction of water flow.

shelving units (Model SR-200, Edsal Manufacturing Co., Inc, Chicago, IL) measuring 2.4 m long by 1.2 m wide by 1.8 m high and constructed with shelves at 35, 96, and 157 cm above the floor (Figure 1) held four experimental units each with two experimental units on the lower shelves and two experimental units on the middle shelves. In selecting the appropriate shelf heights, three factors were considered. First, though the shelving units were designed by the manufacturer to have adjustable shelf heights, the structure of the shelving units required one shelf at 96 cm above the floor. Second, light fixtures (Figure 1-B, C) were to be installed underneath the middle and upper shelves of each shelving unit to provide light for experimental units placed on the lower and middle shelves, respectively. The bottom shelf was placed at a height of 35mm so that the space between the top of the experimental units and the light fixtures was large enough to allow access by experimenters while maximizing the light intensity experienced at the water surface (Figure 1-D) of the experimental units. The top shelf height of 157 cm above the floor resulted in the same clearance for the upper tanks. Minimizing the distance between shelves had the effect of increasing the light intensity experienced at the water level of the experimental units. The shelving units (Figure 2-A) were arranged into three groups of four shelving units so as to maximize usage of the available space (a 4.3 m by 6.1 m laboratory room) while allowing access to all of the experimental units (Figure 2-B).

**Water delivery system**

To minimize the nutrient and temperature variation between experimental units, we designed a water delivery system in which a common water supply recirculated

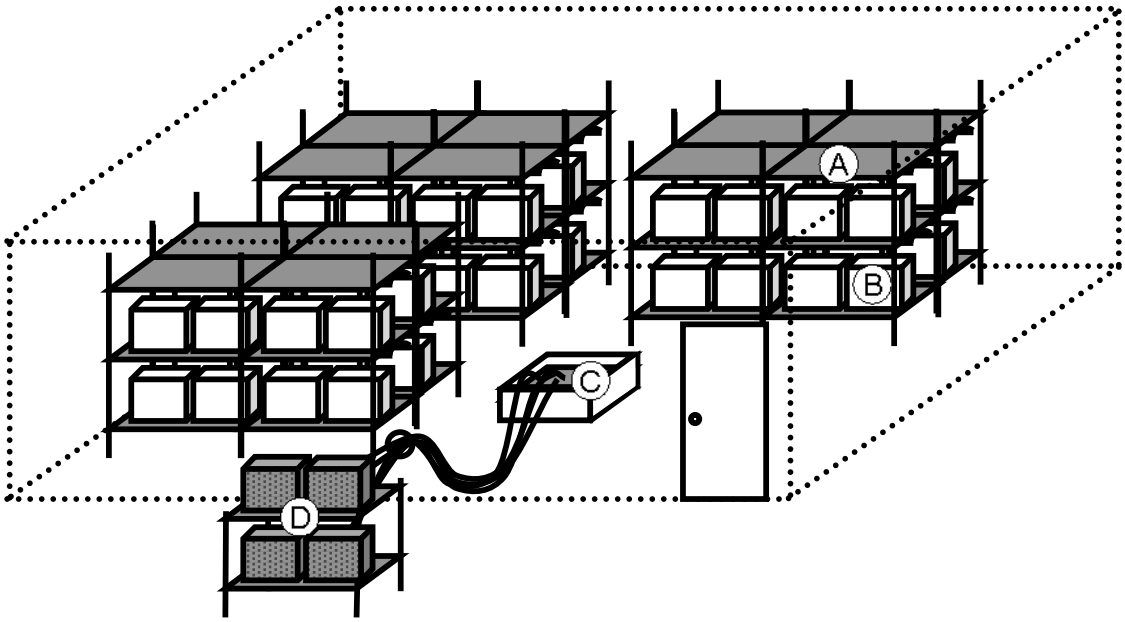


Figure 2. A diagram of the layout of the experimental system including shelving units (A), experimental units (B), the central tank (C) and chillers (D).

between the experimental units (Figure 2-B) and a central tank (Figure 2-C) where temperature was regulated and nutrients were added. Water traveled from this central tank to each experimental unit through a fully parallel water delivery system in which the water flowed through only one experimental unit before returning to the central tank. This was accomplished through a series of branches (Figures 3, 4) that divided the output of a single pump 48 ways to individually supply each experimental unit. This parallel approach avoided the temperature and nutrient gradient that would exist between experimental units if the outflow of one experimental unit became the inflow of another experimental unit in a serial flow design. We further minimized temperature variation between experimental units by taking steps as described below to minimize variation in flow rate through each experimental unit.

A 370 watt centrifugal pump (Aqua Sea 4500, Dolphin Aquarium & Pet Products, Inc., Pensacola, FL) was used to pump water throughout the system. The pump drew water from the central tank through two sponge filters connected by a tee to a 25.4 mm diameter PVC pipe. Water was also carried from the pump to the first split using 25.4 mm diameter PVC (Figure 3-A). We used a flow meter (F-300 series Blue White Industries, Huntington Beach, CA) to detect the flow rate (Figure 3-B) going into the first split (Figure 3-C), where the water flow was divided into three branches through the use of a four-way tee. After the first split, water in each of these primary branches traveled through 25.4 mm diameter PVC to a gate valve (Figure 3-D) on each branch that allowed adjustment of the flow rate for that branch. Water in each of the three branches was then split into two secondary branches at a three way 25.4 mm diameter

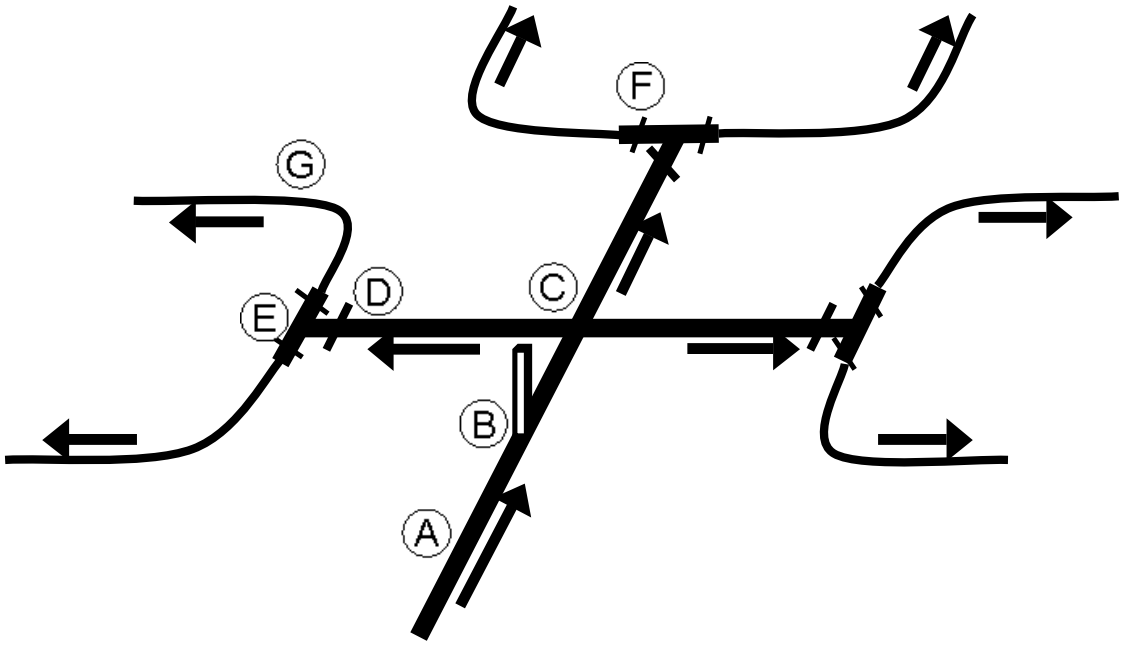


Figure 3. Diagram depicting the first two branches of the water delivery system. Parts are labeled as follows: pipe from pump (A), flow meter (B), first branch point (C), a gate valve on a primary branch (D), one of the three secondary branch points (E), a ball valve on a secondary branch (F), and a secondary branch hose carrying water for eight experimental units (G).

