11-3-2017

Evaluation of Winter Management Methods to Enhance Survival of the Giant Salvinia Weevil, Cyrtobagous salviniae

Lori Robyn Moshman
Louisiana State University and Agricultural and Mechanical College

EVALUATION OF WINTER MANAGEMENT METHODS TO ENHANCE SURVIVAL OF THE GIANT SALVINIA WEEVIL, *CYRTOBAGOUS SALVINIAE*

A Thesis

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Master of Science

in

The Department of Entomology

by
Lori Robyn Moshman
B.S., Cornell University, 2012
December 2017

To Keith Richards: my idol, my inspiration.

Acknowledgements

I am grateful to the faculty, staff, and students in the LSU Department of Entomology who have made me feel a welcome part of the community and made my time here memorable. I give my sincerest appreciation to my major professor Dr. Rodrigo Diaz, who has shown great generosity both personally and professionally and has encouraged me to be a better scientist, researcher, and writer. I am thankful to Dr. Christopher Mudge for his friendliness and support throughout my studies, and for sharing his expertise in working with giant salvinia. I thank Dr. Michael Stout for his clear passion for helping students and his love of entomology, which has been an inspiration to me. I would like to acknowledge the sources of funding that made this study possible: the Louisiana Department of Wildlife and Fisheries (LDWF), the Barataria-Terrebonne National Estuary Program (BTNEP), and the LSU Department of Entomology for providing my graduate assistantship. I wish to thank my lab mates Leslie Avíles, Brad Frazier, Madeline Gill, Balwinder Kaur, Ian Knight, Erick Martin, Adán Orellana, and Zinan Wang for their friendship and assistance during my time at LSU. I especially thank Otto Castillo, Shiloh Judd, Alana Russell, Seth Spinner, Charlie Wahl, and Yi Wang for their tireless help with experiments and willingness to spend long hours with me in the field. I thank my parents, Karen and Stanley Moshman, for their undying love and support for everything I do; my dogs Onyx and Baby, who don't know what I have been doing at school but are far better companions than most humans; and lastly, I thank Alfred Francis Darsey III, a man of many talents whom I am proud to call my best friend.

Table of Contents

Abstract

Giant salvinia (*Salvinia molesta* Mitchell) is a noxious aquatic weed in the southeastern United States. Dense plant mats create ecological and economic consequences by displacing native species and impacting freshwater industries. Biological control programs using the salvinia weevil (*Cyrtobagous salviniae* Calder and Sands, Coleoptera: Curculionidae) have limited efficacy in giant salvinia's northernmost range due to the weevil's low cold tolerance and long-term establishment rates. Spunbonded polypropylene (SBP) fabrics and manipulation of plant density were evaluated as two strategies for managing giant salvinia mats during winter. Greenhouse, laboratory, and field experiments were conducted to determine the suitability of these strategies for enhancing salvinia weevil survival and establishment in Louisiana. Plant quality, weevil survival, and mat temperatures were evaluated for plants that were either covered with insulating fabrics or artificially crowded to low, medium, and high densities. Spunbonded polypropylene fabrics raised mat temperatures by 0.3 to 3.5°C and decreased adult weevil mortality by 36 to 66% in laboratory and field studies in south LA. In simulated cold fronts, SBP covers reduced plant damage by 1.8- to 4.8-times compared with uncovered controls. High density plant mats raised water column temperatures by 0.6 to 1.9°C compared to open water and by 0.6 to 1.0°C compared to low density plant mats. High density plant mats experienced 15% greater adult weevil mortality than medium density plant mats in laboratory experiments at -7°C. Field overwinter survival did not differ among treatments in north and central LA, but in south LA adult and larval population density were 2.2- and 8.3-times greater, respectively, in high density treatments than in low density treatments. Artificial refugia made from SBP are a low-cost alternative to greenhouse films for managing water temperature and are well suited for

outdoor mass-rearing operations. Managing plant density is a potentially useful management strategy for salvinia weevil release sites with mild winter cold exposure. Continued evaluation is recommended to assess the suitability of these refugia for giant salvinia and other weed biological control programs during severe winters in temperate climates.

Chapter 1: Background and Literature Review

1.1. Giant Salvinia Distribution, Biology, and Ecology

Giant salvinia, *Salvinia molesta* Mitchell (Salviniales: Salviniaceae), is a free-floating fern of South American origin, with a history of invasiveness dating to 1939 in Sri Lanka (Williams 1956). This plant is part of the *S. auriculata* complex, which includes *Salvinia auriculata* Aublet, *S. herzogii* de la Sota, *S. biloba* Raddi, and *S. molesta* Mitchell (Mitchell 1972, Mitchell and Thomas 1972). Giant salvinia is the only member of this complex present in the United States (Julien et al. 2002). Another member of the genus, *Salvinia minima* Baker, has invaded the Gulf Coast states of Florida, Georgia, Alabama, Mississippi, Louisiana, and Texas (Jacono et al. 2001). *Salvinia minima,* or common salvinia, may be found in the same environments and occasionally growing alongside giant salvinia, but it does not typically cause the same degree of infestation (Julien et al. 2002). *Salvinia* spp. are characterized by round, light green fronds with dense adaxial trichomes that are highly water repellent (Mitchell and Thomas 1972). *Salvinia* spp. do not possess true roots, but rather modified fronds that hang suspended from a rhizome and absorb nutrients from the water column (Figure 1.1; Mitchell and Thomas 1972).

Mature plants produce reproductive sporocarps that hang adjacent to the "roots"; in giant salvinia, these structures are sterile, therefore the primary mode of reproduction is vegetative (Loyal and Grewal 1966). The complete reproductive unit of *Salvinia* spp. is known as a ramet and consists of two floating leaflets, apical and axillary buds, and the associated rhizome and modified "roots" (Room 1983, Whiteman and Room 1991). Vegetative growth occurs by expansion of terminal or lateral buds with various patterns of branching and fragmentation to

produce new ramets (Room 1983). Plants are kept afloat by spongy aerenchyma tissue and move freely with water currents (Julien et al. 2009).

Figure 1.1. Giant salvinia "roots" are modified submerged leaves that uptake nutrients from the water column. They exhibit high phenotypic plasticity and may vary greatly in length depending on water quality. Photo by L. Moshman.

Giant salvinia and other members of the *S. auriculata* complex are distinguished by fused, egg beater-shaped trichomes. Common salvinia, by contrast, has unfused trichomes, making the two species easy to distinguish with the use of a hand lens. Three growth stages of giant salvinia are recognized: primary, secondary, and tertiary (Mitchell and Thomas 1972). The primary, or invading growth stage, is marked by small, flat leaves (fronds) that can move easily with water current. These are typically found along edges of waterbodies where the current is slower moving. The secondary, or colonizing growth stage, is predominantly flat, has slightly larger fronds than the primary stage, and shows a more complex branching pattern. The tertiary, or mat-forming stage, is marked by vertical growth of the fronds, allowing for tight crowding of the plants without compromising access to sunlight (Coelho et al. 2000). Giant salvinia

infestations are frequently found in canals, lakes, ponds, and other slow-moving waterbodies. Its optimal growth conditions are pH of 6.0 to 7.3 (Gaudet 1973), electrical conductivity of 50 to 1500 µS/cm (Knutson 2012), total nitrogen of 2 to 20 mg/L (Cary and Weerts 1983), and temperature of 24 to 28°C (Cary and Weerts 1983).

Giant salvinia is native to southeastern Brazil (Forno and Harley 1979) and has been reported as an invasive species in more than 21 countries including Australia, Fiji, India, New Guinea, New Zealand, Sri Lanka, South Africa, Trinidad, and the United States (Room et al. 1981, Calder and Sands 1985, Jacono et al. 2001). Giant salvinia was first reported in the United States in 1995 in South Carolina, and was believed to have come from the aquatic plant trade industry (Johnson 1995). A second infestation was reported in Houston, Texas in 1997 (Flores and Wendel 2001). Giant salvinia has spread throughout the southern United States and to date has been reported in 14 states, Guam, Puerto Rico, and the Virgin Islands (Galam et al. 2015, EDDMapS 2017). Giant salvinia is spread by natural phenomena such as floods or by animal activity (Forno and Smith 1999). However, much local dispersal is believed to be human-assisted as ornamentals for water gardens, or unintentionally via contaminated boating equipment. Individual fronds of giant salvinia can easily attach to boats or trailers, making them likely culprits of dispersal to new or isolated water bodies (McFarland et al. 2004).

Giant salvinia causes ecological and economic costs, mostly associated with its thick mat-forming stage. The mats, which can be up to one meter thick (Thomas and Room 1986), block sunlight from penetrating the water column and result in suppression of submersed aquatic vegetation (SAV) (Netten et al. 2010). Submersed aquatic vegetation are important sources of food and habitat for native fauna such as fish, waterfowl, and invertebrates, and their suppression affects community dynamics (Poirrier et al. 2010, Van Driesche et al. 2010). Moreover, absence

of sunlight and SAV in the water column results in a substantial decrease in dissolved oxygen, which can make the water inhospitable to certain species. Dissolved oxygen levels below five mg/L may result in local die-offs or evacuation of fish and wildlife (Chapman and Kimstach 1996, Flores and Carlson 2006).

Economic impacts of giant salvinia infestations largely take the form of impaired access to freshwater sites as a result of boats being unable to penetrate dense mats of vegetation. Industrial and recreational market sectors that rely on boating activity such as freshwater fishing, tourism, and waterfowl hunting suffer as a result of dense infestations (Mitchell and Thomas 1972, Seales et al. 2017). Agricultural commodities such as rice and crawfish are affected from giant salvinia infestation, but farms are limited in control options due to potential harm to the crop (Mudge 2016). Infestation of drinking water reservoirs is also problematic, as herbicides used to control the plant could lead to potential non-target effects or cause public concern (Samuels 2016). Efforts to maintain clear water by management of infestations creates additional expenses in the form of labor, fuel, and chemical control costs (Thomas and Room 1986, McFarland et al. 2004).

1.2. Methods of Control

Giant salvinia is controlled using mechanical, chemical, or biological methods. Each of these methods has its advantages and disadvantages (Madsen 2000). Often, two or more methods may be combined in an integrated management approach for optimal control (McFarland et al. 2004, Julien et al. 2009, Mudge et al. 2013).

1.2.1. Mechanical control

Mechanical control is accomplished by manual removal of plants, or more commonly by machine grinders or harvesters (Madsen 2000). Since giant salvinia reproduces by budding and fragmentation, grinding plants poses a risk of creating living fragments that serve as inoculum once the majority of the infestation has been removed (Madsen 2000). Harvested material must be deposited on land to desiccate or be otherwise properly disposed. Mechanical control is further limited by access, as large machines cannot reach into obstructed areas such as cypress swamps, where giant salvinia is often found (Miller and Wilson 1989, McFarland et al. 2004). At costs of $500 to $2500 per hectare, mechanical removal of aquatic weeds is typically cost-prohibitive (Getsinger et al. 2002).

Lake drawdowns have been used with moderate success for giant salvinia management in Lake Bistineau in north Louisiana (Seales et al. 2017). Water bodies must be drained long enough for giant salvinia plants to desiccate and die, and remaining plants are concentrated into a smaller acreage for chemical treatment (Seales et al. 2017). However, dormant buds may be able to resume growth once water levels are restored (Owens et al. 2004b), and some sections may not drain completely. Timing of drawdowns may be influenced by industry and community pressures (Houston et al. 2017, Seales et al. 2017). Repeated drawdowns have led to occasional fires and elimination of other aquatic vegetation, with potential impact on local species ecology (KTBS 2010, Seales et al. 2017).

1.2.2. Chemical control

Chemical control of giant salvinia relies on the application of registered aquatic herbicides. When applied properly, herbicides can be very effective at controlling giant salvinia,

but may require multiple applications per growing season, especially for thick mats (Miller and Wilson 1989). Chemical control reduces the need for bulk handling that is associated with mechanical control, as the plants die and then sink to the sediment layer where they eventually decompose. As with mechanical methods, herbicide application is limited by hard-to-access sites that may serve as points of re-infestation and require frequent, repeat spray events (Miller and Wilson 1989). Surfactants may be needed to overcome giant salvinia's hydrophobic trichomes for maximum coverage (Nelson et al. 2007). Chemical control of giant salvinia invokes significant costs of fuel, labor, and herbicide products. The Louisiana Department of Wildlife and Fisheries (LDWF) Aquatic Plant Control Program spends an estimated $8 million annually on spray contracts (salaries and chemical costs) to manage giant salvinia and other aquatic vegetation in public waters (J. Day 2016, personal communication). However, chemical control is generally fast-acting and effective for short-term control, and remains the best option for eradicating small infestations (McFarland et al. 2004). The two most commonly used aquatic herbicides for controlling giant salvinia in Louisiana are glyphosate and diquat (Mudge et al. 2016).

1.2.3. Biological control

Biological control of giant salvinia was first attempted in southern Africa in the 1970s using *Paulinia acuminata* (De Geer) (Orthoptera: Acrididae) and *Cyrtobagous singularis* Hustache (Coleoptera: Curculionidae) collected from *Salvinia auriculata* Aublet in Trinidad (Calder and Sands 1985, Schlettwein 1992). However, initial attempts at control were not successful (Schlettwein 1992). To initiate an effective biological control program, an agent is selected from the same area of origin as the invasive target species and tested for host specificity

and potential non-target effects prior to being approved for release in the adventive range. The benefit of classical biological control is that once an agent becomes established, its population will continue to grow and shrink with the pest population over time, thus maintaining sufficient balance to prevent major outbreaks (Van Driesche and Bellows 1996). Biological control of weeds is typically less expensive to implement than mechanical or chemical control, and can result in long-term control over a pest population (Andres 1977). Natural dispersal of agents following release can bring control to areas that would normally be inaccessible. However, biological control programs are rarely effective immediately following the release of an agent; patience or the use of alternative control methods are required during the agent's establishment time, which may take months to years (Van Driesche et al. 2010). The salvinia grasshopper *Paulinia acuminata*, the water lettuce moth *Samea multiplicalis* Guenée (Lepidoptera: Crambidae), and the salvinia weevils *Cyrtobagous singularis* Hustache and *C. salviniae* Calder and Sands (Coleoptera: Curculionidae) have all been evaluated as potential biological control agents for giant salvinia. Of these, only *C. salviniae* was found to be a host-specific natural enemy that can effectively control giant salvinia infestations (Thomas and Room 1986).

1.3. Biological Control of Giant Salvinia Using the Salvinia Weevil

1.3.1. Biology and ecology of the salvinia weevil

The salvinia weevil is a 2-mm aquatic beetle that was first collected on giant salvinia in Joinville, Brazil (Room et al. 1981). The beetle was initially classified as *C. singularis*, but revised to *C. salviniae* after determining that the Brazilian weevil represented a morphologically different species that exerted superior control over giant salvinia than *C. singularis* (Sands 1983, Calder and Sands 1985, Sands and Schotz 1985).