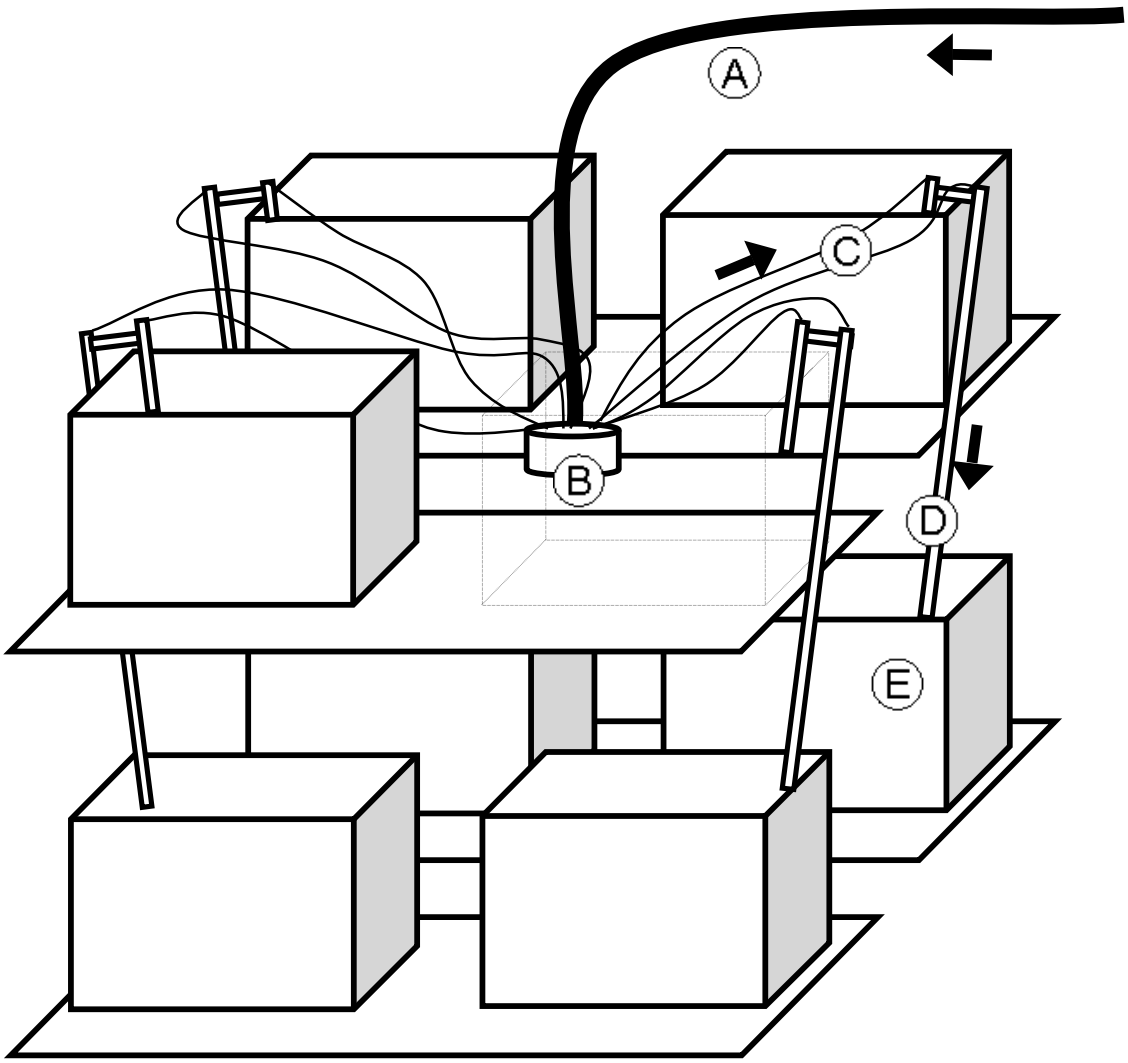


Figure 4. Diagram of a second-level branch and associated third-level branches of the water delivery system. Water travels through hoses (A) from the secondary branch points to an eight way split (B) then through tubing (C) to water delivery pipes (D) and into the experimental units (E). The arrows indicate the direction of water flow.

PVC tee joint (Figure 3-E) so that the pump output was divided into six branches in all. After the tees, the secondary branches were reduced to 13 mm PVC, and 13 mm PVC ball valves (Figure 3-F) on each of these secondary branches to allow further regulation of the flow rate. A 13 mm PVC barbed insert adapter connected the ball valve of each secondary branch to 15 mm diameter rubber heater hoses (Figure 3-G) that carried the water to the final splits.

Each of the six secondary branches (Figures 3-G, 4-A) carried water for eight experimental units through 4.9 m of 13 mm diameter rubber heater hose to an eight outlet drip irrigation hydrant (R154D Bubbler Hydrant, Raindrip Inc, Simi Valley, CA). The hydrants (Figure 4-B) were modified by removing the included screen filters and flow reducers. Each hydrant was connected to its supply hose by a 13 mm PVC barbed insert adapter. The water entering each hydrant then flowed into eight different 80 cm long 6.4 mm diameter vinyl irrigation tubes (Figure 4-C) that each carried water for one experimental unit. Water passed from the hydrants through the 6.4 mm diameter irrigation tubes to a 90º elbow joint and then through another 12 cm section of 6.4 mm diameter irrigation tubing. The 12 cm long sections of 6.4 mm diameter tubing were placed inside the ends of 13 mm diameter PVC pipes (Figure 4-D), which acted as chutes to carry the water below the surface of each experimental unit (Figure 4-E). Each delivery pipe for an upper lever experimental unit was paired with the delivery pipe for the experimental unit directly below it and connected using a short piece of 13 mm PVC and two three-way tees (Figure 4). The short connector of each pair of delivery pipes rested on the edge of the upper level experimental unit so that each pipe hung into its

respective experimental unit. No seal existed between the 6.4mm diameter tubing and the 13 mm diameter delivery pipes. Water simply exited the ends of the 6.4 mm diameter tubing inside of the 13 mm diameter pipes and traveled down the inside surfaces of the pipes to the experimental units. The addition of these delivery pipes allowed water to be delivered to both upper and lower experimental units while exiting the pressurized portion of the delivery system at the same height above the ground for both upper and lower shelf experimental units. The delivery pipes minimized surface disturbance by delivering incoming water below the water surface of each experimental unit while also prevented a siphoning effect that would have existed if the outlet of the 6.4 mm diameter tubing had been below the water surface of the experimental units. Siphoning would have resulted in a higher flow rate to the bottom shelf tanks than to the top shelf tanks when the pump was running. It would also have caused water in the experimental units to be drawn back into the central tank whenever the pump was turned off.

**Balancing water flow**

The single flow meter allowed the water flow rate through each of the six secondary branches to be balanced relative to each other. Using a single flow meter was preferable to using multiple flow meters since it reduced costs and precluded variation in factory calibration between different flow meters from resulting in apparently balanced flow rates between different branches despite identical flow rate measurements. To balance the flow rates of the six secondary branches, all valves regulating flow in the primary and secondary branches were first fully opened. Then two of the primary

branch valves were closed, so that water could only flow through one of the primary branches. The valve of one of the secondary branches still receiving water was then closed, and we recorded the flow rate through the remaining secondary branch. The open secondary branch valve on the open primary branch was then closed, and the secondary branch valve that was previously closed was opened, resulting in a new flow rate. The secondary branch with the higher initial flow rate was then adjusted so that water flowed through it at the same rate as the secondary branch with the lower initial flow rate. The two secondary branches could then be considered to be balanced relative to each other. We then repeated this procedure for the secondary branches of the other two primary branches.

A similar procedure then allowed the three primary branches to be balanced relative to each other. First, recorded the flow rate through each primary branch was recorded when the other two primary branch valves were closed to determine the branch with the slowest flow rate. Then water was allowed to flow only through one of the two faster primary branches. The flow rate was then adjusted in that primary branch to match the flow rate previously recorded for the slowest primary branch. Next, the combined flow rate was recorded as water was allowed to flow through the slowest primary branch and the newly adjusted primary branch simultaneously. The original slowest primary branch was then closed, and water was allowed to flow through the adjusted primary branch and the remaining branch simultaneously. The remaining branch was then adjusted so that the combined flow rate of the second and third primary branches matches the previously noted combined flow rate of the first and second

primary branches. The first primary branch was then fully opened, and the flow rate through the six secondary branches could be expected to be equivalent to each other.

**Water return system**

An overflow hole on the side of each experimental unit allowed water outflow to match inflow. However, to minimize surface disturbance and prevent biological material in each experimental unit from being carried out of the tank, a foam filter (Figure 1-F) was placed below the surface and connected to the side hole by a PVC assembly as shown previously in Figure 1. The assemblies contained a hole open to the air to allow the water level inside the assembly to match the water level in the rest of the tank and prevent siphoning while letting the hole in the side of the tank dictate the water level despite the submerged intake. The filter assembly screwed into a 25.4 mm diameter bulkhead fitting (Figure 1-F) on the inside of the tank. This fitting allowed the outflow to pass through the hole in the side of the experimental units (Figure 5-A) and be connected to a series of collecting pipes (Figure 5-B) and carried back to the central tank. Water from the two adjacent experimental units of the upper shelf of one shelving unit first combined in a 25.4 mm diameter PVC tee fitting. The combined flow then joined the combined output of two experimental units at the same shelf height on the shelving unit adjacent on the long side. The combined flow of four upper shelf experimental units then joined the combined flow from the four experimental units directly below them. Finally, the flow from eight experimental units joined with the combined flow of the eight experimental units adjacent on the short side, and returned to the central tank. The central tank was fitted with a float valve supplied by a reverse

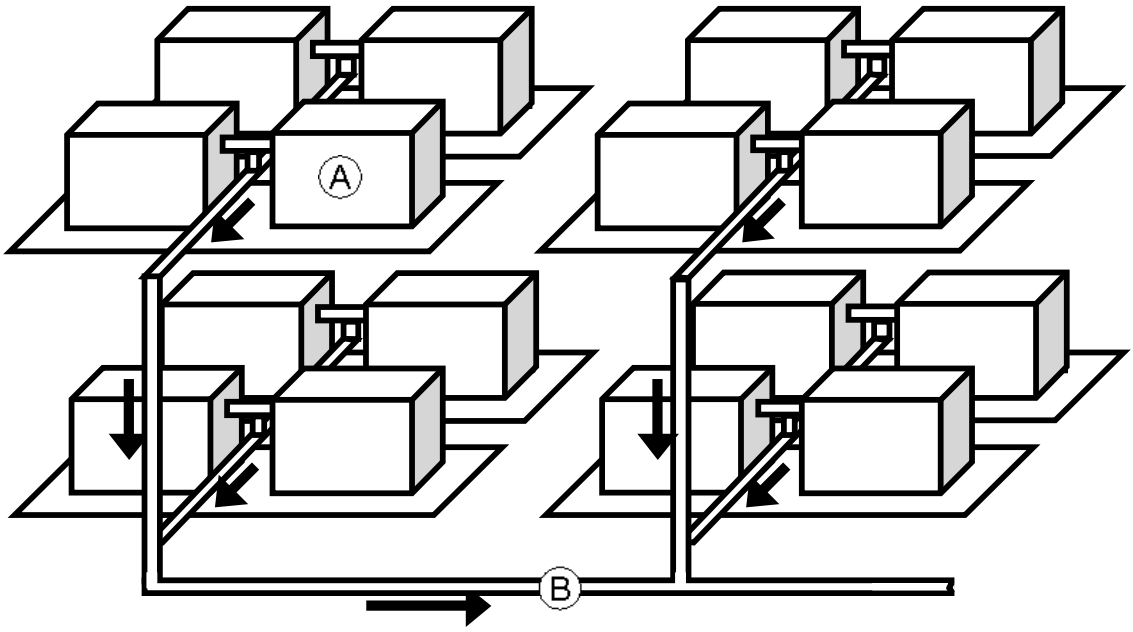


Figure 5. Diagram of one of the three main branches of the water return system. Arrows show direction of flow back to the central tank from the experimental units (A) through the water collection system (B).

osmosis filtration system to replace water lost to evaporation.

**Lighting**

One 1.2 m long electronic ballast fluorescent light fixture (Model E71514, Cooper Lighting, Eufaula, AB) was attached to the underside of the middle and top shelves of each shelving unit so that each pair of experimental units that share a shelf also shared a fluorescent light fixture (Figure 1-B). The fixtures each held two 32 watt T8 fluorescent light bulbs. One was an Octron XP/ECO 850 and one was a 32 watt Octron 950 (Sylvania) and had a reflector shield that extended 15 cm out and 7 cm down to direct light down toward the tanks. To increase the amount of light reaching the water surface, the above-water portion of the inside of each experimental unit was covered with aluminum foil. The fluorescent light fixtures were retrofitted with two incandescent light fixtures (Figure 1-C) so that a 60 watt incandescent light bulb hung down between the fluorescent lamps above each tank as shown in Figure 1. Each modified light fixture was wired so that the electronic ballast and each of the two incandescent sockets were in parallel circuits to each other and to the main power circuit. The modified fixtures were wired so that they had both a male and a female electrical plug, allowing them to be connected end to end. The eight fixtures for each of the three groups of shelving units were powered separately from the fixtures for the other two groups of shelving units.

**Temperature regulation**

Four inline water chillers (Aqua Logic, Inc., San Diego, CA) were installed on a half height shelving unit in a room adjacent to the room containing the rest of the

system. The chillers generated considerable heat while running, and placing them in a separate room prevented the chillers from raising the temperature of the air in the room containing the system. An 8.9 cm diameter hole was cut through the wall and reinforced with a 25 cm long piece of 7.6 cm internal diameter PVC. Fifteen mm heater hose carries water diverted from the main pump or auxiliary pumps through the wall to the chillers and back again. Each chiller was installed in parallel so that water passed through only one chiller before returning to the central tank. Two 370 watt chillers were generally sufficient to maintain water temperatures between 20º C and 30º C, and two additional 560 watt chillers could lower water temperatures below 8º C. Though not always required due to the heat produced by the incandescent lights, a 6000 watt inline spa heater (Vulcan Electric Company, Porter, ME) was also used to raise the water temperature above 35º C.

**System control**

We used the Aquacontroller Pro (Neptune Systems, San Jose, CA.) to continuously monitor the temperature in one arbitrarily selected experimental unit and regulate the chillers and heater to adjust the temperature according to a programmed daily temperature fluctuation. This controller used the open source X10 Powerline Carrier Technology (X10 Ltd., Kowloon, Hong Kong) to turn receiving switches off and on using signals transmitted through the building's power supply. Through its simple programming language, the Aquacontroller Pro could be programmed to turn lights, chillers, heaters and other electrically powered equipment off and on in response to changes in date, time of day, temperature, dissolved oxygen, conductivity or redox

potential. The lights could be programmed to follow a fixed cycle, or the light cycle could vary according to latitude and date. Similarly, the temperature could be held constant, or set to vary with the daylight cycle.

**Data collection and analysis**

To evaluate the system, we set the system to hold the water temperature between 23.4º C and 23.5º C, and then measured for each experimental unit the flow rate of water entering the tank, the temperature of the water leaving the tank and the light intensity at the center of the water surface of the tank. We calculated water flow rates for each experimental unit by recording the amount of time required to fill a 1 liter volumetric flask using water influx of the experimental unit and then performing unit conversions to express the flow rates in liters per hour. Water temperatures were measured using a YSI Inc. (Yellow Springs, OH) Model 85 oxygen, conductivity, salinity, and temperature meter. We measured the light intensity at the center of the water surface of the experimental units using a LI-250 light meter (LI-COR Biosciences, Lincoln, NE). The flow rate, temperature and light intensity measurements were separated into groups representing the six secondary branches and the two shelf heights. For each dependent variable, a two-way ANOVA was performed using these two blocking variables (secondary branch and shelf height). We also recorded the dissolved oxygen at the central tank using the YSI-85 probe.

## Results

The average flow rate (±SE) into each experimental unit was 52.7±0.6 $l\ h^{-1}$ so that a volume of water equal to the volume of each experimental unit passed through

each tank every 0.86±0.01 h, and there were no significant differences among the six secondary branches or the two shelf heights (Table 1). When the system was programmed to hold the water temperature at 23.5ºC while the room temperature was 26.1ºC, the average temperature of each experimental unit was 23.5ºC with a standard error less than 0.1ºC, and there were no significant differences among the six secondary branches or the two shelf heights (Table 1). The average light intensity (±SE) at the center of the water surface of each experimental unit was 129.33±1.77 mmol photon $m^{-2}$ $s^{-1}$, and there were also no significant differences secondary branches or shelf heights. Immediately after the water temperature of the last experimental unit was determined, we found the dissolved oxygen measured at the central tank to be 8.03 mg $l^{-1}$ (94% saturation) and the water temperature to be 23.4ºC.