Salvinia weevils have a life cycle of approximately 55 days and can have 2 to 3 generations in a growing season (Forno et al. 1983, Room et al. 1989b). Female salvinia weevils deposit eggs in small holes or crevices on the salvinia plant, where they hatch in 9 to 18 days depending on temperature. Larvae begin feeding on external plant tissues, then tunnel into the plant rhizome where they continue their development. Third instars pupate amongst the root-like modified fronds and emerge as teneral adults after 10 to 15 days. Adults are sexually mature and begin mating 5 to 26 days after emergence (Forno et al. 1983). Females lay one egg at a time, and oviposit once every 2 to 5 days at 25°C (Forno et al. 1983). Adults may mate multiple times over their lifetime and remain reproductive for up to 38 weeks at 23°C (Sands et al. 1986).

Salvinia weevils are host-specific within the genus *Salvinia* and require permanent contact with the plant to complete their development (Forno et al. 1983). Adults feed on the green fronds, "roots", and rhizome throughout their lifetime, but prefer the nitrogen-rich buds when they are available (Sands et al. 1983). Adult feeding activity results in small, circular scars on leaf tissue and may completely destroy buds over time (Figure 1.2).

Figure 1.2. (a) Minor weevil feeding damage to a terminal bud showing small removed areas of the immature leaf tissue. (b) Severe bud damage showing complete destruction of the bud and youngest leaves. Photos by L. Moshman.

High adult population density can lead to reduced plant growth and extensive tissue damage, but it is the larval tunneling activity that inhibits nutrient uptake from the modified submersed "roots" to the aerial fronds, leading to rapid plant death and sinking (Forno et al. 1983). In a population from Brisbane, Australia, larvae ceased to complete development below 17°C, and adult feeding activity ceased below 13°C or above 33°C (Forno et al. 1983, Sands et al. 1983). Although salvinia weevils may feed on any *Salvinia* species, a slightly smaller *C. salviniae* ecotype that prefers feeding on *S. minima* has been reported in Louisiana and Florida (Madeira et al. 2006, Parys and Johnson 2013).

1.3.2. History of biocontrol programs using the salvinia weevil

The salvinia weevil was first successfully used to combat giant salvinia growing in Lake Moondarra, Queensland, Australia in 1980. After unsuccessful attempts to eradicate giant salvinia using extensive herbicide applications, 1,500 adult salvinia weevils were released onto the 400 hectare infestation and within seven months had reduced the infestation size by 80% (Room et al. 1981). Since then, salvinia weevils have been released as biocontrol agents in over eighteen countries including Australia, South Africa, Papua New Guinea, and Sri Lanka (Room and Thomas 1985, Room et al. 1989a, Cilliers 1991. Julien et al. 2009). The first salvinia weevil release to control giant salvinia in the United States occurred in 2001 in the Toledo Bend Reservoir and Lake Texana in Texas and Louisiana (Tipping and Center 2003). This weevil population originated from Joinville, Brazil (26°S, 48°W), and was imported to the United States via Australia (Room et al. 1981, Tipping and Center 2003, Julien 2012). To date, all subsequent weevil releases made in the United States have come from this same population (Russell et al. 2017).

1.3.3. Mass rearing and release of salvinia weevils in Louisiana

Louisiana's salvinia weevil rearing program began in 2007 and is managed by the LSU AgCenter and LDWF. Under the current partnership, giant salvinia and salvinia weevils are reared year-round in outdoor open-earthen ponds (Sanders et al. 2012, Wahl et al. 2016). These ponds are located at 30°N latitude in Iberia, Iberville, and Lafayette Parishes. The ponds are managed for pests and fertility and are harvested yearly for statewide distribution to public and private agencies (C. Wahl 2017, personal communication). Monitoring procedures have been standardized for assessing weevil population density and effectiveness of releases over time (Wahl et al. 2016).

Giant salvinia biocontrol in Louisiana has seen variable results depending upon yearly weather patterns, location of weevil release sites, and timing of releases (Tipping et al. 2008, Obeysekara et al. 2015). Mild winters have resulted in reduced cold mortality of giant salvinia and increased plant growth rates during the spring and summer (Owens et al. 2004a). Sites in north Louisiana such as Lake Bistineau have not reported successful control despite the use of integrated strategies including water level drawdowns and herbicide applications in conjunction with biological control (Seales et al. 2017). Greater success has been found in southern Louisiana, where giant salvinia mats have sunk with 99% biomass reduction achieved 21 months following initial salvinia weevil release (Tipping et al. 2008). Weevil population density in relation to the level of the giant salvinia infestation determines how quickly an infestation can be controlled; optimal control occurs when the weevil population reaches a density of 100 adult weevils or more per kilogram of fresh salvinia, resulting in necrosis and eventual loss of buoyancy (sinking) of the plant mat (Ireland et al. 2012). Weevil establishment following releases is paramount to the success of a biological control program, and most cases of poor

control can be attributed to poor agent establishment (Bale 2005, Grevstad et al. 2012, Parys and Johnson 2013).

1.3.4. Limitations to weevil establishment and integrated management techniques

The success or failure of weevil establishment following agent releases may be due to either timing of the release, the physical environment, or climatic factors. Timing of releases is an important predictor of how the weevil population will perform over time. Spring releases are typically recommended because they allow the weevil population ample time to feed and reproduce before the end of the growing season (Sullivan and Postle 2010, Nachtrieb 2013). Releases made in the peak of summer are unlikely to have much impact on rapidly growing giant salvinia mats, and may not give the weevils sufficient time to build their population before the onset of winter. Well established plant mats likewise do not produce as many fresh buds as active new growth (Coelho et al. 2005). Ideal release sites have certain physical attributes, such as stable water depth and minimal disturbance to the water surface. Events such as periodic drought and rapid water current can hinder local weevil establishment following release; however, these may be managed by selecting only suitable areas for release, or by irrigating when necessary and using containment booms to minimize water movement (Sanders et al. 2012). Finally, climatic factors are unavoidable but important determiners of long-term weevil establishment. Annual floods have been known to wash salvinia weevils downstream, resulting in the loss of locally established populations (Schooler et al. 2011). Unanticipated cold fronts or heat waves may stress salvinia weevil populations, resulting in levels of mortality that could hinder long-term establishment (Allen et al. 2012).

In temperate areas, winter conditions may prove unsuitable to weevil establishment despite favorable conditions during spring and summer, and result in yearly losses (Nachtrieb 2013). In these situations, annual spring releases may be necessary to restore weevil populations lost over the winter, coupled with an integrated management approach. Biological control using salvinia weevils can be successfully paired with mechanical or chemical methods to result in superior control than either method by itself. Management guidelines often recommend using mechanical or chemical methods to thin giant salvinia mats prior to weevil release to promote weevil establishment over a greater proportion of the infestation (Sullivan and Postle 2012). Herbicides are typically compatible with biological control as long as some healthy plants are reserved for the salvinia weevils to feed on. As salvinia weevils feed within or between plant tissue, they are seldom directly exposed to herbicides, and if exposed do not suffer high rates of mortality, making chemical control a safe approach for thinning out large infestations (Mudge et al. 2013).

1.4. Importance of Winter Management for Giant Salvinia and Salvinia Weevils

Giant salvinia and the salvinia weevil have a tropical to subtropical native distribution, and have had to adapt to a temperate climate to establish in coastal Australia, South Africa, and the United States (Allen et al. 2014). In temperate regions, winter conditions are a crucial factor in determining how these species will perform in their adventive range (Bale 2005, Obeysekara et al. 2015). Informed winter management decisions can benefit biological control programs for giant salvinia by increasing the likelihood of salvinia weevil establishment during winter and early spring. To study such practices, it is necessary to understand the overwintering biology of giant salvinia and the salvinia weevil.

Room (1986) reported that giant salvinia ceases growth below temperatures of 5°C, while Whiteman and Room (1991) reported that the plant is killed when ice forms within the plant tissue, occurring 2 to 3 hours after exposure below -3°C. Giant salvinia may overwinter in either the primary, secondary, or tertiary stages (Owens et al. 2004a). Where winter conditions are mild, tertiary stage mats may persist until the spring. During severe winters, tissue damage may result in plant death and sinking of a majority of the tertiary biomass, leaving only small fragments of primary or secondary stage growth to re-infest water bodies in the spring (Owens et al. 2004a). Surviving fragments of giant salvinia typically overwinter in backwater areas that are protected from winter elements such as wind, rain, and rapid currents (Tipping and Center 2003). Recent evidence suggests that giant salvinia may have increased susceptibility to aquatic herbicides immediately preceding severe winter cold fronts (Mudge and Sartain 2018).

Understanding the effect of freezing temperatures on the salvinia weevil is important for predicting survival of salvinia weevil populations during mild or severe winters. Mortality may result directly from ice nucleation in freeze avoidant insects (Bale and Hayward 2010), or indirectly through nutritional deficiency caused by lack of food or poor host plant quality (Mukherjee et al. 2014). Decrease in the population of overwintering adults may be exacerbated by reduced longevity due to cold temperature stress, decreased reproductive fecundity, and reduced survival of egg and larval stages (Bale 2005). Experiments on salvinia weevil thermal limits demonstrated that adults do not feed below a threshold temperature of 13°C (Forno et al. 1983), and oviposition and larval development cease below 19°C and 17°C, respectively (Sands et al. 1983, Hennecke and Postle 2006). Repeated cold exposures at the chill coma threshold of 4°C, the temperature at which neuromuscular coordination is completely lost, were shown to progressively reduce salvinia weevil survival and feeding activity, indicating that seasonal

temperature fluctuations can increase physiological stress and reduce performance (Obeysekara et al. 2015). Chill coma recovery time, the time required by an insect to regain neuromuscular coordination following exposure to nonlethal cold temperatures, was used to characterize the cold tolerance of four *C. salviniae* populations, showing that average recovery time could differ by as much as 15 minutes between populations (Mukherjee et al. 2014). Laboratory studies defined critical thermal minimum and maximum for *C. salviniae*—the temperatures at which individuals cannot walk from loss of coordination—to be 12.5°C and 42.4°C, respectively, together representing the optimal temperature range for weevil activity (Allen et al. 2014).

1.5. Support for Winter Management Techniques

Increasing heat retention within salvinia mats may make microhabitats more favorable to overwintering salvinia weevils and their host plants, preventing cold damage to plants and increasing salvinia weevil survival by reducing time spent at or below freezing temperatures. When giant salvinia plants overwinter in backwater areas, they are often protected by overhanging structures such as tree trunks and tall vegetation which can buffer effects from the environment to increase water temperatures (Tipping and Center 2003). Artificial methods of insulating plant mats can simulate these buffering conditions to provide a more favorable microclimate for overwintering salvinia weevils and reduce sources of physiological stress including repeated cold exposures and declining host plant quality (Obeysekara et al. 2015). These conditions can improve weevil survival during the winter, allowing for greater population increases in the spring that would improve early season control of reemerging giant salvinia infestations.

Covering giant salvinia mats with an insulating material can raise mat temperatures without requiring additional energy expenditure. Artificial refugia can be constructed as practical means of protecting salvinia weevils during severe winters by increasing microhabitat temperatures within giant salvinia mats. At the Lewisville Aquatic Ecosystem Research Facility (LAERF) in Lewisville, TX, cold frames placed over weevil culture boxes during severe weather improved overwintering and elevated spring temperatures, allowing weevils to reach peak population levels earlier in the year (Nachtrieb 2013). Studies on Lake Bistineau near Shreveport, LA have evaluated weevil overwinter survival using mulches and plastics as protective insulating materials (Micinski 2014).

Nonwoven fabric made from spunbonded polypropylene (SBP), or row cover, may have potential as an artificial refuge. In agricultural applications, row cover protects plants from frost by reducing heat loss from convection, resulting in warming of the insulated area (Olle and Bender 2010). Spunbonded polypropylene also provides a barrier to insects and wind while allowing sufficient penetration of light and water (Avril 2001). In strawberry plots, SBP fabrics increased temperatures by 1 to 3°C compared to uncovered plots (Hochmuth et al. 1986). In cranberry bogs, SBP covers increased canopy temperature by 3 to 5°C and 10 to 12°C during cool days and warm days, respectively, and maintained soil temperatures 2 to 5°C warmer than uncovered plots (Patten and Wang 1993). Additional benefits of SBP fabrics including frost protection, insect damage prevention, and higher leaf number have been documented in various fruit and vegetable systems (Olle and Bender 2010). In aquatic systems, opaque SBP fabrics are utilized for control of aquatic macrophytes as floating shades (Dawson and Hallows 1983) and benthic barriers (Cooke 1980, Madsen 2000, Hofstra and Clayton 2012). These fabrics inhibit plant growth primarily by light reduction, but may also increase rates of plant decomposition by

raising temperature (Dawson and Hallows 1983). Applications of lighter SBP fabrics in aquatic plant protection have not been previously explored.

Using SBP row cover in aquatic systems may produce similar benefits of cold protection to giant salvinia and the salvinia weevil during cold fronts as has been documented in terrestrial systems. Evaluation of these options may reveal an optimal fabric type for cold protection and enhancement of winter microclimate for salvinia weevils overwintering within giant salvinia mats. As a winter management strategy, row cover can be used to supplement outdoor weevil rearing operations or to create protected nurseries to maintain weevil populations through cold weather. The greatest utility of this method could be maintaining outdoor weevil rearing ponds in temperate northern areas that are more susceptible to periodic frosts throughout the winter.

Crowding plants to increase density is another approach for raising temperatures within a mat. Floating plant mats modify microclimates by reflecting solar radiation, reducing surface evaporation, and reducing mixing of water layers, resulting in thermal stratification beneath the mat (Dale and Gillespie 1976). Air spaces in leaves of floating vegetation form a layer of insulation over the water surface, reducing heat loss to the environment (Room and Kerr 1983). Floating aquatic plants increase water surface temperatures compared with open water (Reeder 2011). In the genus *Salvinia*, increased plant density has been shown to promote vertical growth of tissues to maximize photosynthetic capacity (Coelho et al. 2000). Vertical arrangement of giant salvinia fronds into the tertiary growth stage results in larger, more robust plants that are preferable to adult salvinia weevils for feeding and oviposition (Tipping and Center 2005).

Crowding giant salvinia plants to increase surface coverage and reduce spaces of open water is hypothesized to have a net warming effect on the system, which will benefit the overwintering salvinia weevil population. Manipulating plant density with containment booms or

floating nurseries can increase overall mat temperatures and improve microclimate conditions for weevil overwintering. Booms can be used on a large scale to contain salvinia weevil populations, and may be adjusted as needed to produce a desired plant density. This is particularly useful in open water systems that are susceptible to natural plant thinning during winter from currents and flooding events. Floating nurseries may likewise be used in outdoor salvinia weevil rearing operations to constrict the movement of giant salvinia and maintain a desired density of tertiary stage plants.

The main objective of this research is to investigate two strategies, SBP fabrics and increasing plant density, for overwinter management of floating giant salvinia mats. These strategies are hypothesized to contribute to the dual purpose goal of conserving plant quality and increasing salvinia weevil survival during winter.

1.6. References

Allen, J. L, S. Clusella-Trullas, and S. L. Chown. 2012. The effects of acclimation and rates of temperature change on critical thermal limits in *Tenebrio molitor* (Tenebrionidae) and *Cyrtobagous salviniae* (Curculionidae). J. Insect Physiol. 58: 669-678.

Allen, J. L, S. Clusella-Trullas, and S. L. Chown. 2014. Thermal tolerance of *Cyrtobagous salviniae*: a biocontrol agent in a changing world. BioControl 59: 357-366.

Andres, L. A. 1977. The economics of biological control of weeds. Aquat. Bot. 3: 111-123.