## Discussion

The most expensive parts of the described system are the chillers. A recirculating system with no chilling capacity could be constructed for under $5,000 US in materials. The addition of two 370 W chillers raisees the total cost to just over $7,000 US. The further addition of two 560 W chillers raisees the total cost to less that $11,000 US. Once constructed, very few parts should be expected to suffer significant wear. In two years of nearly continuous operation, three significant equipment failures occurred. In one instance, a heater coil burned out, resulting in a $100 loss, and in another two chiller coil casings cracked due to ice formation, resulting in an $800 US loss. Each of these situations was the result of operational errors that left the devices running without

Table 1. System performance analysis and measurements. For each system measurement, a two-way full factorial ANOVA with an interaction term was performed to test for the differences in each system measurement between the six secondary branches and two shelf heights.

| Measure | $F_{(11,36)}$ of model | P of model | $\bar{x}$ ±SE |
|---|---|---|---|
| Flow Rate (l $h^{-1}$) | 0.884 | 0.563 | 52.7±0.6 l $h^{-1}$ |
| Temperature (°C) | 0.808 | 0.632 | 23.5±<0.1 ºC |
| Light Intensity (-) | 1.323 | 0.252 | 129.44±1.77 mmol $m^{-2}s^{-1}$ |

adequate water flow, and could have been avoided by the addition of low flow protectors ($100 US each) to automatically turn off such flow sensitive devices when necessary. Maintenance entailed flushing the foam filters in place at the pump inlet to remove debris and clearing the 6.4 mm irrigation tubing, which occasionally became clogged with debris despite the filters. These pieces of tubing were easily cleared with high pressure air.

The system can be modified to meet the needs of a diverse array of aquatic studies. The number of experimental units could be increased or decreased with ease. Similarly, the chilling or heating capacity could be increased through the use of additional chillers or heaters. As the water level in an experimental unit is the result of the height of the drainage hole placed in the side of that unit, the water level in the experimental units could be set at construction to any desired level. A mechanism could also be built to allow adjustment of the water level after construction. Also, additional pieces of equipment could be added to regulate pH, conductivity, and REDOX potential to desired levels.

As described, the system was capable of maintaining very similar temperature and lighting conditions among the 48 experimental units. The common water supply allowed abiotic factors like water quality, dissolved nutrient availability, and temperature to be held constant across experimental units. The system was thus well suited to study various ecological parameters at desired levels of integration, and to assess the impacts of abiotic factors on these ecological parameters. Additionally, experiments could be conducted at any time of year and conditions typical of any season

could be reproduced. Since it was designed to study the free floating aquatic weeds *S. molesta* and *S. minima*, the system could easily be used for further studies of these weeds and other free floating plants like water lettuce, (*Pistia stratiotes* L.), and duckweed (*Spirodela polyrhiza* (L.) Shleid.) as well as rooted plants with floating leaves like parrotfeather (*Myriophyllum aquaticum* (Vell.) Verd.) or hydrilla (*Hydrilla verticillata* (L. f.) Royle). and submerged, rooted species like Eurasian water-milfoil (*Myriophyllum spicatum* L.). Of particular interest would be studies of natural enemies of these species which can result in a sustainable, low input method of control.

Elevated oxygen levels such as the 8.03 $mgl^{-1}$ (94% saturation) measured at the central tank of our system are a prerequisite to the study of aquatic organisms like fish, crustaceans, and mollusks. Such high saturation was maintained through the mixing of air and water in the delivery pipes that carry water from the irrigation tubing to the tanks, and within the gravity driven water return system. Also, since metabolism rate is directly related to ambient temperature in ectothermic organisms, the homogeneity between experimental units and range of potential temperatures that the system can produce make it a powerful tool for the study of many aquatic organisms.

# CHAPTER III

# IMPLICATIONS OF NATURAL ENEMY STRAIN DIFFERENCES FOR BIOLOGICAL CONTROL OF *Salvinia* SPECIES IN THE UNITED STATES

## Introduction

Giant salvinia (*Salvinia molesta* Mitchell), is a floating aquatic weed that first gained widespread recognition in the 1950's, when the Lake Kariba reservoir was formed in Africa. Growing rapidly on nutrients leached from the recently submerged farmland, giant salvinia already established in the rivers feeding into this lake quickly expanded to cover 1000 $km^2$ of the 5544 $km^2$ reservoir (Marshall and Junor 1981). Classical biological control efforts against giant salvinia were begun in the 1960's (Bennett 1966), but were hindered due to misidentification of the plants as *Salvinia auriculata* Aublet.

The first successful biological control effort against giant salvinia occurred in 1980 in Australia (Room et al. 1981) through the release and establishment of a weevil (Coleoptera: Curculionidae) then identified as *Cyrtobagous singularis* Hustache that had been collected from giant salvinia's native range in southern Brazil. The released agent was later recognized as a previously unidentified species and given the name *Cyrtobagous salviniae* Calder and Sands (Calder and Sands 1985). The success of this species in Australia has been followed by the redistribution of Australian *C. salvinia* for control of giant salvinia infestations in Papua New Guinea (Thomas and Room 1986),

India (Joy et al. 1985), Namibia, (Giliomee 1986), South Africa (Cilliers 1991), and Senegal (Pieterse et al. 2003) among others.

In the species description of *C. salviniae*, its native range was described as stretching across much of South America on members of the *S. auriculata* complex that includes giant salvinia (Calder and Sands 1985). Calder and Sands (1985) also noted the existence of a population of *C. salviniae* in Florida on common salvinia, *Salvinia minima* Baker, and found that specimens collected from Florida were significantly smaller than specimens collected elsewhere. Common salvinia was considered a native of Florida, but a study of herbarium records by Jacono et al. (2001) showed that the species was not naturalized in Florida before the 1930's. Jacono et al. (2001) also suggested that the presence of *C. salviniae* in Florida may explain why common salvinia is much less aggressive there than in other states (Jacono et al. 2001). However, a genetic study (Goolsby et al. 2000) suggested that the *Cyrtobagous* weevils in Florida may actually be a different species than those released in Australia, though a definitive study of the species status of the two populations has not been published to date. Without such information, the Australia and Florida *Cyrtobagous* populations are considered here as different strains of a single species, *C. salviniae*.

The presence of two *Salvinia* species in the US and the availability of two different natural enemy populations combine to present a more complicated challenge for biological control than has been previously faced with giant salvinia control efforts. In the US, a successful biological control program must provide an acceptable level of control over both giant salvinia and common salvinia in monocultures and also in mixed

infestations. However, with no comparative studies of the host ranges of the two strains of *C. salviniae*, the possibility must be considered that both strains of *C. salviniae* might be required to control a mixed infestation. Unfortunately, the uncertain species status of the Florida *Cyrtobagous* population suggests the possibility of post-zygotic reproductive isolation between weevils from the Australia and Florida populations. Reproductive isolation of this kind would adversely effect the population growth rate of *Cyrtobagous* weevils if they were allowed to interbreed as in a multiple strain release scenario. This concern could be avoided if one of the *Cyrtobagous* populations could be identified that could be expected to effectively utilize both *Salvinia* species to complete development. A biological control program involving only one weevil strain might also be significantly simpler and less expensive to implement since only one strain would need to be reared for releases.

In addition to concerns about host range differences between the two strains of *C. salviniae*, differences in response to various temperatures could also influence the development of a successful biological control program. For example, though the Australian strain of *C. salviniae* has been used to successfully control giant salvinia in tropical regions, there are still some questions about its effectiveness in more temperate regions. Female Australian strain *C. salviniae* stop laying eggs at 21 °C and eggs must be above 19 °C in order to hatch (Cilliers 1991). In contrast, though giant salvinia grows fastest at 30 °C, it can continue to grow at 12 °C (Room 1986). These factors may explain why colder temperatures have slowed the time to control giant salvinia in field releases. Cilliers (1991) reported that control was achieved four years after initial

releases of Australia strain weevils in the more temperate Southeastern Cape of South Africa, while the salvinia mats in the more tropical regions of the country were controlled in only one or two years. Similarly, when *C. salviniae* was released in Australia, control was achieved within two years of the initial release except at two temperate sites in the southern part of the country (Room et al. 1984). In contrast to the work studying the influence of temperature on Australian giant salvinia, there have been no studies involving temperature and the Florida strain of *C. salviniae*. Here we present a comparison of the Australia and Florida strains of *C. salviniae* in a temperature controlled laboratory experiment designed to determine the most appropriate release candidate for *Salvinia* infestations in the US.