Avril, D. 2001. An innovative approach to spunbond agricultural crop cover. J. Ind. Text. 30: 311-319.

Bale, J. S. 2005. Effects of temperature on the establishment of non-native biocontrol agents: the predictive power of laboratory data, pp. 593-602. *In* M. S. Hoddle (ed.), Second International Symposium on Biological Control of Arthropods (ISBCA), vol. II. Publication FHTET-2005-08. USDA Forest Service, Morgantown, WV.

Bale, J. S., and S.A.L. Hayward. 2010. Insect overwintering in a changing climate. J. Exp. Biol. 213: 980-994.

Calder, A. A., and D.P.A. Sands. 1985. A new Brazilian *Cyrtobagous* Hustache (Coleoptera: Curculionidae) introduced into Australia to control *Salvinia*. Journal of the Australian Entomological Society 24: 57-64.

Cary, P. R., and P.G.J. Weerts. 1983. Growth of *Salvinia molesta* as affected by water temperature and nutrition. I. Effects of nitrogen level and nitrogen compounds. Aquat. Bot. 16: 163-172.

Chapman, D., and V. Kimstach. 1996. Selection of water quality variables, pp. 74-133. *In* D. Chapman (ed.), Water quality assessments: a guide to the use of biota, sediments, and water in environmental modeling. E & FN Spon, London, UK.

Cilliers, C. J. 1991. Biological control of water fern, *Salivinia molesta* (Salviniaceae), in South Africa. Agric. Ecosyst. Environ. 37: 219-224.

Coelho, F. F., F. S. Lopes, and C. F. Sperber. 2000. Density-dependent morphological plasticity in *Salvinia auriculata* Aublet. Aquat. Bot. 66: 273-280.

Coelho, F. F., F. S. Lopes, and C. F. Sperber. 2005. Persistence strategy of *Salvinia auriculata* Aublet in temporary ponds of Southern Pantanal, Brazil. Aquat. Bot. 81: 343-352.

Cooke, C. D. 1980. Covering bottom sediments as a lake restoration technique. Water Resour. Bul. 16: 921-926.

Dale, H. M., and T. Gillespie. 1976. The influence of floating vascular plants on the diurnal fluctuations of temperature near the water surface in early spring. Hydrobiologia 49: 245-256.

Dawson, F. H., and H. B. Hallows. 1983. Practical applications of a shading material for macrophyte control in watercourses. Aquat. Bot. 17: 301-308.

EDDMapS. 2017. Early detection & distribution mapping system. The University of Georgia - Center for Invasive Species and Ecosystem Health. Available online at http://www.eddmaps.org/; last accessed July 27, 2017.

Flores, D., and L. E. Wendel. 2001. Proposed field release of the salvinia weevil, *Cyrtobagous salviniae* Calder and Sands (Curculionidae: Coleoptera) a host-specific biological control agent of giant salvinia, *Salvinia molesta* D.S. Mitchell (Salviniaceae: Polypodiophyta) a federal noxious weed indiginous to southeast Brazil. USDA / APHIS / PPQ / CPHST, Mission Plant Protection Center, Mission, TX. http://www.salvinia.org/LCR/proposedrelease.pdf.

Flores, D., and J. W. Carlson. 2006. Biological control of giant salvinia in East Texas waterways and the impact on dissolved oxygen levels. J. Aquat. Plant Manage. 44: 115-121.

Forno, I. W., and P. A. Smith. 1999. Management of the alien weed, *Salvinia molesta*, in the wetlands of the Okavango, Botswana, pp. 159-166. *In* Streever, W. (ed.), An International Perspective on Wetland Rehabilitation. Kluwer Academic Publishers, Dordrecht, Netherlands.

Forno, I. W., D.P.A. Sands, and W. Sexton. 1983. Distribution, biology and host specificity of *Cyrtobagous singularis* Hustache (Coleoptera: Curculionidae) for the biological control of *Salvinia molesta*. Bull. Entomol. Res. 73: 85-95.

Galam, D., J. Silva, D. Sanders, and J. H. Oard. 2015. Morphological and genetic survey of giant salvinia populations in Louisiana and Texas. Aquat. Bot. 127: 20-25.

Getsinger, K. D., A. G. Poovey, W. F. James, R. M. Stewart, M. J. Grodowitz, M. J. Maceina, and R. M. Newman. 2002. Management of Eurasian watermilfoil in Houghton Lake, Michigan: workshop summary. Environmental Lab. TR-02-24. US Army Corps of Engineers, Engineer Research and Development Center, Vicksburg, MS.

Grevstad, F. S., E. M. Coombs, and P. B. McEvoy. 2012. Revisiting release strategies in biological control of weeds: are we using enough releases? pp. 368-376. *In* Y. Wu et al. (eds.), Proceedings of the XIII international symposium on biological control of weeds, September 11-16, 2011, Waikoloa, Hawaii, USA. U.S. Forest Service Forest Health Technology Enterprise Team, Morgantown, WV.

Hennecke, B., and L. A. Postle. 2006. The key to success: an investigation into oviposition of the salvinia weevil in cool climate regions, pp. 780-783. *In* C. Preston, J. H. Watts, and N. D. Crossman (eds.), Proceedings of the 15th Australia Weeds Conference. Weed Management Society of South Australia, Adelaide.

Hochmuth, G. J., S. R. Kostewicz, S. J. Locascio, E. E. Albreghts, C. M. Howard, and C. D. Stanley. 1986. Freeze protection of strawberries with floating row covers. Proc. Fla. State Hortic. Soc. 99: 307-311.

Hofstra, D. E., and J. S. Clayton. 2012. Assessment of benthic barrier products for submerged aquatic weed control. J. Aquat. Plant Manage. 50: 101-105.

Houston, K., J. Seales, J. Sibley, and E. Thames. 2017. Lake Bistineau: lake history & management issues. Waterbody Management Plan Series Part VI-A. Louisiana Department of Wildlife and Fisheries, Office of Fisheries, Inland Fisheries Section, Baton Rouge, LA.

Ireland, P. A., A. Knutson, and L. Gregory. 2012. Rearing the salvinia weevil in outdoor tanks at Caddo Lake, Texas, pp. 25-36. *In* A. Knutson and J. Nachtrieb (eds.), A guide to mass rearing the salvinia weevil for biological control of giant salvinia. Texas A&M AgriLife Extension Service, Special Publication ESP-475. http://cise.tamu.edu/media/355740/gs_manual_10-15-12.pdf.

Jacono, C. C., T. R. Davern, and T. D. Center. 2001. The adventive status of *Salvinia minima* and *S. molesta* in the southern United States and the related distribution of the weevil *Cyrtobagous salviniae*. Castanea 66: 214-226.

Johnson, D. 1995. Giant salvinia found in South Carolina. Aquatics 17: 22.

Julien, M. 2012. *Salvinia molesta* D.S. Mitchell – salvinia, pp. 518-525. *In* M. Julien, R. McFadyen, J. Cullen (eds.), Biological control of weeds in Australia. CSIRO Publishing, Collingwood, Australia.

Julien, M. H., T. D. Center, and P. W. Tipping. 2002. Floating fern (*Salvinia*), pp. 17-32. *In* R. Van Driesche, B. Blossey, M. Hoddle, S. Lyon, R. Reardon (eds.), Biological control of invasive plants in the eastern United States. USDA Forest Service Publ. FHTET-2002-04, Forest Health Technology Enterprise Team, Morgantown, WV.

Julien, M. H., M. P. Hill, and P.W. Tipping. 2009. *Salvinia molesta* D.S. Mitchell (Salviniaceae), pp. 378-407. *In* R. Muniappan, G. V. Reddy, and A. Raman (eds.), Biological control of tropical weeds using arthropods. Cambridge University Press, Cambridge, UK.

Knutson, A. 2012. Water quality parameters important to the growth of giant salvinia, pp. 9-12. *In* A. Knutson and J. Nachtrieb (eds.), A guide to mass rearing the salvinia weevil for biological control of giant salvinia. Texas A&M AgriLife Extension Service, Special Publication ESP-475. http://cise.tamu.edu/media/355740/gs_manual_10-15-12.pdf

KTBS 3 News Team. 2010. Three fires still smoldering at Lake Bistineau. https://www.ktbs.com/news/three-fires-still-smoldering-at-lake-bistineau/article_865a849b-0db7-5f0d-8e71-90f76f2fbf08.html (Accessed 11 July 2017).

Loyal, D. S., and R. K. Grewal. 1966. Cytological study on sterility in *Salvinia auriculata* Aublet with a bearing on its reproductive mechanism. Cytologia 31: 330-338.

Madeira, P. T., P. W. Tipping, D. E. Gandolfo, T. D. Center, T. K. Van, and C. W. O'Brien. 2006. Molecular and morphological examination of *Cyrtobagous* sp. collected from Argentina, Paraguay, Brazil, Australia, and Florida. BioControl 51: 679-701.

Madsen, J. D. 2000. Advantages and disadvantages of aquatic plant management techniques. Environmental Lab. MP-00-1. US Army Corps of Engineers, Engineer Research and Development Center, Vicksburg, MS.

McFarland, D. G., L. S. Nelson, M. J. Grodowitz, R. M. Smart, and C. S. Owens. 2004. *Salvinia molesta* D. S. Mitchell (giant salvinia) in the United States: a review of species ecology and approaches to management. Environmental Lab. SR-04-2. US Army Corps of Engineers, Engineer Research and Development Center, Vicksburg, MS.

Micinski, S. 2014. S-1058 annual report for 2014 and plans for 2015, pp. 23-26. *In* Minutes, S1058 Biological Control of Arthropod and Weed Pests in the Southern United States. Southeastern Branch Meeting of the Entomological Society of America, 15 March 2015, Biloxi, MS.

Miller, I. L., and C. G. Wilson. 1989. Management of salvinia in the Northern Territory. J. Aquat. Plant Manage. 27: 40-46.

Mitchell, D. S. 1972. The Kariba weed: *Salvinia molesta*. Brit. Fern Gaz. 10: 251-252.

Mitchell, D. S., and P. A. Thomas. 1972. Ecology of water weeds in the neotropics. UNESCO, Paris, France.

Mudge, C. R. 2016. Evaluation of topramezone and benzobicyclon for activity on giant salvinia. APCRP Technical Notes Collection. ERDC/APCRP-CC-21. U.S. Army Engineer Research and Development Center, Vicksburg, MS.

Mudge, C. R., and B. T. Sartain. 2018. Influence of winter on herbicide efficacy for control of giant salvinia (*Salvinia molesta*). J. Aquat. Plant Manage. *In Press*.

Mudge, C. R., N. E. Harms, and J. G. Nachtrieb. 2013. Interactions of herbicides, surfactants, and the giant salvinia weevil (*Cyrtobagous salviniae*) for control of giant salvinia (*Salvinia molesta*). J. Aquat. Plant Manage. 51: 77-83.

Mudge, C. R., A. J. Perret, and J. R. Winslow. 2016. Evaluation of foliar herbicide and surfactant combinations for control of giant salvinia at three application timings. J. Aquat. Plant Manage. 54: 32-26.

Mukherjee, A., A. Knutson, D. A. Hahn, and K. M. Heinz. 2014. Biological control of giant salvinia (*Salvinia molesta*) in a temperate region: cold tolerance and low temperature oviposition of *Cyrtobagous salviniae*. BioControl 59: 781-790.

Nachtrieb, J. G. 2013. Mass-rearing *Cyrtobagous salviniae* for biological control of giant salvinia: field release implications. J. Aquat. Plant Manage. 51: 122-126.

Nelson, L. S., L. M. Glomski, and D. N. Gladwin. 2007. Effect of glyphosate rate and spray volume on control of giant salvinia. J. Aquat. Plant Manage. 45: 58-61.

Netten, J.J.C., G.H.P. Arts, R. Gylstra, E. H. van Nes, M. Scheffer, and R.M.M. Roijackers. 2010. Effect of temperature and nutrients on the competition between free-floating *Salvinia natans* and submerged *Elodea nuttallii* in mesocosms. Fund. Appl. Limnol. 177: 125-132.

Obeysekara, P. T., A. Knutson, A. Mukherjee, and K. M. Heinz. 2015. Repeated cold exposure effects on mortality and feeding activity of the salvinia weevil, *Cyrtobagous salviniae* (Coleoptera: Curculionidae). Environ. Entomol. 44: 1590-1598.

Olle, M., and I. Bender. 2010. The effect of non-woven fleece on the yield and production characteristics of vegetables. Agraarteadus 1: 24-29.

Owens, C. S., R. M. Smart, and R. M. Stewart. 2004a. Low temperature limits of giant salvinia. J. Aquat. Plant Manage. 42: 91-94.

Owens, C. S., R. M. Smart, and G. O. Dick. 2004b. Regeneration of giant salvinia from apical and axillary buds following desiccation or physical damage. J. Aquat. Plant Manage. 42: 117-119.

Parys, K. A., and S. J. Johnson. 2013. Biological control of common salvinia (*Salvinia minima*) in Louisiana using *Cyrtobagous salviniae* (Coleoptera: Curculionidae). Fla. Entomol. 96: 10-18.

Patten, K. D., and J. Wang. 1993. Spunbonded polypropylene fabric covers can enhance cranberry establishment and production. HortScience 28: 1202.

Poirrier, M. A., K. Burt-Utley, J. F. Utlety, and E. A. Spalding. 2010. Submersed aquatic vegetation of the Jean Lafitte National Historical Park and Preserve. Southeast. Nat. 9: 477-486.

Reeder, B. C. 2011. Assessing constructed wetland functional success using diel changes in dissolved oxygen, pH, and temperature in submerged, emergent, and open-water habitats in the Beaver Creek Wetlands Complex, Kentucky (USA). Ecol. Eng. 37: 1772-1778.

Room, P. M. 1983. 'Falling apart' as a lifestyle: the rhizome architecture and population growth of *Salvinia molesta*. J. Ecol. 71: 349-365.

Room, P. M. 1986. Equations relating growth and uptake of nitrogen by *Salvinia molesta* to temperature and the availability of nitrogen. Aquat. Bot. 24: 43-59.

Room, P. M., and J. D. Kerr. 1983. Temperatures experienced by the floating weed *Salvinia molesta* Mitchell and their prediction from meteorological data. Aquat. Bot. 16: 91-103.

Room, P. M., and P. A. Thomas. 1985. Nitrogen and establishment of a beetle for biological control of the floating weed *Salvinia* in Papua New Guinea. J. Appl. Ecol. 22: 139-156.

Room, P. M., K.L.S. Harley, I. W. Forno, and D.P.A. Sands. 1981. Successful biological control of the floating weed salvinia. Nature 294: 78-80.

Room, P. M., G. A. Gunatilaka, P. Shivanathan, and I.V.S. Fernando. 1989a. Control of *Salvinia molesta* in Sri Lanka by *Cyrtobagous salviniae*, pp. 285-290. *In* E.S. Delfosse (ed.), Proceedings of the VII International Symposium on Biological Control of Weeds, Rome, Italy.

Room, P.M., M. H. Julien, and I. W. Forno. 1989b. Vigorous plants suffer most from herbivores: latitude, nitrogen and biological control of the weed *Salvinia molesta*. Oikos 54: 92-100.