## Materials and Methods

### Experimental system

We built the indoor experimental system described in Chapter II and which consisted of a temperature-controlled central tank from which water was pumped to 48 different 45.5 l tanks which served as the experimental units in the system. A constant water level was maintained in the experimental units using an overflow on the side of each tank. To prevent plant or insect material from clogging the overflow or being transported to the other tanks, the intake of each overflow was located beneath the water surface and screened with a foam filter. The turnover rate of water moving between the central tank and each experimental unit was 52.7±0.6 l $h^{-1}$ ($\bar{x}$ ±SE ), which was fast enough for each unit to experience the same water temperature and water chemistry conditions. Each experimental unit received light from a 32 watt fluorescent bulb and a 60 watt

incandescent bulb resulting in an average light intensity (±SE) of 129.44±1.77 mmol photosynthetically active radiation $m^{-2}$ $s^{-1}$ during periods of illumination.

**Experimental design**

Three consecutive 60 day experiments were conducted at three different water temperature regimes derived from recorded surface water temperature measurements collected by the US Geological Survey during summer 2002 and winter of 2001-2002 at Hubbard Creek Reservoir (32°50'N, 98°58'W) near Breckenridge, TX (Gandara 2003b) and summer 2002 at Lake Charlotte (29°52'N, 94°43'W) near Anahuac, TX (Gandara 2003a). These sites were chosen as representing the cooler and warmer ends of the US water temperatures *Salvinia molesta* has been shown to be able to survive (Whiteman and Room 1991). For each week of each experiment, the daytime water temperature was regulated to approximate the weekly average of recorded daily maximum water temperatures for the corresponding lake temperature measurements, and the nighttime water temperature was regulated to approximate the weekly average of the recorded daily minimum water temperatures. The experiments conducted using summer and winter surface water temperatures at the Hubbard Creek reservoir resulted in average temperatures of 26.5 ºC and 8.0º C, respectively, while the experiment conducted using summer surface water temperatures from Lake Charlotte resulted in an average temperature of 31.4 ºC. For each experiment, the daily photoperiods were regulated to reproduce the photoperiods experienced when the temperature data were collected by taking into account the latitudes of the lakes being simulated and the dates when the

original water temperature data were collected. The water temperatures used for each experiment are shown in Figure 6.

Each experiment was conducted in a 2x3 randomized full factorial design with two plant species factor levels (giant salvinia and common salvinia) and three weevil strain factor levels (Australia weevils, Florida weevils, no weevils). To start each experiment, a partial covering of salvinia was added to each experimental unit. Mitchell and Tur (1975) described three growth phonologies among salvinia species including a fragile form with relatively longer narrower rhizomes and relatively smaller unfolded leaves that lay flat on the water surface. This growth form, referred to as the "primary" growth form, tends to occur in open water. Mitchell and Tur (1975) also described a "tertiary" growth form characterized by relatively shorter and wider rhizomes and larger leaves that remain folded and upright with the midline at the water surface and the rest of the leave above the water. Transitional phonologies between the primary and tertiary growth forms were considered "secondary." For our experiments, tertiary growth form plants were placed in an uncrowded situation in each tank and allowed to grow. New growth first developed in the primary growth form until crowding resulted in the appearance of the tertiary growth form. Two weeks after the mat growth form had been observed in all 48 tanks, two mating pairs of adult weevils were added to each tank except the controls. Mating pairs were isolated by removing pairs of adult weevils from the colonies as they were observed in the act of copulation.

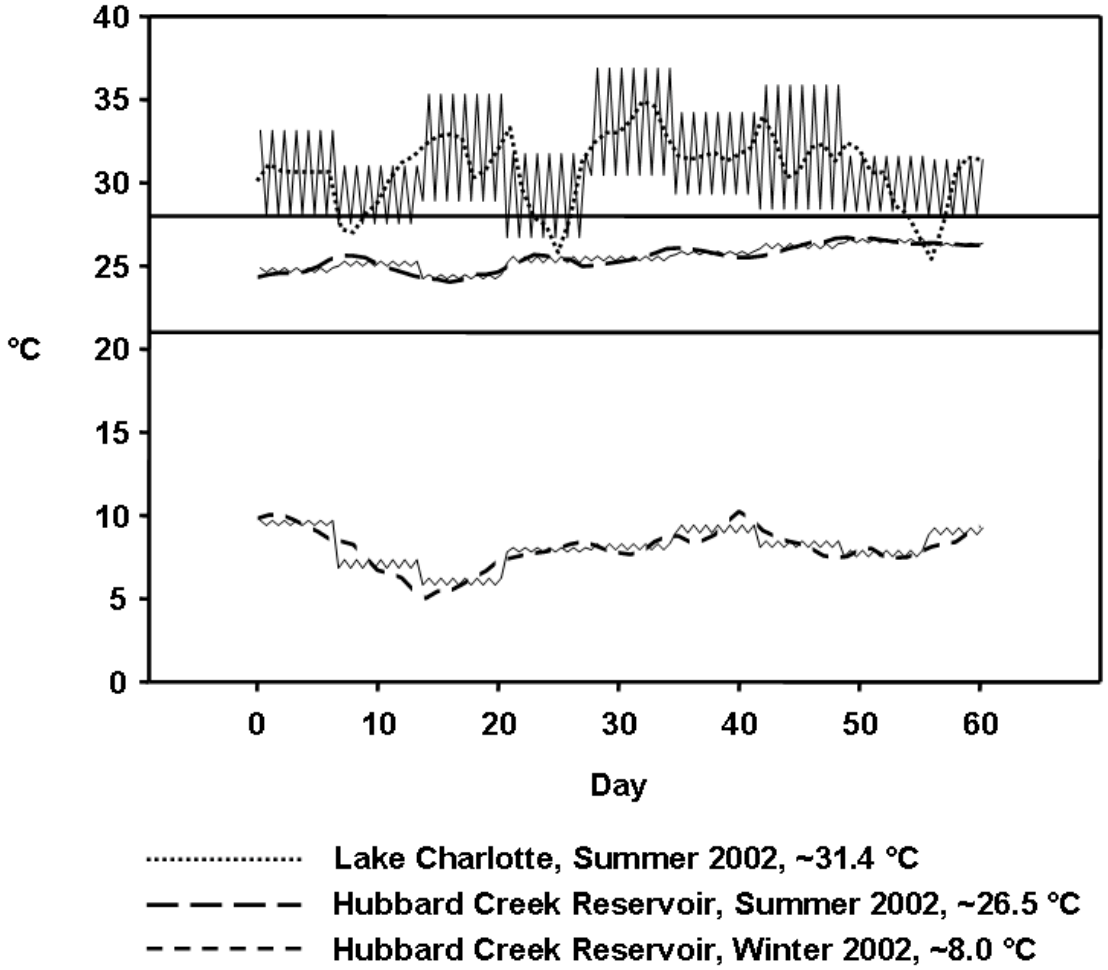


Figure 6. Mean daily surface water temperatures recorded at lakes in Texas within the potential range of *Salvinia* species in the United States. Daily highs and lows used in the experiments were based on weekly averages of highs and lows recorded at the corresponding lake in the corresponding season and are represented by the continuous lines associated with each mean lake surface water temperature. The average temperature experienced by the plants and weevils inthe temperature profiles derived from Lake Charlotte during the summer of 2002, Hubbard Creek Reservoir during the summer of 2002 and Hubbard Creek Reservoir during the winter of 2002 were 31.4, 26.5 and 8.0 ºC, respectively.

The plants were grown in reverse osmosis filtered water to which nutrients were added to produce the following initial concentrations: 4.5 ppm N from urea, 0.5 ppm N from nitrate, 5.0 ppm $P_2O_5$, 5.0 ppm $K_2O$, 10 ppm $Mg^{2+}$, 20 ppm $Ca^{2+}$, 1.1 ppm Fe, 9ppm $Na^{+}$. These target concentrations were obtained by the addition of appropriate quantities of Peters® 20-20-20All Purpose Plant Food (United Industries Corporation, St. Louis, MO), $MgSO_4*7H_2O$, $CaCl_2$, Green Light Company (San Antonio, TX) Iron and Soil Acidifier, and NaCl. A complete list of nutrient additions is given in Table 2. Nutrients as described above were first added to reverse osmosis filtered tap water just before the addition of *Salvinia* to the experimental units at the start of each experiment, and were all replenished any time a nutrient deficiency began to appear in the plants.

**Insect colonies**

The USDA APHIS PPQ Pest Detection, Diagnostics and Management Laboratory in Edinburg, TX provided 400 adult representatives of the Australia strain of *C. salviniae* on January 27, 2003. The USDA ARS Invasive Plant Research Laboratory in Fort Lauderdale, FL provided 250 adult representatives of the Florida strain of *C. salviniae* on June 24, 2003. We subsequently maintained laboratory colonies of Australian strain *C. salviniae* on giant salvinia and Florida strain *C. salviniae* on common salvinia at the Texas A&M University Biological Control Facility in College Station, TX. Adult weevils for the experiments were obtained from the colonies we maintained in College Station, except in the case of the Australia weevils for the 24 ºC temperature experiment. In that case, 40 Australia weevils obtained directly from the

Table 2. Concentrations of plant and insect nutrients added to the water in the experimental system. Concentrations of elemental nutrients with chemical form in parentheses are for the element rather than the entire chemical.

| Nutrient | mg/l |
|---|---|
| N (Urea) | 4.5 |
| N ($NO_3$) | 0.5 |
| $P_2O_5$ | 5 |
| $K_20$ | 5 |
| $Mg^{2+}$ | 10 |
| $SO_4^{2-}$ | 47 |
| $Ca^{2+}$ | 20 |
| $Cl^-$ | 49 |
| $Fe^{2+}$ | 1 |
| $Cu^{2+}$ | 0.27 |
| $Zn^{2+}$ | 0.27 |
| B ($H_3BO_3^-$) | 0.005 |
| $Mn^{2+}$ | 0.01 |
| $Mo^+$ | 0.0001 |
| $Na^+$ | 9 |

Pest Detection, Diagnostics and Management Laboratory in Edinburg, TX were used to supplement 24 Australia weevils obtained from the colony maintained in College Station, TX.

**Data collection**

Immediately before adding weevils to each tank, and every three days thereafter for the next 60 days, one 9cm diameter core was removed from each tank at a randomly determined position within the vegetation mat. Samples were isolated from the rest of the mat using a 9 cm diameter, hollow, open-ended cylinder of ¼ inch hardware cloth that was pressed into the mat. The samples were dried over 13 cm weigh boats (Fisher Scientific International Inc., Hampton, NH) containing water and a small piece of salvinia to extract adult weevils and larvae in a method derived from Boland and Room (1983). The adult weevils aggregated on the piece of salvinia in the water below the sample, and were then counted and returned to the tanks. The larvae dropped into the water and sank to the bottom of the weigh boat, from which they were counted and discarded.

Studies of the Australia strain of *C. salviniae* have found total development times between 29 and 36 days (Sands et al. 1986). Thus, we used the total number of larvae collected over the first 33 days as a measure of the reproductive output of the released weevils, while the total number of larvae collected over the entire 60 days of experiment represents reproductive output from both the released weevils and their progeny. We also compared the total number of adults collected over the first 33 days of the experiment to detect any differences in mortality among released weevils, and the total

number of adults collected over the entire 60 days to detect differences in survivorship to adulthood between the two weevil strains.

At the end of the experiment, we took a digital image of the salvinia mat in each tank using an Olympus (Olympus America Inc., Melville, NY) 314R 1.3 megapixel digital camera. The images were processed to convert all healthy areas of salvinia in the image to white pixels and all necrotic areas of salvinia in the image to black pixels. To do this we used freely available image editing software (Irfanview© version 3.91) to perform a series of image manipulation procedures to each image. First, the blue component of the red, green, blue color definition for each pixel was set to the maximum value and the image was saved in an uncompressed file format. Then the saturation of each image was raised to the maximum value and again saved in an uncompressed file format. The color saturation was raised to full three more times this way. These steps accentuated the apparent difference between the healthy green sections of the image and the necrotic sections. Then color balance of each image was then adjusted to remove all red and blue, resulting in an image in which all healthy plant growth was represented by bright green pixels and necrotic plant material was black. The images were then converted to black and white images with black pixels representing necrotic areas and white pixels representing healthy areas of the tanks, and the proportion of the black pixels in each image was then determined. The increase in necrosis due to weevil feeding was calculated for each weevil release tank using the following equation:

$$\%D_{tpsi} = \left(P_{tpsi} - \overline{P}_{tpc}\right) * 100 / \overline{P}_{tpc}$$

where $\%D_{tpsi}$ is the percent increase in visibly necrotic plant tissue that can be attributed to feeding by strain *s* in the $i^{th}$ replicate and of the treatment combination of temperature profile *t* and plant species *p*. $P_{tpsi}$ is the proportion of necrotic plant tissue visible in the $i^{th}$ replicate of the treatment combination of temperature profile *t*, plant species *p*, and weevil strain *s*. $\overline{P}_{tpc}$ is the average proportion of necrotic plant tissue visible in replicates of the no weevil control treatment for the combination of temperature profile *t* and plant species *p*.

**Data analysis**

The two weevil strains were compared in the total number of larvae collected through days 33 and 60 as well as the total number of adults collected through days 33 and 60 and the percent increase in visible necrosis at the end of the experiment. We used independent samples t-tests at each combination of temperature profile and plant species except in the case of combinations with non-normal distribution as indicated by the Shapiro-Wilk test for normality and combinations with normal distributions but unequal variances as indicated by Levene's test for equality of error variance. We substituted the Mann-Whitney U test in cases of departures from normality. In cases of unequal variance, we calculated the degrees of freedom for the *t* statistic using the unequal variances assumption method which produces non-integer degrees of freedom.

## Results

**Larval counts**

We found considerable variation in larval weevil counts between sample consecutive sample dates at both summer temperature profiles as shown in Figure 7.

This likely arises from larval densities near the detection threshold of our sampling method, which resulted in many samples containing zero larvae. When feeding on common salvinia, the two weevil strains did not differ significantly in the number of larvae collected over the first 33 days (Figure 8) of the experiment at either the 31.4 ºC ($U_{8,8}$ = 31; $p$ = 0.92) or 26.5 ºC ($t_{14}$ = 0.54; $p$ = 0.60) temperature profiles. Neither was there a significant difference ($U_{8,8}$ = 31; $p$ = 0.92) between the two strains in the number of larvae collected over the first 33 days when feeding on giant salvinia at the 31.4 ºC temperatures, but significantly fewer ($t_{14}$ = 2.671; $p$ = 0.018) Florida than Australia larvae were collected over the first 33 days when feeding on giant salvinia at the 26.5 ºC temperatures. No larvae of either strain were collected under the winter conditions on either plant species. After 60 days, there were no significant differences between the two weevil strains in the total number of larvae collected from common salvinia at either the 31.4 ºC profile ($t_{8,5}$ = -1.76; $p$ = 0.11) or at the 26.5 ºC profile ($t_{14}$ = 1.45; $p$ = 0.17). Neither was there a significant difference between the two weevil strains in the total number of larvae collected from giant salvinia at either the 31.4 ºC profile ($U_{8,8}$ = 21.5; $p$ = 0.27) or at the 26.5 ºC profile ($t_{14}$ = 1.76; $p$ = 0.10).