Russell, A., S. Johnson, X. Cibils, F. McKay, L. Moshman, P. Madeira, Z. Blair, and R. Diaz. 2017. Surveys in Uruguay and Argentina reveal *Cyrtobagous salviniae* (Coleoptera: Curculionidae) populations adapted to survive temperate climates in southeastern USA. Biological Control 107: 41-49.

Samuels, S. 2016. Salvinia in Shreveport's drinking water supply. https://www.ktbs.com/news/salvinia-in-shreveport-s-drinking-water-supply/article_a7d9cc47-388b-5abe-8dcb-f279e81d64b5.html (Accessed 10 July 2017).

Sands, D.P.A. 1983. Identity of *Cyrtobagous* sp. (Coleoptera: Curculionidae) introduced into Australia for biological control of salvinia. J. Aust. Ent. Soc. 22: 200.

Sands, D.P.A., and M. Schotz. 1985. Control or no control: a comparison of the feeding strategies of two salvinia weevils, pp. 551-556. *In* E.S. Delfosse (ed.), Proceedings of the VI International Symposium on Biological Control of Weeds, 19-25 August 1984, Vancouver, Canada. Agriculture Canada, Ottawa.

Sands, D.P.A., M. Schotz, and A. S. Bourne. 1983. The feeding characteristics and development of larvae of a salvinia weevil *Cyrtobagous* sp. Entomol. Exp. Appl. 34: 291-296.

Sands, D.P.A., M. Schotz, and A. S. Bourne. 1986. A comparative study on the intrinsic rates of increase of *Cyrtobagous singularis* and *C. salviniae* on the water weed *Salvinia molesta*. Entomol. Exp. Appl. 42: 231-237.

Sanders, D., W. Lorio, and K. Whitehead. 2012. Rearing the salvinia weevil in ponds, pp. 47-51. *In* A. Knutson and J. Nachtrieb (eds.), A guide to mass rearing the salvinia weevil for biological control of giant salvinia. Texas A&M AgriLife Extension Service, Special Publication ESP-475. http://cise.tamu.edu/media/355740/gs_manual_10-15-12.pdf.

Schlettwein, C.H.G. 1992. Aquatic weeds and their management in southern Africa: biological control of *Salvinia molesta* in the Eastern Caprivi, pp. 173-187. *In* T. Matiza and H.N. Chabwela (eds.), Proceedings of the Southern African Development Coordination Conference, 3-5 June 1991, Gaborone, Botswana. IUCN, Gland, Switzerland.

Schooler, S. S., B. Salau, M. H. Julien, and A. R. Ives. 2011. Alternative stable states explain unpredictable biological control of *Salvinia molesta* in Kakadu. Nature 470: 86-89.

Seales, J., Houston, K., J. Sibley, and E. Thames. 2017. Lake Bistineau: waterbody evaluation & recommendations 2017. Waterbody Management Plan Series Part VI-B. Louisiana Department of Wildlife and Fisheries, Office of Fisheries, Inland Fisheries Section, Baton Rouge, LA.

Sullivan, P. R. and L. A. Postle. 2010. Low temperature reproduction of *Cyrtobagous salviniae*: good news for biological control of salvinia in a temperate climate. J. Aquat. Plant Manage. 48: 92-96.

Sullivan, P., and L. Postle. 2012. Salvinia biological control field guide. New South Wales Department of Primary Industries, NSW, Australia. http://www.dpi.nsw.gov.au/__data/assets/pdf_file/0008/425807/Salvinia-biological-control-field-guide.pdf.

Thomas, P. A., and P. M. Room. 1986. Taxonomy and control of *Salvinia molesta*. Nature 320: 581-584.

Tipping, P. W., and T. D. Center. 2003. *Cyrtobagous salviniae* (Coleoptera: Curculionidae) successfully overwinters in Texas and Louisiana. Fla. Entomol. 86: 92-93.

Tipping, P. W., and T. D. Center. 2005. Influence of plant size and species on preference of *Cyrtobagous salviniae* adults from two populations. Biol. Control 32: 263-268.

Tipping, P. W., M. R. Martin, T. D. Center, and T. M. Davern. 2008. Suppression of *Salvinia molesta* Mitchell in Texas and Louisiana by *Cyrtobagous salviniae* Calder and Sands. Aquat. Bot. 88: 196-202.

Van Driesche, R., and T. S. Bellows, Jr. 1996. Biological control. Kluwer Academic Publishers, Boston, MA.

Van Driesche, R. G., R. I. Carruthers, T. Center, M. S. Hoddle, J. Hough-Goldstein, L. Morin, L. Smith, D. L. Wagner, et al., 2010. Classical biological control for the protection of natural ecosystems. Biol. Control Supplement 1, S2–S33.

Wahl, C., L. Moshman, and R. Diaz. 2016. How to rear giant salvinia weevils in outdoor ponds. LSU AgCenter Pub. 3551. Louisiana State University Agricultural Center, Baton Rouge, LA.

Whiteman, J. B., and P. M. Room. 1991. Temperatures lethal to *Salvinia molesta* Mitchell. Aquat. Bot. 40: 27-35.

Williams, R. H. 1956. *Salvinia auriculata* Aubl.: the chemical eradication of a serious aquatic weed in Ceylon. Tropical Agriculture 33: 145-157.

Chapter 2: Assessment of Row Cover Fabrics for Winter Protection of Giant Salvinia and Salvinia Weevils

2.1. Introduction

In classical biological control, introducing biological control agents into new areas can lead to a mismatch between climatic conditions of the native and adventive ranges including temperature, photoperiod, and timing of generations (Bale 2005, Grevstad and Coop 2015). Winters in temperate climates pose a risk for agents native to tropical and semitropical areas (Bale 2005). In these situations, agent populations thrive during most of the year but suffer winter losses due to direct mortality, slow population growth, and reduced fecundity (McClay 1996). Such has been observed in the alligatorweed flea beetle *Agasicles hygrophila* Selman and Vogt. (Coleoptera: Chrysomelidae), an introduced agent for alligatorweed (*Alternanthera philoxeroides* (Mart.) Griseb.) (Buckingham 2002), and the water hyacinth weevils *Neochetina eichorniae* Warner and *N. bruchi* Hustache (Coleoptera: Curculionidae), biocontrol agents of water hyacinth (*Eichornia crassipes* (Mart.) Solms) (Manning 1979). In these systems, additional measures are required to ensure the survival and continued growth of the agent population. These may include annual releases (Buckingham 2002), establishment of greenhouse colonies (Manning 1979, Parys and Johnson 2013), and establishment of nursery areas (Manning 1979).

Giant salvinia, *Salvinia molesta* Mitchell, is a floating aquatic fern that is considered an invasive species in over 21 countries including Australia, Fiji, India, New Guinea, New Zealand, Sri Lanka, South Africa, Trinidad, and the United States (Room et al. 1981, Calder and Sands 1985, Jacono et al. 2001). Since the early 2000s giant salvinia has spread throughout the southern United States and to date has been reported in 14 states, Guam, Puerto Rico, and the

Virgin Islands (Galam et al. 2015, EDDMapS 2017). Giant salvinia is native to southeastern Brazil and is believed to have spread primarily through the ornamental plant trade (Forno and Harley 1979, McFarland et al. 2004). Dense plant mats that can reach up to one meter thick (Thomas and Room 1986) contribute to impaired boat access, declining water quality, and reduced native species diversity (McFarland et al. 2004). Control is achieved through mechanical, chemical, or biological means (Miller and Wilson 1989).

The salvinia weevil, *Cyrtobagous salviniae* Calder and Sands (Coleoptera: Curculionidae), is a 2-mm aquatic beetle associated with plants in the genus *Salvinia* (Forno et al. 1983). Under optimal temperatures, salvinia weevils mature from egg to adult in 55 days and can complete 2 to 3 generations in a growing season (Forno et al. 1983, Room et al. 1989). Larvae feed upon the plant's nitrogen-rich tissues and tunnel inside its underwater rhizome, causing the plant to fragment and sink (Forno et al. 1983). Adult weevils feed on the young buds and lay eggs in small holes or crevices. Oviposition occurs within the range of 19 to 37°C, and outside of this range, only adults can survive until favorable conditions return (Forno et al. 1983). Biological control of giant salvinia using the salvinia weevil has resulted in excellent control of giant salvinia with minimal associated costs and greater long-term results than chemical or mechanical means (Sullivan et al. 2011).

In the northern limits of giant salvinia's range in the United States, salvinia weevils are subject to winter losses resulting in failure to establish and poor control of infestations (Mukherjee et al. 2014). Mass rearing facilities have been established to aid in the production and distribution of weevils, but these require winter cold frames (Nachtrieb 2013) or are limited to southern regions where year-round outdoor rearing is possible (Sanders et al. 2012). Overwinter establishment has been reported as far north as the Toledo Bend Reservoir (32°N),

where cold fronts can occasionally reach as low as -9.4°C (Tipping and Center 2003, USDA PHZM 2012).

Giant salvinia biocontrol programs can benefit from winter protection methods that raise temperatures and enhance microclimate to achieve increased overwinter establishment of the salvinia weevil. In outdoor fish ponds, precise overwintering temperature control can be achieved with electronic control systems, but is energy-demanding and can cost as much as $13.80 per day to operate (Hall et al. 2002). Solar-passive thermal refugia made from insulating materials are cost-effective, requiring no additional energy input (Putegnat 2013). Greenhouse plastics have been explored as portable thermal refugia for outdoor fish ponds (Putegnat 2013) and are utilized in semi-permanent greenhouses for mass rearing salvinia weevils in north Texas (Ireland et al. 2012, Nachtrieb 2013). Spunbonded polypropylene (SBP) fabrics are widely used as row covers for terrestrial crops (Olle and Bender 2010) and have been used as benthic barriers to manage submersed aquatic vegetation (Cooke 1980, Hofstra and Clayton 2012). However, SBP fabrics have not been thoroughly evaluated as alternative materials for artificial overwintering refugia in an aquatic setting. At approximately $\$0.10/m^2$, SBP is a fraction of the cost of greenhouse film sold for $\$1.40/m^2$ (Growers Supply 2014). An advantage of SBP fabric is its porosity, which prevents water puddling and allows more air exchange than greenhouse film (Wells and Loy 1993). The goal of this study was to explore SBP as a thermal refuge for overwintering salvinia weevils in a floating plant mat. I hypothesized that SBP covers would increase mat temperatures by creating an insulated air layer that reduced the temperature gradient at the air-water interface, thus resulting in reduced plant and weevil mortality and increased overwinter establishment of salvinia weevil populations.

The specific objectives of this research were: (1) to determine which SBP type is most suitable for enhancing biological control of giant salvinia during winters in Louisiana; (2) to evaluate whether seasonal use of SBP row cover produces any negative effects on the growth and development of giant salvinia and salvinia weevils; (3) to assess how giant salvinia mat temperature is affected by seasonal row cover use; and (4) to assess how salvinia weevil survival and reproduction are affected by seasonal row cover use.

2.2. Materials and Methods

2.2.1. Greenhouse evaluation of three SBP fabrics on giant salvinia and salvinia weevil growth and development

To determine whether long-term use of SBP row cover posed negative growth effects on weevil-inhabited giant salvinia as a result of increased temperature or decreased light penetration, a greenhouse experiment was conducted at the Louisiana State University Research Greenhouse Facility in Baton Rouge, LA. The first replication of this experiment tested three fabric types plus an uncovered control and was conducted from January through March 2016 (Year 1). A second replication tested only the heaviest fabric against an uncovered control and was conducted from November 2016 through January 2017 (Year 2).

White plastic buckets (19 L, 37 cm height x 30 cm diameter) were filled with 15 L of water sourced from a greenhouse giant salvinia colony grown in stock tanks. Each bucket was provisioned with enough weevil-free, tertiary stage giant salvinia to cover approximately 80% of the water surface, or 350 to 360 g fresh weight. Plants were sourced from the LSU greenhouse colony (Year 1) or outdoor nurseries from a shallow pond in the LSU Reproductive Biology Center, Saint Gabriel, LA (Year 2). All buckets were arranged randomly on a greenhouse bench.

The greenhouse was heated with natural gas radiant heaters and cooled using evaporative cooling pads to maintain an ambient temperature between 22 to 28°C.

In Year 1, each bucket was randomly assigned a treatment of uncovered (control) or one of three row cover treatments: light (19.0 g/m^2), medium (30.5 g/m^2), and heavy (49.5 g/m^2). Eighty total buckets were used, with 20 replicates per treatment. In Year 2, the experiment was simplified to evaluate only the heavy and control treatments. (n = 20). The heavy treatment was chosen over the light and medium treatments for further evaluation in Year 2 based on its likelihood to provide the greatest cold protection without sacrificing long-term plant quality. One layer of fabric was secured over each bucket using rubber bands and remained in place for the duration of the experiment (eight weeks), maintaining an air space between the fabric and the plant material. All fabric was obtained from AgFabric, Wellco Industries, Vista, CA. Each bucket was fertilized to 2 mg/L nitrogen biweekly using Miracle-Gro® Water Soluble Lawn Food (36-0-6, The Scotts Company LLC, Marysville, OH) (Eisenberg and Johnson 2012). Reverse osmosis water was added weekly to replace water lost through evaporation. All buckets were sprayed every two weeks with the insecticide *Bt* var. *kurstaki* (Javelin, 0.3 grams/liter) to control feeding damage by *Samea multiplicalis* Guenée (Lepidoptera: Crambidae). Water quality parameters pH, electrical conductivity (Years 1 and 2), and dissolved oxygen (Year 2 only) were monitored weekly using handheld probes (HANNA® Instruments, Carrollton, TX).

After four weeks, 10 buckets were randomly selected from each treatment and were harvested. Giant salvinia fresh weight biomass (live plants plus collected root sediment) were measured from each bucket immediately after harvest, and dry weight biomass were obtained after drying plants completely in an oven at 65°C for a minimum of 72 hours. At this time each remaining bucket was inoculated with 20 adult salvinia weevils from the LSU greenhouse colony

to investigate long-term effects of the fabric treatments on weevil survival and fecundity. Weevils were obtained by live extraction through Berlese funnels equipped with 60-W lamps and collected onto small portions of weevil-free giant salvinia in Whirl-Pak® bags (Nasco, Fort Atkinson, WI) using a method modified from Boland and Room (1983). Extracted weevils were placed into each bucket using soft forceps. Voucher specimens are deposited in the Louisiana State Arthropod Museum.

A final harvest of the remaining buckets was made four weeks post-inoculation. All remaining plants were placed into Berlese funnels and all salvinia weevil adults plus F1 larvae were extracted into 95% ethanol over a period of 24 hours or until plants were dry to the touch. Giant salvinia was dried for an additional 24 hours in an oven at 65°C to ensure complete water removal before final biomass determination. Dry weight biomass, specific leaf area (SLA), leaf phenolic content, and tissue carbon:nitrogen (C:N) content were measured following each harvest event. A wet weight-dry weight linear regression was constructed from extra plants collected on Day 1 to calculate the change in dry weight over time.

Specific leaf area, which estimates leaf area per unit mass (cm^2/mg leaf tissue; Diaz et al. 2011) was obtained from each bucket on Day 1 of the experiment and at each harvest event. The first four fronds located directly behind the youngest developing bud leaves were selected and removed at their base, then pressed flat using a piece of glass (Figure 2.1). Total leaf area was determined from scale-calibrated photographs using the computer program ImageJ 1.49v (National Institutes of Health, USA). Fronds were placed between paper towel sheets and dried in an oven at 65°C for 72 hours before weighing. The SLA was calculated by dividing total leaf area by the dry weight of each frond.

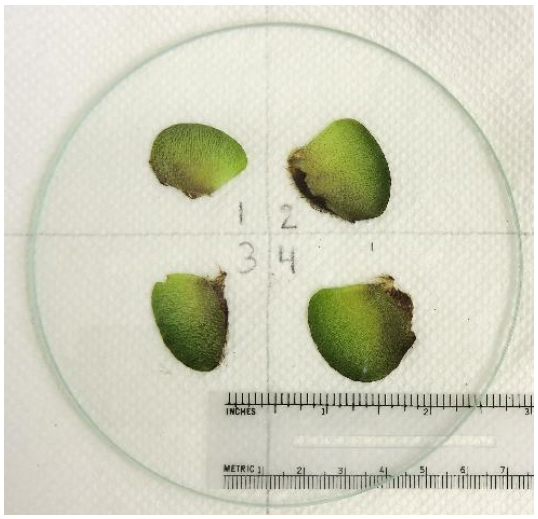

Figure 2.1. Measurement of specific leaf area using fresh giant salvinia fronds pressed under glass.

Total leaf phenolic content was determined from five randomly selected replicates from each treatment at the first and final harvests using a Folin-Ciocalteu assay (Waterman & Mole 1994). Twenty-five milligrams of dried giant salvinia fronds were crushed and extracted into 5 mL of 50% methanol for 7 days. An 80 µL aliquot of the methanol extract was added to a glass test tube (27 mL) and mixed with 2.65 mL distilled water to obtain a final volume of 2.75 mL. A volume of 500 µL 1N Folin-Ciocalteu reagent (Sigma-Alrich Co. LLC, St. Louis, MO) was also added to each test tube. After 2 minutes of incubation, 500 µL of 20% sodium carbonate (Na_2CO_3) solution was added to each test tube and vortexed. After 90 minutes, the contents of each test tube was poured into a clear polystyrene cuvette and absorbance was read in a UV-visible spectrophotometer at 720 nm for maximum absorption of blue products of the chemical reaction (UV-1601, Shimadzu Scientific Instruments, Columbia, MD) (Stout et al. 1998). Each sample was assayed twice and the two absorbance readings were averaged.

Tissue C:N content was analyzed from whole dried plants in five randomly selected replicates from each treatment at the first and final harvests. Percent carbon and nitrogen were

determined from subsamples of ground dried plant material using dry combustion by a LECO CN analyzer (LECO Corporation, St. Joseph, MI). All C:N analysis was performed by the LSU AgCenter Soil Testing and Plant Analysis Laboratory.

Weevil variables collected included adult survival, adult reproductive status, number of F1 larvae, and proportion of damaged terminal buds. Adult survival was measured by counting the number of adult weevils out of the initial 20 recovered in the final Berlese extraction. All adults were then dissected and categorized by reproductive status (male, female parous, or female nonparous). Females were classified as either parous or nonparous based on the presence or absence of fully developed eggs in the body cavity, and proportion of parous females out of total females was calculated. This measurement was selected to determine whether the covered treatments created stressful conditions that could affect female fecundity (Eisenberg 2011). Number of F1 larvae recovered from extractions was totaled for each bucket. At the final harvest, ten terminal buds were haphazardly selected from each bucket and inspected for signs of weevil feeding damage including scarred circular lesions and necrotic tips without the presence of frass.

Light intensity and water temperature were measured at 30-minute intervals over the course of the experiment by HOBO Pendant® data loggers (Onset Computer Corporation, Bourne, MA) floating at the plant level. Data loggers were placed in two randomly selected replicates in each treatment and the resultant curves were averaged. A single data logger recorded ambient greenhouse temperature throughout the 8 week experiment.

Due to differences in plant source, number of treatments, and time of year, results from Year 1 and Year 2 were analyzed separately. Biomass increase, SLA, total phenolics, and tissue C:N from the first and final harvest events were analyzed using two-way ANOVA to compare

the main effects of harvest date and treatment, and the interaction effect of harvest date*treatment on each dependent variable. Variables found to be significant at $\alpha = 0.05$ were further analyzed using a post-hoc LSMeans Student's *t*-test. Adult weevil survival, F1 larval recovery, proportion of parous females, and proportion of buds damaged were analyzed using one-way analysis of variance (ANOVA) (Year 1) or pooled *t*-tests (Year 2). Average temperature and light intensity curves for each treatment were likewise analyzed using ANOVA (Year 1) or pooled *t*-tests (Year 2). All statistical analyses were carried out in JMP® Pro 13.0.0 (SAS Institute Inc., Cary, NC, 2016).

2.2.2. Laboratory evaluation of three SBP fabrics on plant quality and weevil survival following acute cold exposure

To determine the level of protection resulting from three row cover fabrics on plant tissue damage and weevil mortality during acute cold exposures, a laboratory experiment was conducted in climate-controlled growth chambers (Thermo Fisher Scientific, Marietta, OH, USA). Translucent plastic containers (1.2 L, 16 cm x 16 cm x 9 cm) were filled with 950 mL of tap water and provisioned with one weevil-free tertiary stage giant salvinia plant of similar length and width, approximately 10 g fresh weight, obtained from the Louisiana State University Research Greenhouse Facility in Baton Rouge, LA. Salvinia weevils were extracted from the greenhouse colony using Berlese funnels and placed twenty at a time onto fresh plants. Plants and weevils were acclimated for 72 hours at the feeding threshold of 13°C (Forno et al. 1983) in a laboratory growth chamber, as done previously by Hennecke and Postle (2006) and Russell et al. (2017). A photoperiod of 10:14h [light:dark (L:D)] was used to simulate winter conditions. Each container was randomly assigned to one of four fabric treatments: light (19.0 g/m^2), medium (30.5 g/m^2), heavy (49.5 g/m^2), and no fabric (control) (n = 6). One fabric layer was

draped over each container, and the containers were placed inside a growth chamber set to -7°C in total darkness for 36 hours to simulate a winter cold front. One HOBO Pendant® data logger recorded water surface temperature every 15 minutes in a randomly selected replicate from each treatment. Additional data loggers were used to monitor the air temperature of each growth chamber to ensure that the chambers maintained constant temperatures. After 36 hours of exposure at -7°C, the containers were returned to 13°C over a period of 12 hours and maintained at 10:14h (L:D) for three days before evaluation of plant tissue damage and weevil survival. Five additional uncovered containers were maintained at a constant 13°C with no cold exposure for the duration of the experiment as a control for natural weevil mortality.

Plant tissue damage resulting from cold exposure was assessed on a qualitative scale of 0 to 5, where 0 indicates healthy plants showing no signs of browning; 1 indicates mostly green plants with less than 10% browning of leaf edges; 2 indicates mostly green plants with 11 to 30% browning of leaf edges and some discoloration of outer leaves; 3 indicates 31 to 50% leaf browning and loss of integrity (limpness) of leaf edges with partial browning of terminal bud; 4 indicates 51 to 99% leaf browning and loss of integrity in two or more leaves with partial browning of terminal bud; and 5 indicates dead plants with complete necrosis and loss of integrity in the leaves and buds (Figure 2.2). Damage scores were averaged for each treatment and analyzed using one-way ANOVA with Tukey post-hoc tests at $\alpha = 0.05$.

Weevil mortality was assessed 72 hours post-exposure. All plants were destructively searched to recover as many weevils as possible out of the original 20. Weevils were removed from the plants using soft-tipped forceps and placed onto a moist paper towel. Weevils that were unable to make coordinated movements after 2 hours of observation at 25°C were recorded as dead. The percentage of dead weevils found was averaged for each treatment and analyzed using

one-way ANOVA with post-hoc Tukey tests carried out at $\alpha = 0.05$. Average water temperature over the 36-hour acute cold exposure period was analyzed for all four treatments using ANOVA.

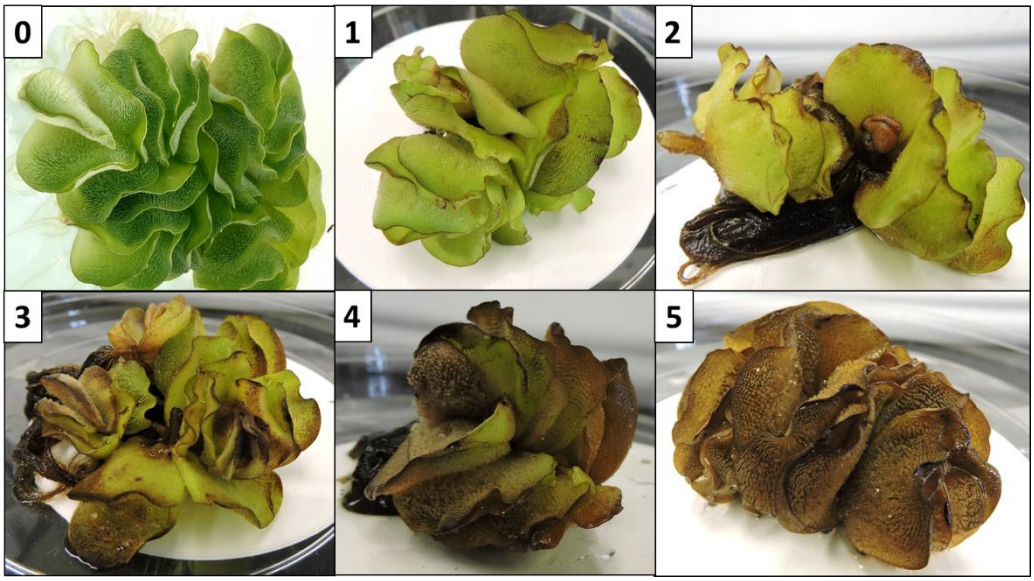

Figure 2.2. Qualitative scale used to assess giant salvinia damage following cold exposure. A rating of 0 indicates healthy plants showing no signs of browning; 1 indicates mostly green plants with less than 10% browning of leaf edges; 2 indicates mostly green plants with 11 to 30% browning of leaf edges and some discoloration of outer leaves; 3 indicates 31 to 50% leaf browning and loss of integrity (limpness) of leaf edges with partial browning of terminal bud; 4 indicates 51 to 99% leaf browning and loss of integrity in two or more leaves with partial browning of terminal bud; and 5 indicates dead plants with complete necrosis and loss of integrity in the leaves and buds.

To measure tissue damage that occurred during a less severe cold front (-3°C) under the same four treatments (light, medium, and heavy fabrics plus uncovered control), the experiment was replicated using weevil-free giant salvinia. Containers with plants and water were prepared and acclimated as previously described, without the addition of salvinia weevils, as previous experiments determined low rates of weevil mortality at this temperature (L. Moshman, unpublished data). Containers were randomly assigned to one of four treatments (n = 6) and placed in a growth chamber at -3°C for two successive exposures of 14 hours each, with three

days in between exposures. After each exposure, plants were returned to 25°C over a period of 12 hours and evaluated after 72 hours using the plant damage scale described above. Plant damage scores were analyzed using ANOVA with post-hoc Tukey tests carried out at $\alpha = 0.05$.

2.2.3. Effect of SBP fabrics on giant salvinia water temperature during acute cold exposure

To determine the effect of three row cover fabrics on giant salvinia root zone temperature under a simulated overnight cold exposure, a laboratory experiment was conducted in climate-controlled growth chambers. Translucent plastic containers (1.2 L, 16 cm x 16 cm x 9 cm) were filled with 950 mL of tap water and provisioned with 120 to 140 g (wet weight) of weevil-free, tertiary stage giant salvinia plants obtained from the Louisiana State University Research Greenhouse Facility in Baton Rouge, LA. A second, identical container holding 200 mL water served as a water bath to provide a layer of insulation around the sides and bottom. Containers were acclimated for 72 hours at 13°C in a growth chamber at 10:14h (L:D) to simulate winter conditions. After acclimation, containers were placed four at a time into a second growth chamber held at 0°C in total darkness. A type K thermocouple (REED Instruments, Wilmington, NC) was placed 4 cm beneath the water surface of each container.

For each trial, three containers were draped with a single-layer fabric treatment: light (19.0 g/m^2), medium (30.5 g/m^2), or heavy (49.5 g/m^2), and one remained uncovered as a control. Temperature curves were generated by logging water temperature every 5 minutes for a minimum of 14 hours. A HOBO Pendant® data logger was placed in each growth chamber and checked to ensure that the chambers maintained constant temperatures. At the conclusion of ten trials, temperature was averaged over the 14 hour exposure period. Temperature averages for each treatment were analyzed using one-way ANOVA ($\alpha = 0.05$).

2.2.4. Field assessment of heavy SBP fabric in outdoor ponds

To determine the effect of seasonal row cover application on giant salvinia plant quality and salvinia weevil overwintering, a field experiment was conducted in floating giant salvinia nurseries. Two ponds in north and south Louisiana were selected as field sites: Eddie D. Jones Park, Keithville, in Caddo Parish, LA (32.26°N, -93.93°W), and the LSU Reproductive Biology Center, Saint Gabriel, in Iberville Parish, LA (30.27°N, -91.10°W). Keithville (hereafter north LA) is located in USDA plant hardiness zone 8b, with average annual minimum winter temperature of -9.4 to -6.7°C. Saint Gabriel (hereafter south LA) is located in zone 9a, with average annual minimum winter temperature of -6.7 to -3.9°C (USDA PHZM 2012). These sites were selected to demonstrate how winter climatic differences may influence management strategies.

Ten floating nurseries were constructed at each site from 1.2-m diameter plastic wading pools (General Foam Plastics Corporation, Norfolk, VA) with bottoms removed to allow water and nutrient exchange in and out of the pools. Each pool was held afloat by two 1.4-m polystyrene pool noodles. Weevil-infested tertiary stage giant salvinia plants obtained from an outdoor pond were placed in the pools at a rate of 4.8 kg fresh weight per pool. Initial adult weevil density was determined by Berlese extraction of ten 0.5-kg samples from the original plant source. All nurseries were covered with a nylon organza (1-mm mesh) to prevent plant or weevil escape. Next, nurseries were divided into treatments of uncovered (control, mesh only) or covered (mesh plus fabric) in a completely randomized design (n = 5). Covered treatments were tightly covered with heavy row cover fabric (49.5 g/m^2) (AgFabric, Wellco Industries, Vista, CA) and secured with zip ties. In north LA, a boom was deployed to contain all ten floating nurseries to a sectioned area of an isolated pond (Figure 2.3a). In south LA, each

nursery was anchored in place using rope and cinder blocks (Figure 2.3b). Plants were sprayed monthly with the insecticide *Bt* var. *kurstaki* (Javelin, 0.3 grams/liter) to control feeding damage by *S. multiplicalis* (Wang et al. 2016).

Figure 2.3. (a) Setup of weevil nurseries inside boom in north LA (Keithville, 32.26°N, -93.93°W). (b) Weevil nurseries anchored with cinder blocks in south LA (Saint Gabriel, 30.27°N, -91.10°W).

Nurseries were established from November to December 2016 and were sampled once monthly from January through April 2017. Water quality was monitored monthly by measuring pH, electrical conductivity, and dissolved oxygen using handheld meters (HANNA® Instruments, Carrollton, TX), and water nitrate samples were analyzed in the laboratory using a nitrate ion electrode (Vernier Software & Technology, Beaverton, OR).