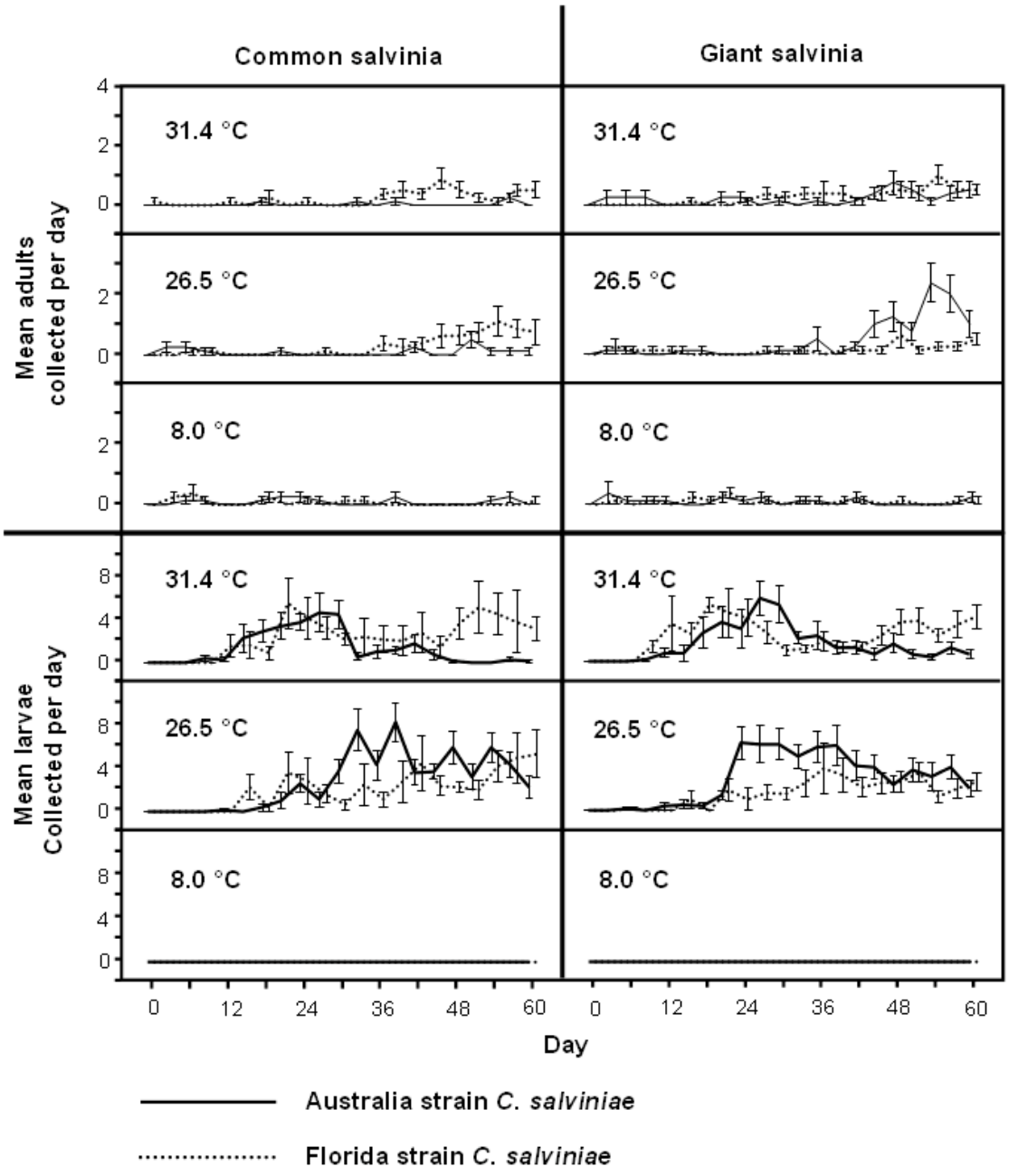


Figure 7. Mean (± SE) adults and larvae collected per day of each weevil strain at each combination of plant species and temperature profile. Data were collected through day 60 of the experiment at three day intervals beginning at day 3.

**Adult counts**

We also found considerable variation in adult weevil counts between sample consecutive sample dates at both summer temperature profiles as shown in Figure 7. The two weevil strains did not differ significantly in the number of adults (Figure 8) collected over the first 33 days on common salvinia at 31.4 ºC ($U_{8,8}$ = 26; $p$ = 0.44), 26.5 ºC ($U_{8,8}$ = 22.5; $p$ = 0.26) or 8.0 ºC ($t_{14}$ = -0.55; $p$ = 0.59). Neither did the two strains differ significantly in the number of adults (Figure 8) collected over the first 33 days on giant salvinia at 31.4 ºC ($U_{8,8}$ = 31.5; $p$ = 0.96), 26.5 ºC ($U_{8,8}$ = 27; $p$ = 0.57) or 8.0 ºC ($t_{14}$ = 0.40; $p$ = 0.69). However, when feeding on common salvinia, significantly more Florida than Australia strain adults were collected over the whole 60 days of the experiment under both the 26.5 ºC ($t_{8.7}$ = -2.956; $p$ = 0.017) and 31.4 ºC ($U_{8,8}$ = 0.5; $p$ < 0.001) temperatures. There were no significant differences between the two weevil strains in the number of adults collected over the entire 60 days of the 8 °C temperature profile on common salvinia ($t_{14}$ = 0.55; $p$ = 0.59) or giant salvinia ($t_{14}$ = 0.28; $p$ = 0.78). When feeding on giant salvinia, significantly fewer ($t_{14}$ = 3.959; $p$ = 0.001) Florida strain adults were collected over the entire 60 days of the 26.5 ºC temperature profile, though no difference was observed at the 31.4 ºC profile ($t_{14}$ = -0.72; $p$ = 0.48).

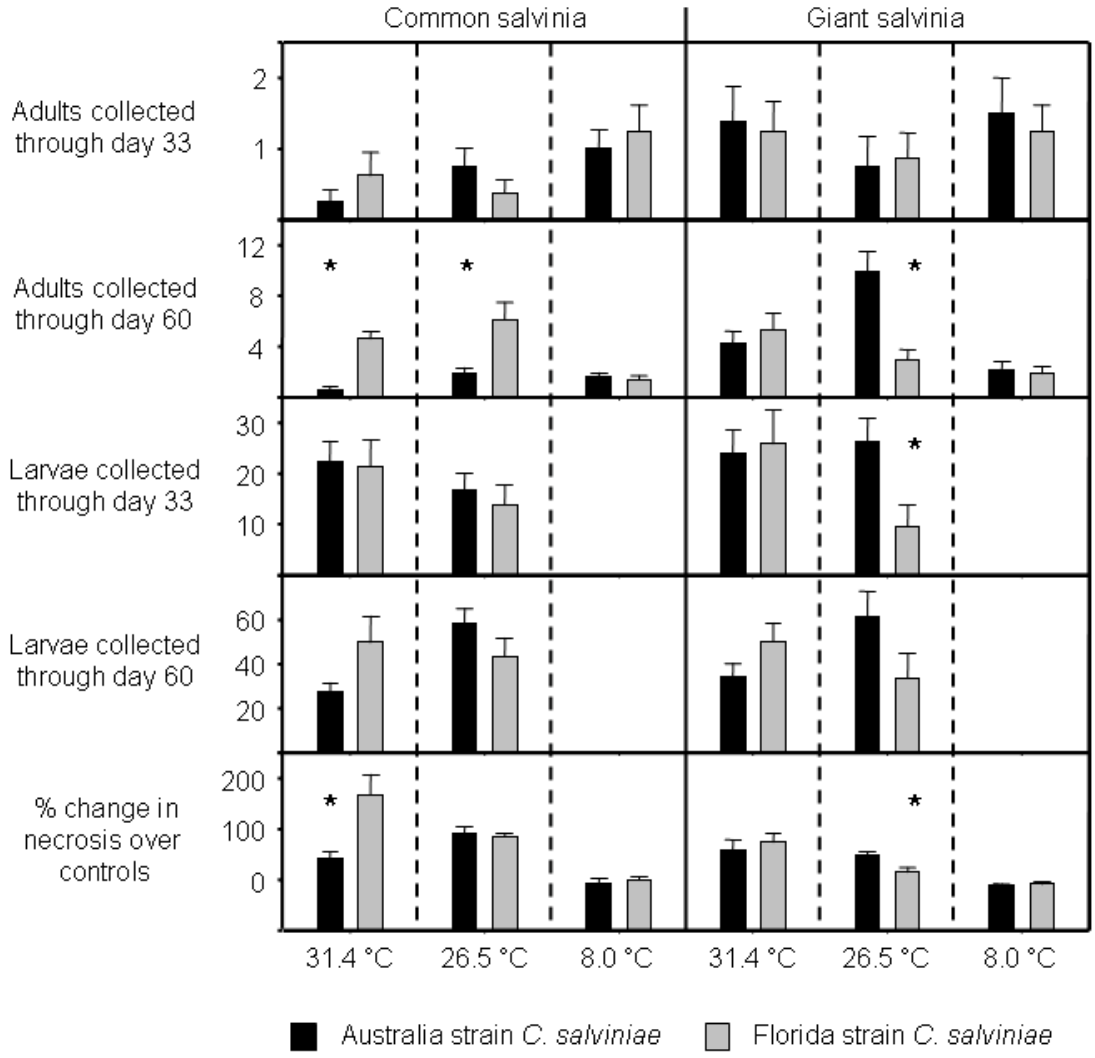


Figure 8. Mean and standard error of the total number of adults and larvae of each weevil strain on each plant species collected through the 33rd and 60th day of each temperature profile, and the mean and standard error of the percent change in necrosis over the average amount proportion of necrosis visible in the control tanks of each plant species at each temperature profile. Asterisks denote response variables and treatment combinations where significant ($p < 0.05$) differences between the two weevil strains were observed.