Each month, samples of 0.5 kg giant salvinia were collected from each pool and placed into Berlese funnels to extract salvinia weevil adults and larvae for determination of weevil population density. An additional five plants per nursery were selected haphazardly and observed for terminal bud feeding damage and number of adult weevils present. Feeding damage was determined by presence or absence of characteristic tissue scarring on the terminal bud of each plant. Entire plants were inspected for the presence of adult weevils and the total

number of weevils per five plants was counted. New plants were selected for each sampling event. On the final sampling date in April 2017, percent green was visually estimated to the nearest 10% for each replicate and plant tissue samples were sent to the LSU AgCenter Soil Testing and Plant Analysis Laboratory for carbon and nitrogen analysis. Adult and larval weevil density, number of damaged buds, and number of weevils observed per five plants were analyzed using a two-way ANOVA to compare the main effects of date and treatment, and the interaction effect of date*treatment on each dependent variable. Variables found to be significant at $\alpha < 0.05$ were further analyzed using a post-hoc LSMeans Student's *t*-test. Percent green and C:N ratio were analyzed after the final sampling event using a two-sample *t*-test.

Temperature data were recorded at 30 minute intervals at each site. Four HOBO Pendant® data loggers were deployed in the center of the plant mat at the water surface in four replicates from each treatment. One data logger was deployed at each site to record air temperature data. The four temperature curves generated for each treatment at each site were averaged and compared using a two-tailed *t*-test. The total number of exposures at or below 0°C, mean air temperature during these exposures, average duration of exposures, and cumulative duration of exposure were analyzed by filtering air temperature data in JMP® Pro 13.0.0.

2.3. Results

2.3.1. Greenhouse evaluation of three SBP fabrics on giant salvinia and salvinia weevil growth and development

A greenhouse experiment was conducted to evaluate the effects of SBP fabrics on giant salvinia quality and salvinia weevil survival and reproduction over a two-month period. Giant salvinia remained weevil-free under each of the fabric treatments plus uncovered control for the first four weeks, and twenty salvinia weevils per replicate were allowed to feed on plants during

the last four weeks. In Year 1, light (19.0 g/m^2), medium (30.5 g/m^2), and heavy (49.5 g/m^2) fabrics plus an uncovered control were evaluated. In Year 2, only heavy fabric and the uncovered control were evaluated.

In Year 1, ambient greenhouse temperature averaged 28.4°C with a minimum temperature of 20.7°C and maximum of 41.7°C. Water pH averaged 4.8 ± 0.0 and electrical conductivity averaged 46.1 ± 1.1 µS/cm (means ± SE). Despite weekly additions of RO water (pH 7 to 9), pH of the water in all buckets remained low as the result of the giant salvinia mat lowering the pH over time (Owens et al. 2005). A full-factorial effects test found no significant difference among treatments in plant biomass increase, SLA, leaf phenolic content, or C:N content. However, all parameters excluding biomass increase differed by date (Table 2.1). No significant difference was detected in adult weevil survival (14.1 ± 0.9 adults recovered), F1 larvae (1.7 ± 0.4 recovered), proportion of parous females (0.2 ± 0.0), or proportion of buds damaged (0.6 ± 0.0).

Table 2.1. Plant quality parameters (mean ± SE) for giant salvinia harvested before (first) and after (final) feeding by salvinia weevils (*Cyrtobagous salviniae*) in Year 1.

Parameter	**First harvest**[a]	**Final harvest**	***F***	**df**	***P***
Biomass increase (%)	60.0 ± 5.7	73.6 ± 6.3	2.3	1, 52	0.1377
SLA (cm^2/mg)	0.4 ± 0.0	0.3 ± 0.0	67.2	1, 232	<0.0001*
Total phenolics (nmoles/mg)	176.4 ± 7.9	326.2 ± 21.8	52.3	1, 32	<0.0001*
C:N	30.1 ± 1.0	27.5 ± 0.6	4.5	1, 32	0.0409*

[a]Values are averaged for all treatments: light (19.0 g/m^2), medium (30.5 g/m^2), and heavy (49.5 g/m^2) cover plus uncovered control.

Plant mat temperature under covered treatments was 0.7 to 1.3°C greater than the uncovered control (24.5 ± 2.6°C), with light and medium cover treatments having the highest average temperatures (25.7 and 25.8°C, respectively) over one month of data collection. Light intensity was 19% greater in the uncovered control (8671 ± 399 lux) than the heavy cover treatment (7268 ± 316 lux). Light and medium covered treatments were intermediate (8215 and 8531 lux, respectively) between the uncovered and heavy cover treatments (Table 2.2).

Table 2.2. Average water temperature and light intensity (mean ± SE) below various row cover fabrics covering giant salvinia, plus an uncovered control, in a greenhouse trial from January 13 through February 13, 2016 (Year 1).

Treatment[a]	**Temperature (°C)**[b]	**Light intensity (lux)**[c]
No cover	24.5 ± 2.6**c**	8671 ± 399**a**
Light	25.7 ± 3.2**a**	8215 ± 352**ab**
Medium	25.8 ± 3.1**a**	8531 ± 362**ab**
Heavy	25.2 ± 2.6**b**	7268 ± 316**b**

[a]Light, 19 g/m^2 spunbonded polypropylene fabric; medium, 30.5 g/m^2; heavy, 49.5 g/m^2.
[b]One-way ANOVA F = 67.3; df = 3, 5948; $P < 0.0001$.
[c]One-way ANOVA F = 3.1; df = 3, 5948; $P = 0.0254$.
Means with different letters within a column denote significant differences between treatments according to Tukey HSD at $\alpha < 0.05$.

In Year 2, ambient greenhouse temperature averaged 25.7°C with a minimum temperature of 20.7°C and maximum of 45.1°C. Water pH averaged 7.4 ± 0.0, electrical conductivity 517.8 ± 6.0 µS/cm, and dissolved oxygen 2.7 ± 0.2 mg/L. Dry weight biomass increase was 11% greater in the uncovered control (3% increase) than in the heavy fabric treatment (8% decrease) (F = 37.5; df = 1, 36; $P < 0.0001$). Specific leaf area and total phenolics did not differ between treatments, but did differ by date of harvest, as occurred in Year 1 (Table

2.3). The C:N ratio did not differ significantly in the first harvest (17.2 ± 0.2), but in the final harvest was 12% greater in the uncovered control (19.3 ± 0.3) than in the heavy fabric treatment (17.2 ± 0.1); this was apparent as a treatment*harvest date interaction (F = 19.4, df = 1, 16, P = 0.0004). Separate analyses of carbon and nitrogen revealed that the greater C:N ratio in the final harvest of uncovered plants was due to lower nitrogen content, as opposed to higher carbon content.

Table 2.3. Plant quality parameters (mean ± SE) for giant salvinia harvested before (first) and after (final) feeding by salvinia weevils (*Cyrtobagous salviniae*) in Year 2.

Parameter	**First harvest**	**Final harvest**	***F***	**df**	***P***
Biomass increase (%)					
No cover	-1.3 ± 1.5**a**	7.8 ± 1.4**a**	20.6	1, 36	<0.0001
Heavy cover	-12.1 ± 2.6**b**	-4.3 ± 1.7**b**			
SLA (cm^2/mg)					
No cover	0.6 ± 0.0	0.5 ± 0.0	30.9	1, 156	<0.0001
Heavy cover	0.6 ± 0.0	0.5 ± 0.0			
Total phenolics (nmoles/mg)					
No cover	98.5 ± 17.7	75.3 ± 24.3	5.1	1, 16	0.0381
Heavy cover	148.9 ± 22.6	72.4 ± 23.0			
C:N					
No cover	17.2 ± 0.3	19.3 ± 0.3**a**	20.6	1, 16	0.0003
Heavy cover	17.2 ± 0.2	17.2 ± 0.1**b**			

Means with different letters within a column denote significant differences between treatments according to LSMeans Student's *t*-test at $\alpha < 0.05$.

Adult salvinia weevil survival, number of F1 larvae recovered, and proportion of parous females did not differ between treatments in Year 2 (Table 2.4). Terminal bud damage was 20% greater in the heavy fabric treatment (90% damaged) than the uncovered control (70%) (t = -3.0, df = 18, P= 0.0073). As in Year 1, both temperature and light intensity differed significantly between treatments during a one-month period from November 6 through December 6, 2016 (Table 2.5). On average, the heavy cover treatment was 0.8°C warmer than the uncovered control (t = -8.3, df = 2880, $P < 0.0001$) and had 20% lower light intensity (t = 2.8, df = 2880, P = 0.0057).

Table 2.4. Recovery, reproduction, and feeding impact (mean ± SE) of salvinia weevils feeding on giant salvinia under uncovered or covered conditions in a greenhouse experiment (Year 2).

Parameter	**Uncovered**[a]	**Covered**	***t***	**df**	***P***
Adult survival (number recovered)	17.5 ± 0.8	18.0 ± 0.3	-0.6	18	0.5496
F1 larvae recovered	8.3 ± 1.6	7.6 ± 1.6	0.3	18	0.7615
Proportion parous females	0.7 ± 0.1	0.6 ± 0.1	0.6	18	0.5335
Proportion buds damaged	0.7 ± 0.1	0.9 ± 0.0	-3.0	18	0.0073*

[a]Uncovered, no fabric; covered, 49.5 g/m^2 spunbonded polypropylene fabric.

Table 2.5. Average temperature and light intensity (mean ± SE) below row cover fabric covering giant salvinia in a greenhouse trial from November 6 through December 6, 2016 (Year 2).

Treatment	**Temperature (°C)**	**Light intensity (lux)**
No cover	25.1 ± 0.1**b**	4912 ± 224**a**
Heavy cover	25.9 ± 0.1**a**	4084 ± 199**b**

Means with different letters within a column denote significant differences between treatments according to Tukey HSD at $\alpha < 0.05$.

2.3.2. Laboratory evaluation of three SBP fabrics on plant quality and weevil survival following acute cold exposure

Plastic containers filled with water and giant salvinia were covered with light (19.0 g/m^2), medium (30.5 g/m^2), and heavy (49.5 g/m^2) fabrics, plus an uncovered control, and exposed to simulated cold fronts at -7°C and -3°C for periods of up to 36 hours. Prior to the -7°C exposure, twenty adult salvinia weevils were added to each replicate. Three days after exposure, plants were evaluated for tissue damage using a qualitative scale and weevil mortality was determined.

Plants in the uncovered treatment suffered complete tissue mortality following the -7°C exposure (5.0 ± 0.0) and had a 67% higher damage rating than the 13°C control (3.0 ± 0.0). Damage in the uncovered treatment did not differ significantly from the three fabric treatments with an average rating of 4.1 ± 0.2 (F = 6.5; df = 4, 21; P = 0.0014; Figure 2.4a).

After a 14-hour exposure on fresh plants at -3°C, visible plant damage was 3.0- to 6.0-times greater in the uncovered control (3.0 ± 0.3) than the three covered treatments (average 0.8 ± 0.1) (F = 26.8; df = 3, 20; P < 0.0001). After a second 14-hour exposure occurring 72 hours later on the same plants, the uncovered control (4.0 ± 0.0) had 1.8-times greater damage than the light and medium cover treatments (2.2 ± 0.2) and 4.8-times greater damage than the heavy cover treatment (0.8 ± 0.2) (F = 29.6; df = 3, 20; P < 0.0001; Figure 2.4b).

After 36 hours at -7°C, the three row cover treatments combined resulted in 34% lower salvinia weevil mortality compared with the uncovered control (F = 4.8; df = 4, 21; P = 0.0066). Light and medium fabrics each had 36% lower mortality than the uncovered control (46.5 ± 12.7%) and equivalent mortality to the 13°C control (7.5 ± 3.6%). Mortality under heavy fabric (16.1 ± 6.0%) was intermediate between the uncovered and 13°C controls (Figure 2.5). Water surface temperature was 3.3 to 3.5°C warmer under fabric covers (1.1 ± 0.2°C) than in uncovered controls (-2.3 ± 0.2°C) (F = 26.4; df = 3, 576; P <0.0001).

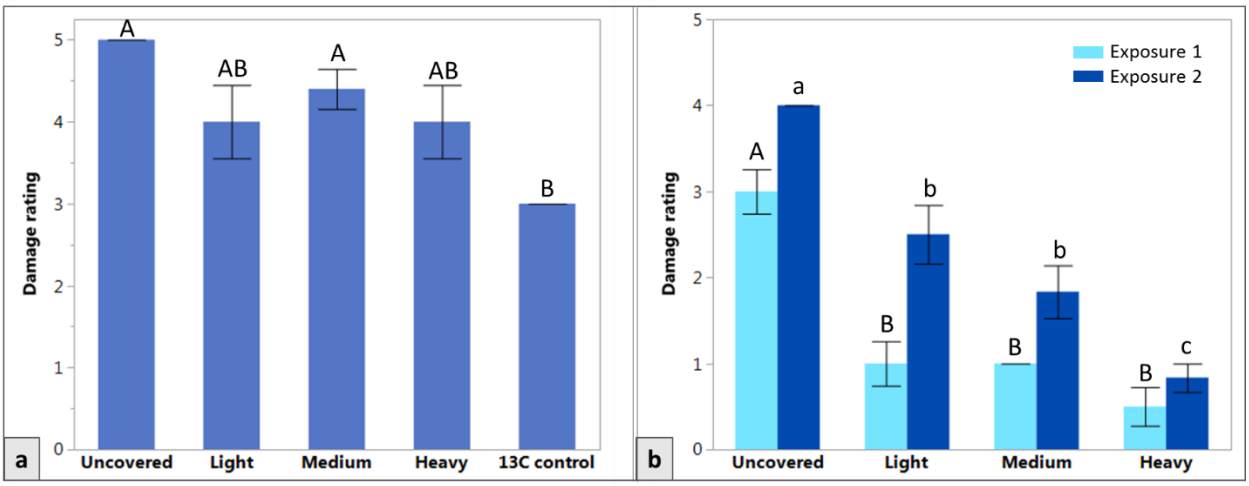

Figure 2.4. Plant damage rating three days after (a) a 36-hour exposure at -7°C and (b) two 14-hour exposures at -3°C for plants that were uncovered or covered with light, medium, and heavy fabrics. Ratings of 0 indicate healthy plants and ratings of 5 indicate complete necrosis and loss of leaf integrity. Bars with different letters are statistically significant according to Tukey HSD at $\alpha < 0.05$.

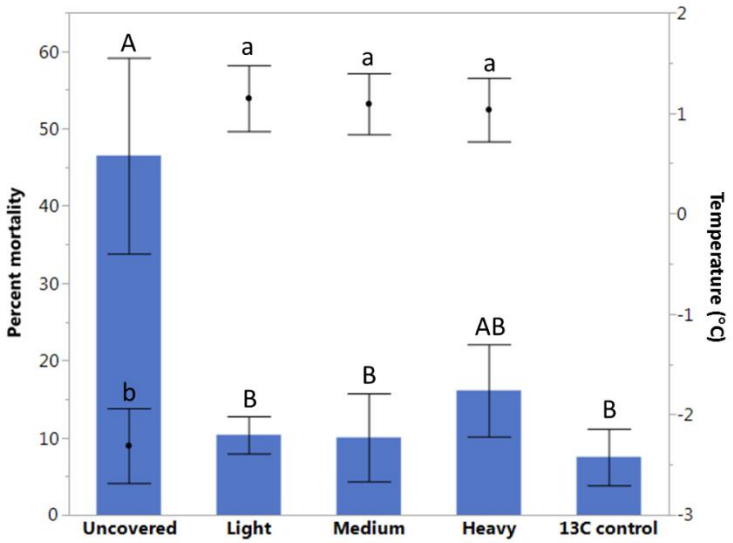

Figure 2.5. Adult salvinia weevil mortality (blue bars) and water surface temperature (black dots) for uncovered plants or plants covered with light, medium, or heavy fabrics after a 36-hour exposure at -7°C, plus uncovered plants that did not experience a cold exposure (13°C control). Bars with different letters within a given parameter (mortality or temperature) are statistically significant according to Tukey HSD at $\alpha < 0.05$.

2.3.3. Effect of SBP fabrics on giant salvinia water temperature during acute cold exposure

Plastic containers filled with water and giant salvinia were covered with light (19.0 g/m^2), medium (30.5 g/m^2), and heavy (49.5 g/m^2) fabrics, plus an uncovered control, and were exposed to simulated cold fronts at 0°C for periods of 14 hours in a laboratory growth chamber. Thermocouples placed 4 cm below the water surface recorded temperature every five minutes. The effect of the fabric treatments on water column temperature was small, and average water temperature did not differ significantly between the three fabric cover treatments and the uncovered control (3.7 ± 0.1°C, mean ± SE; F = 2.4; df = 3, 672; P = 0.0627).

2.3.4. Field assessment of heavy SBP fabric in outdoor ponds

Floating nurseries were established in outdoor ponds in north and south Louisiana from November through December 2016. At each pond site, nurseries were filled with weevil-infested giant salvinia and covered with either 1-mm nylon mesh (control) or heavy (49.5 g/m^2) fabric plus nylon mesh (n = 5). Each nursery was sampled monthly from January through April 2017 to determine changes in weevil population density and plant quality over time.

In north LA, initial salvinia weevil density in December was 54.9 ± 5.7 adults/kg (mean ± SE) and 3.0 ± 1.7 larvae/kg giant salvinia. In south LA, initial densities in November were 205.5 ± 11.6 adults/kg and 48.0 ± 8.5 larvae/kg giant salvinia. Average water quality parameters for each site are reported in Table A.1. In north LA, adult and larval weevil population density differed significantly by date but not by treatment (adult density, F = 18.4; df = 3, 32; P < 0.0001; larval density, F = 23.9; df = 3, 32; P < 0.0001). From the time of placement until the final sampling in April, average adult weevil density decreased by 91% and larval weevil density increased by 494% (Figure 2.6a). The number of damaged buds was 1.7-times greater under the

heavy cover treatment (2.0 ± 0.2, out of 5) than the uncovered control (1.2 ± 0.2) (F = 7.5; df = 1, 40; P = 0.0093). The number of weevils observed per five plants differed by date, but not by treatment, with the most weevils (0-2 weevils observed per five plants) occurring in January and February (F = 7.0; df = 4, 40; P = 0.0002). Percent green was 36% greater in covered plants (86 ± 2.4%) than uncovered plants (50 ± 3.2%) (t = -9.0, df = 8, P < 0.0001). The C:N ratio was 24% greater in covered plants (27.1 ± 0.6) than uncovered plants (21.8 ± 0.8) (t = -5.3, df = 8, P = 0.0007).

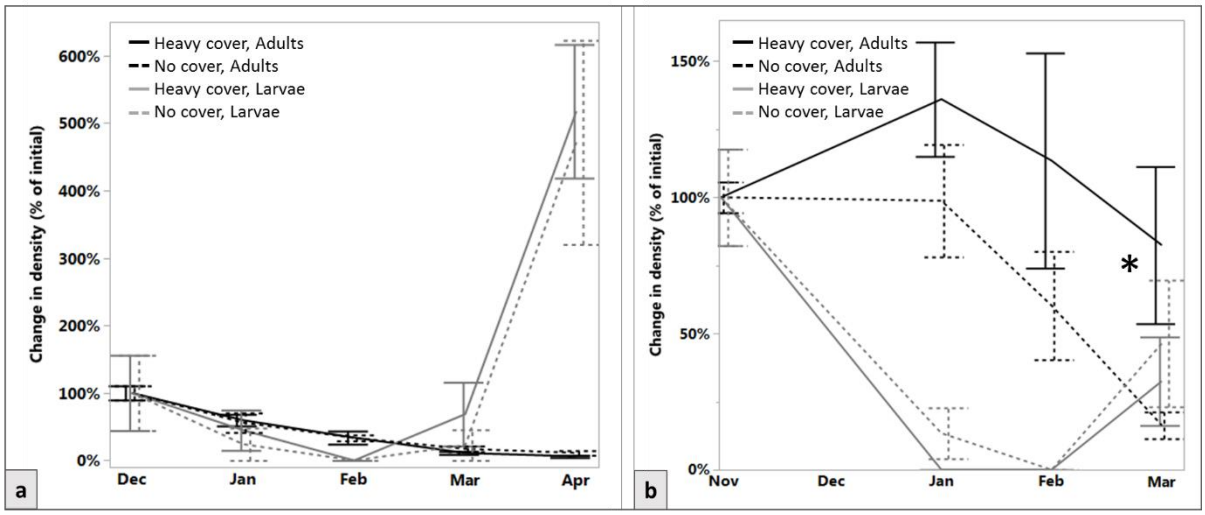

Figure 2.6. Change in adult (black) and larval (grey) salvinia weevil population density of uncovered and covered giant salvinia mats in (a) north LA and (b) south LA. Asterisk indicates significant difference between treatments according to two-way ANOVA at α < 0.05.

In south LA, adult and larval weevil population density differed significantly by date (adult density, F = 5.0; df = 2, 23; P = 0.0155; larval density, F = 5.6; df = 2, 23; P = 0.0106), with only adult density differing by treatment (F = 11.0; df = 1, 23; P = 0.0030). Monthly sampling was affected by flooding events that occurred in the pond from late March to April and April data could not be collected. At the last sampling in March, adult weevil population density

was five times greater under heavy cover (169.4 ± 59.2 weevils/kg, mean ± SE) than in the uncovered control (33.6 ± 10.1 weevils/kg), marking an 18% decrease from the initial population in covered nurseries compared to an 84% decrease in the control. Average larval population density decreased by 61% from November to March in both treatments combined (Figure 2.6b). The number of damaged buds differed by date, but not by treatment, with the greatest number of terminal buds damaged (4 out of 5) in January (F = 15.5; df = 4, 40; $P < 0.0001$). The high damage activity observed in January was likely due to the much larger adult weevil population present in this month, which declined over time to result in less new damage observed as sampling progressed. Nearly twice as many adult weevils per five plants were observed in covered nurseries (2.5 ± 0.8) as in uncovered nurseries (1.4 ± 0.6) over the sampling period (F = 4.6; df = 1, 40; P = 0.0389). Due to the flooding disturbance that occurred at this site, final estimates of percent green and C:N content were not attainable.

Table 2.7. Air temperature analysis from north and south LA outdoor ponds. Data were filtered to show exposure temperature and duration during times when air temperature ≤ 0°C.

Parameter	**North LA**	**South LA**
Air max. (°C)	30.4	30.3
Air min. (°C)	-9.1	-5.1
Average air (°C)	14.1 ± 0.1	16.3 ± 0.1
# times ≤ 0°C	11	6
Average exposure temp. (°C)	-3.2 ± 0.2	-2.1 ± 0.2
Average exposure duration (hours)	8.8 ± 1.9	5.5 ± 2.4
Cumulative duration (hours)	97	33

In north LA, water temperature in the center of the plant mat was 0.3°C greater in covered nurseries (16.9 ± 0.1°C) than uncovered nurseries (16.6 ± 0.1°C), with the greatest differences occurring in March and April (t = -3.5, df = 12478, P = 0.0005). In south LA, water temperature did not differ between the covered and uncovered treatments (mean temperature 18.2 ± 0.1°C; t = -0.2, df = 12480, P = 0.8301). Air temperature analysis indicated 11 discrete periods of cold exposure below 0°C in north LA, versus 6 discrete periods in south LA. The total duration below 0°C was 97 hours in north LA, versus only 33 hours in south LA (Table 2.7).

2.4. Discussion

2.4.1. Giant salvinia plant quality and mat surface temperature under SBP fabrics

The effect of SBP on plant carbon and nitrogen differed depending on seasonal conditions. In the warm-weather greenhouse study, heavy SBP covers reduced giant salvinia biomass and C:N ratio compared with uncovered controls. In north LA, SBP covers increased C:N ratio compared to the control. Increased C:N ratio in water hyacinth (*E. crassipes*) was associated with increased plant biomass and decreased C:N ratio was associated with faster decomposition following winter frost damage (Center and Dray 2010). In giant salvinia, uncovered plants in the greenhouse study may have experienced increased C:N as an effect of greater biomass increase compared to the covered treatment. Likewise, uncovered plants in north LA may have experienced reduced C:N as a function of nitrogen release following decomposition of frost-damaged tissue in the early spring. Covering plants with heavy fabric significantly reduced light penetration, and may have contributed to increased bud damage and reduced plant biomass observed under this treatment.

Plant damage assessments provided strong evidence that SBP fabrics reduce tissue damage to giant salvinia during winter cold fronts. As has been reported in the terrestrial systems of strawberries (Hochmuth et al. 1986), cranberries (Patten and Wang 1983), and other fruit and vegetable crops (Olle and Bender 2010), SBP can be a useful tool in aquatic systems to prevent and reduce plant damage during cold exposures.

Thermal properties of SBP may be less predictable in aquatic environments than in terrestrial environments due to differences in the way water conducts heat compared to soil. In greenhouse and laboratory studies, SBP fabrics raised plant mat surface temperatures by 0.7 to 3.5°C due to direct insulation of the mat to prevent heat dissipation. A greater temperature increase was achieved in controlled laboratory settings that were minimally disturbed by moving air currents, which could influence rates of heat loss from the plant-water interface. In the field, SBP increased mat temperatures by only 0.3°C, likely a result of combined external factors such as wind speed, water flow, and thermal inertia of the water body. These observations are in agreement with findings by Dale and Gillespie (1977) and Kumar and Arakeri (2015) that demonstrated slow-moving water trends toward increased stratification and greater surface heat retention than water that is agitated by wind or mixing of strata. The SBP did not increase mat temperature in south LA, which may be due to greater water movement and mixing at this site (L. Moshman, personal observation). In aquatic environments, SBP should therefore produce the most benefit in slow-moving water, such as non-fluvial systems that are protected from wind. This complements observations by Tipping and Center (2003) and Sullivan et al. (2011) that giant salvinia and salvinia weevils overwinter best in backwater areas with minimal current and protection from the elements.

2.4.2. Salvinia weevil mortality is reduced by SBP row covers during acute cold exposure

In a laboratory trial, SBP covers reduced weevil mortality by 36% compared to an uncovered control during a single 36-hour cold exposure. In the field, the south LA pond experienced a cumulative exposure of 33 hours below 0°C, similar to what was tested in the laboratory, whereas the north LA pond experienced a cumulative exposure of 97 hours, nearly three times as long. Moreover, average below-freezing exposures in north LA were colder and longer in duration than those experienced in south LA. Heavy SBP cover reduced adult weevil mortality by 66% in south LA, but winter conditions in north LA were likely too severe for the cover to make a notable difference in adult weevil survival.

It is uncertain whether SBP row cover would prevent total loss of salvinia weevil populations during a severe winter in north LA. The winter of 2016-2017 was considered a mild winter and despite high adult mortality in north LA, the salvinia weevil population was able to rebound and produce large numbers of F1 larvae. A greater number of repeated cold exposure cycles and longer cumulative duration of exposures during a severe winter could lead to higher mortality rates and reduce the likelihood of overwintering, as predicted by Obeysekara et al. (2015).

2.4.3. Comparison of SBP with other overwintering methods

Managing water temperature during winter is advantageous for protecting aquatic organisms from cold fronts and increasing growth rates in early spring (Nachtrieb 2013, Putegnat 2013). Maintenance of natural and artificial refugia for overwintering biocontrol agents can increase re-establishment rates following winter losses and can result in faster control of target species (Manning 1979). During the early establishment of water hyacinth weevils in Louisiana,

natural refuge colonies were maintained in warmer areas of the state to stock weevil populations that were lost during severe winters (Manning 1979). Alligatorweed flea beetles were likewise imported from southern overwintering sites to replace annual losses in colder areas (Buckingham 2002). Creation of artificial refugia in cold climates can reduce the need for transportation of agents and aid overwinter population establishment. Whereas high-precision, electronically controlled geothermal systems can be costly to operate (Hall et al. 2002), SBP fabrics are cost-effective and offer similar advantages to greenhouse films as solar-passive thermal refugia (Putegnat 2013).

2.4.4. SBP practical uses and recommendations for aquatic applications

In the current studies, SBP did not negatively affect giant salvinia or salvinia weevils with seasonal use up to four months. However, there are certain limits to SBP's practicality in the field. Water splashing or debris from overhead vegetation can obscure covers and reduce light transmittance, which may negatively affect plant quality over time. Spunbonded polypropylene requires a support structure to prevent sagging and wind disturbance. Covering a large water body could negatively affect organisms that rely on open water for food and habitat, and pose a risk of entanglement for fish, birds, humans, and other wildlife. Constructing a portable "floating greenhouse" (Putnegat 2013) may be a good approach for anchoring and supporting SBP for extended field use.

Refugia constructed from SBP can be easily adapted to mass-rearing operations for biological control agents in aquatic environments. Where agents are threatened by winter cold fronts, SBP covers can be placed immediately before a predicted cold front and removed when temperatures warm. Use of mobile support frames facilitates rapid deployment of SBP with minimal labor required. A single layer of SBP may not produce a sufficient temperature increase

to enhance salvinia weevil overwintering during severe winters in north LA, which have historically resulted in near-100% mortality (Obeysekara et al. 2015). Future studies should evaluate two or more layers of SBP during severe winters in north LA. Natural materials such as pine straw mulch may likewise provide cold-weather protection for salvinia weevils (Micinski 2014) and would be good candidates for continued future evaluation.

2.5. References

Bale, J. S. 2005. Effects of temperature on the establishment of non-native biocontrol agents: the predictive power of laboratory data, pp. 593-602. *In* M. S. Hoddle (ed.), Second International Symposium on Biological Control of Arthropods (ISBCA), vol. II. Publication FHTET-2005-08. USDA Forest Service, Morgantown, WV.

Boland, N. P., and P. M. Room. 1983. Estimating population density of a *Cyrtobagous* sp. (Coleoptera: Curculionidae) on the floating weed salvinia using Berlese funnels. J. Aust. Entomol. Soc. 22: 353-354.

Buckingham, G. R. 2002. Alligatorweed, pp. 5-15. *In* R. Van Driesche, B. Blossey, M. Hoddle, S. Lyon, R. Reardon (eds.), Biological control of invasive plants in the eastern United States. USDA Forest Service Publ. FHTET-2002-04, Forest Health Technology Enterprise Team, Morgantown, WV.