**Plant damage**

Significantly more ($U_{8,8}$ = *12; p = 0.036*) plant damage was observed at the end of the experiment from Florida strain weevils feeding on common salvinia at the 31.4 ºC temperature profile, but the two strains did not differ in the amount of plant damage observed on common salvinia at the 26.5 ºC ($U_{8,8}$ = 29; $p$ = 0.75) and 8 °C ($t_{14}$ = -0.57; $p$ = 0.57) temperature profiles (Figure 8). When feeding on giant *salvinia*, the Australia strain caused significantly more ($U_{8,8}$ = 9; $p$ = 0.016) visible plant damage at the 26.5 ºC profile, but the two strains did not differ significantly in the amount of visible plant damage at the 31.4 ºC ($U_{8,8}$ = 25; $p$ = 0.46) and 8 °C ($t_{14}$ = -0.58; $p$ = 0.57) profiles.

## Discussion

These experiments demonstrate the greater ability of the Florida strain of *C. salviniae* to utilize common salvinia as a host plant compared to the Australia strain. On common salvinia, we collected similar numbers of larvae of both weevil strains through day 33 at both summer temperature profiles, indicating similar oviposition, egg hatch, and larval survival rates between the two strains. However, significantly more Florida strain adults were collected from common salvinia at both summer temperature profiles over the entire 60 days. This cannot be attributed to a slower developmental rate by the Australia weevils on common salvinia because a light brown, newly emerged Australia strain adult was collected from a common salvinia tank on day 27 of the 26.5 ºC experiment. Since the number of adults collected to day 33 did not differ, the larger number of Florida strain adults collected to day 60 must be attributed to a much lower rate of survivorship to adulthood by Australia strain weevils on common salvinia with

the major mortality occurring in the late larval or pupal stage. These results suggest the Florida strain of *C. salviniae* as the more appropriate release candidate for common salvinia on account of the difference in survivorship between the two strains and equal or greater degree of plant damage observed on common salvinia from Florida weevils compared to Australia weevils.

When comparing the two strains on giant salvinia, at the 31.4 ºC temperature profile, there were no significant differences between the two strains in the number of adults collected to days 33 or 60, the number of larvae collected to days 33 or 60, and weevil induced visible plant damage. At the 26.5 ºC temperature profile, the Florida strain produced significantly fewer larvae to day 33 than did the Australia strain. Thus, even with similar developmental and survivorship rates, there should have been fewer Florida strain adults collected through day 60 from giant salvinia at the 26.5 ºC temperature profile simply because there were fewer Florida larvae to complete development to adulthood. The lesser degree plant damage resulting from Florida weevil feeding at this temperature profile reflects this smaller weevil population.

These differences between the two strains may reflect true temperature-dependent differences in host acceptance of giant salvinia by the initially released Florida weevils, resulting in a lower rate of oviposition and subsequent lower measured larval and adult counts, or may be the result of differences in individual weevil health. It should be noted that size of the Florida weevil colony had dwindled just before the start of the 26.5 ºC temperature profile so that almost every weevil in the colony was required to start the experiment, while the Australia weevils for that profile were derived partly

from a younger, growing colony to make up for a small number of Australia weevils available from the laboratory colony. Even if the two strains were equally able to utilize *S. molesta* at any temperature profile, it would not be surprising for Florida weevils from a declining colony to not compare favorably with Australia weevils primarily from a strong, growing colony.

Tipping and Center (2005) found that host plant preference for both strains was determined not by plant species, but by plant size and that larger plants were preferred especially by the Australia strain (or as they term it, the Brazilian). They also found that Australian strain weevils were significantly larger than Florida strain weevils and suggested that more pronounced preference for larger plants by the Australia strain is likely related to the strain's larger size relative to the Florida strain. The poorer performance of Australia *C. salviniae* on common salvinia that we observed is thus likely related to the significantly smaller size (Tipping and Center 2005) of tertiary growth common salvinia compared to tertiary growth giant salvinia.

The Australia strain of *C. salviniae* has a well established record of successful control of giant salvinia infestations in many regions of the world (Room et al., 1981; Joy et al. 1985; Giliomee 1986; Thomas and Room 1986; Chilliers 1991; Pieterse et al. 2003). However, our experiments simulating Texas environments have shown that Florida strain *C. salviniae* is the more appropriate release candidate for common salvinia and could possibly be as successful in controlling giant salvinia as the Australia strain. Efforts to use biological control against infestations of giant salvinia in the US should thus include field evaluations of the Florida strain of *C. salviniae.* If such releases prove

successful, biological control efforts against *Salvinia* species in the US could be greatly simplified by rearing and redistributing only the Florida strain for control of both common salvinia and giant salvinia since the Australia strain cannot be considered an appropriate release candidate for common salvinia infestations.

# CHAPTER IV

## CONCLUSION

Giant salvinia and common salvinia are continuing to increase in distribution in the US despite biological, chemical and mechanical control attempts and public education campaigns. However, there have been significant reductions in giant salvinia infestations at several Australia *C. salviniae* release sites in Texas (Jacono and Richerson 2005). Reductions in common salvinia infestations have also been achieved through releases of Florida *C. salviniae* in Louisiana (Jacono and Richerson 2005). However, the uncertain species status of the two strains and the ability of Florida *C. salviniae* to utilize and damage giant salvinia at least in some cases continues to cloud the *Salvinia* biological control picture. These issues continue the legacy of taxonomic uncertainty that has hampered giant salvinia biological control at two previous stages when then undescribed *S. molesta* was incorrectly identified as *S. auriculata* and again when then undescribed *C. salviniae* was incorrectly identified as *C. singularis.* Years of persistent research into the system were required to locate and recognize a successful natural enemy of giant salvinia. In light of these past challenges, it is a relative luxury to now have two different natural enemies with potential to control giant salvinia infestations. The discovery in Florida *C. salviniae* of an effective natural enemy of common salvinia is also fortuitous in light of the low survivorship rates of Australia *C. salviniae* on common salvinia described in Chapter III.

Several aspects of this research could have been improved. Weevil strain comparisons might yield greater discriminatory power if an effort was expended to standardize the age of the adults released into the tanks so that only young, healthy adults were used. Such efforts would have either eliminated the observed reduction fecundity of Florida *C. salviniae* on giant salvinia at the 26.5 ºC temperature profile or allowed for greater confidence that the observation represented a true difference between the weevil strains.

Finally, two further studies are needed in order to clarify the appropriate course or courses of action for using biological control to suppress *Salvinia* infestations in the US. First, field releases should be conducted with Florida weevils to determine whether they can effectively control giant salvinia field infestations. If Florida *C. salviniae* failed to control giant salvinia, a two strain strategy would be justified in which Florida weevils would be released exclusively for common salvinia infestations and Australia weevils would be released exclusively for giant salvinia infestations. Conversely, Florida weevils were shown to effectively control giant salvinia infestations, it would allow the possibility of rearing, releasing and redistributing a single weevil strain for all *Salvinia* infestations in the US. In addition to simplifying rearing procedures and avoiding taxonomic questions, a single strain control strategy would allow greater lay participation in redistributing the weevils from release sites to new infestations. Rather than needing to train willing but inexperienced redistribution program participants in DNA identification techniques, the subtle size differences between the two strains, and how to differentiate common salvinia from giant salvinia, it would be enough for them to

know that small black weevils feeding on either *Salvinia* species could be collected and released on any other *Salvinia* infestation encountered.

The current situation in which both strains are being released against *Salvinia* species whose distributions overlap makes eventual contact between the two strains in the field inevitable. An immediate switch to a single strain control strategy would reduce the degree of interaction between the two strains, while a continued two strain control strategy would result in an increasing the frequency of encounter and the plants spread to more drainages and more weevil releases are made.

To address questions about the possible result of the Florida and Australia strain weevils encountering each other in the field, a cross mating study should be conducted to determine whether there are any behavioral or genetic reproductive isolation mechanisms between the two strains and how interbreeding might influence the level of control of both plant species. Such a study would examine mate acceptance, fecundity by cross mated females, hybrid survival rates to adulthood relative to the parental strains, viability of offspring resulting from backcrosses of hybrids to the parental strains, and ability of hybrids and backcrosses to utilize common salvinia and giant salvinia for development to adulthood. The findings of such a study would provide information about the risks to long term *Salvinia* control posed by encounters between the two weevil strains.

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