Calder, A. A., and D.P.A. Sands. 1985. A new Brazilian *Cyrtobagous* Hustache (Coleoptera: Curculionidae) introduced into Australia to control *Salvinia*. Journal of the Australian Entomological Society 24: 57-64.

Center, T. D., and F. A. Dray, Jr. 2010. Bottom-up control of water hyacinth weevil populations: do the plants regulate the insects? J. Appl. Ecol. 47: 329-337.

Cooke, C. D. 1980. Covering bottom sediments as a lake restoration technique. Water Resour. Bul. 16: 921-926.

Dale, H. M., and T. Gillespie. 1976. The influence of floating vascular plants on the diurnal fluctuations of temperature near the water surface in early spring. Hydrobiologia 49: 245-256.

Dale, H. M., and T. Gillespie. 1977. Diurnal fluctuations of temperature near the bottom of shallow water bodies as affected by solar radiation, bottom color and water circulation. Hydrobiologia 55: 87-92.

Diaz, R., C. Aguirre, G. S. Wheeler, S. L. Lapointe, E. Rosskopf, and W. A. Overholt. 2011. Differential performance of tropical soda apple and its biological control agent *Gratiana boliviana* (Coleoptera: Chrysomelidae) in open and shaded habitats. Environ. Entomol. 40: 1437-1447.

EDDMapS. 2017. Early detection & distribution mapping system. The University of Georgia - Center for Invasive Species and Ecosystem Health. Available online at http://www.eddmaps.org/; last accessed September 3, 2017.

Eisenberg, L. J. 2011. The reproductive morphology and physiological age-grading of female *Cyrtobagous salviniae*, the salvinia weevil. M.S. Thesis 4299, Louisiana State University, Baton Rouge. http://digitalcommons.lsu.edu/gradschool_theses/4299.

Eisenberg, L. J., and S. J. Johnson. 2012. Rearing the salvinia weevil in the greenhouse, pp. 37-46. *In* A. Knutson and J. Nachtrieb (eds.), A guide to mass rearing the salvinia weevil for biological control of giant salvinia. Texas A&M AgriLife Extension Service, Special Publication ESP-475. http://cise.tamu.edu/media/355740/gs_manual_10-15-12.pdf.

Forno, I. W., and K.L.S. Harley. 1979. The occurrence of *Salvinia molesta* in Brazil. Aquat. Bot. 6: 185-187.

Forno, I. W., D.P.A. Sands, and W. Sexton. 1983. Distribution, biology and host specificity of *Cyrtobagous singularis* Hustache (Coleoptera: Curculionidae) for the biological control of *Salvinia molesta*. Bull. Entomol. Res. 73: 85-95.

Galam, D., J. Silva, D. Sanders, and J. H. Oard. 2015. Morphological and genetic survey of giant salvinia populations in Louisiana and Texas. Aquat. Bot. 127: 20-25.

Grevstad, F. S., and L. B. Coop. 2015. The consequences of photoperiodism for organisms in new climates. Ecol. Appl. 25: 1506-1517.

Growers Supply. 2014. Hydroponic systems, greenhouses & high tunnels, gardening & nursery supplies. Dyersville, IA. www.growerssupply.com/. (Accessed 5 September 2017).

Hall, S. G., J. Finney, R. P. Lang, and T. R. Tiersch. 2002. Design and development of a geothermal temperature control system for broodstock management of channel catfish *Ictalurus punctatus*. Aquacult. Eng. 26: 277-289.

Hennecke, B., and L. A. Postle. 2006. The key to success: an investigation into oviposition of the salvinia weevil in cool climate regions, pp. 780-783. *In* C. Preston, J. H. Watts, and N. D. Crossman (eds.), Proceedings of the 15th Australia Weeds Conference. Weed Management Society of South Australia, Adelaide.

Hochmuth, G. J., S. R. Kostewicz, S. J. Locascio, E. E. Albreghts, C. M. Howard, and C. D. Stanley. 1986. Freeze protection of strawberries with floating row covers. Proc. Fla. State Hortic. Soc. 99: 307-311.

Hofstra, D. E., and J. S. Clayton. 2012. Assessment of benthic barrier products for submerged aquatic weed control. J. Aquat. Plant Manage. 50: 101-105.

Ireland, P. A., A. Knutson, and L. Gregory. 2012. Rearing the salvinia weevil in outdoor tanks at Caddo Lake, Texas, pp. 25-36. *In* A. Knutson and J. Nachtrieb (eds.), A guide to mass rearing the salvinia weevil for biological control of giant salvinia. Texas A&M AgriLife Extension Service, Special Publication ESP-475. http://cise.tamu.edu/media/355740/gs_manual_10-15-12.pdf.

Jacono, C. C., T. R. Davern, and T. D. Center. 2001. The adventive status of *Salvinia minima* and *S. molesta* in the southern United States and the related distribution of the weevil *Cyrtobagous salviniae*. Castanea 66: 214-226.

Kumar, N., and J. H. Arakeri. 2015. Natural Convection Driven Evaporation from a water surface. Procedia IUTAM 15: 108-115.

Manning, J. H. 1979. Establishment of waterhyacinth weevil populations in Louisiana. J. Aquat. Plant. Manage. 17: 39-41.

McClay, A. S. 1996. Biological control in a cold climate: temperature responses and climatic adaptation of weed biocontrol agents, pp. 377-383. *In* V. C. Moran and J. H. Hoffman (eds.), Proceedings of the IX international symposium on biological control of weeds, 19-26 January 1996. Stellenbosch, South Africa, University of Cape Town.

McFarland, D. G., L. S. Nelson, M. J. Grodowitz, R. M. Smart, and C. S. Owens. 2004. *Salvinia molesta* D. S. Mitchell (giant salvinia) in the United States: a review of species ecology and approaches to management. Environmental Lab. SR-04-2. US Army Corps of Engineers, Engineer Research and Development Center, Vicksburg, MS.

Micinski, S. 2014. S-1058 annual report for 2014 and plans for 2015, pp. 23-26. *In* Minutes, S1058 Biological Control of Arthropod and Weed Pests in the Southern United States. Southeastern Branch Meeting of the Entomological Society of America, 15 March 2015, Biloxi, MS.

Miller, I. L., and C. G. Wilson. 1989. Management of salvinia in the Northern Territory. J. Aquat. Plant Manage. 27: 40-46.

Mukherjee, A., A. Knutson, D. A. Hahn, and K. M. Heinz. 2014. Biological control of giant salvinia (*Salvinia molesta*) in a temperate region: cold tolerance and low temperature oviposition of *Cyrtobagous salviniae*. BioControl 59: 781-790.

Nachtrieb, J. G. 2013. Mass-rearing *Cyrtobagous salviniae* for biological control of giant salvinia: field release implications. J. Aquat. Plant Manage. 51: 122-126.

Obeysekara, P. T., A. Knutson, A. Mukherjee, and K. M. Heinz. 2015. Repeated cold exposure effects on mortality and feeding activity of the salvinia weevil, *Cyrtobagous salviniae* (Coleoptera: Curculionidae). Environ. Entomol. 44: 1590-1598.

Olle, M., and I. Bender. 2010. The effect of non-woven fleece on the yield and production characteristics of vegetables. Agraarteadus 1: 24-29.

Owens, C. S., R. M. Smart, D. R. Honnell, and G. O. Dick. 2005. Effects of pH on growth of *Salvinia molesta* Mitchell. J. Aquat. Plant Manage. 43: 34-38.

Parys, K. A., and S. J. Johnson. 2013. Biological control of common salvinia (*Salvinia minima*) in Louisiana using *Cyrtobagous salviniae* (Coleoptera: Curculionidae). Fla. Entomol. 96: 10-18.

Patten, K. D., and J. Wang. 1993. Spunbonded polypropylene fabric covers can enhance cranberry establishment and production. HortScience 28: 1202.

Penman, H. L. 1948. Natural evaporation from open water, bare soil and grass. Proc. R. Soc. A 193: 120-145.

Putegnat, J. W. 2013. Design and field-test of solar-passive thermal refugia for overwintering Mozambique tilapia (*Oreochromis mossambicus*) in central Texas. M.S. Thesis, Texas A&M University, College Station.

Room, P. M., K.L.S. Harley, I. W. Forno, and D.P.A. Sands. 1981. Successful biological control of the floating weed salvinia. Nature 294: 78-80.

Room, P.M., M. H. Julien, and I. W. Forno. 1989. Vigorous plants suffer most from herbivores: latitude, nitrogen and biological control of the weed *Salvinia molesta*. Oikos 54: 92-100.

Russell, A., S. Johnson, X. Cibils, F. McKay, L. Moshman, P. Madeira, Z. Blair, and R. Diaz. 2017. Surveys in Uruguay and Argentina reveal *Cyrtobagous salviniae* (Coleoptera: Curculionidae) populations adapted to survive temperate climates in southeastern USA. Biological Control 107: 41-49.

Sanders, D., W. Lorio, and K. Whitehead. 2012. Rearing the salvinia weevil in ponds, pp. 47-51. *In* A. Knutson and J. Nachtrieb (eds.), A guide to mass rearing the salvinia weevil for biological control of giant salvinia. Texas A&M AgriLife Extension Service, Special Publication ESP-475. http://cise.tamu.edu/media/355740/gs_manual_10-15-12.pdf

Stout, M. J., R. A. Brovont, and S. S. Duffey. 1998. Effect of nitrogen availability on expression of constitutive and inducible chemical defenses in tomato, *Lycopersicon esculentum*. J. Chem. Ecol. 24: 945-963.

Sullivan, P. R., L. A. Postle, and M. Julien. 2011. Biological control of *Salvinia molesta* by *Cyrtobagous salviniae* in temperate Australia. Biol. Control 57: 222-228.

Thomas, P. A., and P. M. Room. 1986. Taxonomy and control of *Salvinia molesta*. Nature 320: 581-584.

Tipping, P. W., and T. D. Center. 2003. *Cyrtobagous salviniae* (Coleoptera: Curculionidae) successfully overwinters in Texas and Louisiana. Fla. Entomol. 86: 92-93.

USDA PHZM, 2012. Agricultural Research Service, U.S. Department of Agriculture. http://planthardiness.ars.usda.gov. (Accessed 6 October 2017).

Wang, Y., S. Brown, and R. Diaz. 2016. Water lettuce moth. LSU AgCenter Pub. 3538. Louisiana State University Agricultural Center, Baton Rouge, LA.

Waterman, P. G., and S. Mole. 1994. *Analysis of Phenolic Plant Metabolites*. Blackwell Scientific Publications, Oxford, England.

Wells, O. S., and J. B. Loy. 1993. Rowcovers and high tunnels enhance crop production in the northeastern United States. Horttechnology 3: 92-95.

Chapter 3: Assessment of Plant Density Manipulation for Winter Management of Giant Salvinia and Salvinia Weevils

3.1. Introduction

Biological control agents of weeds in temperate climates can be adversely affected by incongruities between the native and adventive ranges, particularly when agents originate from tropical to semitropical climates (Bale 2005). Stressors to introduced agents, including extreme heat, cold, or change in photoperiod, could lead to undesirable consequences such as slow growth and reduced fecundity (McClay 1996, Grevstad and Coop 2015). In aquatic systems, agents are furthermore susceptible to flooding events that may displace introduced populations, reducing their ability to establish (Schooler et al. 2011). Several classical biological control programs in the United States have been impacted by failure of an agent to establish in certain regions due to climatic limitations. These include control of alligatorweed (*Alternanthera philoxeroides* (Mart.) Griseb.) by the alligatorweed flea beetle *Agasicles hygrophila* Selman and Vogt. (Coleoptera: Chrysomelidae) (Buckingham 2002), and control of water hyacinth (*Eichornia crassipes* (Mart.) Solms) by the water hyacinth weevils *Neochetina eichorniae* Warner and *N. bruchi* Hustache (Coleoptera: Curculionidae) (Manning 1979). In some cases, agents can adapt to new ranges and increase their population over time (McClay 1996). In other cases, interventions such as mass rearing, annual releases, and seasonal management strategies may be required (Manning 1979, Nachtrieb 2013, Parys and Johnson 2013).

Giant salvinia (*Salvinia molesta* Mitchell), a floating fern native to southeastern Brazil, is considered an invasive species in over 21 countries worldwide (Room et al. 1981, Calder and Sands 1985, Jacono et al. 2001). In the United States, giant salvinia has been reported in 14 states, Guam, Puerto Rico, and the Virgin Islands within the past quarter century (Galam et al.

2015, EDDMapS 2017). Vegetative mats can double in size in as little as 36 hours under favorable conditions (Johnson et al. 2010). Persistent mats can negatively affect water quality, reduce native species diversity, and interfere with recreational or agricultural activities (McFarland et al. 2004). Giant salvinia management has been achieved through mechanical, chemical, and biological control (Miller and Wilson 1989).

The salvinia weevil, *Cyrtobagous salviniae* Calder and Sands (Coleoptera: Curculionidae), is a biological control agent of giant salvinia that can achieve control in as little as three months, up to a year or more in temperate climates (Room et al. 1981, Tipping et al. 2008). Salvinia weevils are less expensive than chemical or mechanical control measures and exert greater long-term control when utilized under favorable conditions (Sullivan et al. 2011). Salvinia weevils only feed on plants in the genus *Salvinia* and can develop from egg to adult in 42 to 68 days at 25.5°C (Forno et al. 1983). Adults can actively feed between 13 to 33°C, whereas larval development is limited to the smaller range of 17 to 31°C (Forno et al. 1983, Sands et al. 1983). Adult weevils feed on young nitrogen-rich buds and lay eggs in small holes or crevices (Forno et al. 1983). Young larvae feed on external tissues whereas older instars tunnel inside the rhizome, causing necrosis and eventual sinking of plant mats (Forno et al. 1983).

Failure of salvinia weevils to establish in temperate climates has been previously attributed to cold winter temperatures and declining host plant quality (Mukherjee et al. 2014, Obeysekara et al. 2015). As documented in biocontrol programs using the water hyacinth weevils *N. eichorniae* and *N. bruchi* (Manning 1979), winter losses of salvinia weevils can be compensated for by releasing weevils from warmer "refuge" areas to repopulate plant mats (Sullivan et al. 2011). In the United States, mass rearing of salvinia weevils in outdoor ponds is

possible year-round in southernmost regions (below 32°N latitude), and is achieved in northern regions with the use of winter cold frames (Nachtrieb 2013). Severe winters can decimate overwintering weevil populations; however, overwinter establishment has been reported as far north as the Toledo Bend Reservoir (32°N), where cold fronts can reach as low as -9.4°C (Tipping and Center 2003, USDA PHZM 2012).

Enhancement of overwintering microhabitat for salvinia weevils can benefit giant salvinia biological control programs in temperate climates. Alternative methods of heating aquatic systems have been previously explored in outdoor fish ponds; however, precise temperature control is energy-demanding and can cost as much as $13.80 per day to operate (Hall et al. 2002). Solar-passive thermal refugia have been explored as cost-effective means of raising pond temperature, but may be limited to small managed areas and can vary in effectiveness depending on construction materials and external environmental conditions (Putegnat 2013). Increasing plant density to raise mat temperatures is a potentially useful management approach that has not been previously evaluated in aquatic weed biological control programs. This method is of interest to land managers who already utilize containment devices to prevent spreading of local giant salvinia infestations.

The effect of floating aquatic plants on mat and water temperature has been studied in eutrophic ditches (Driever et al. 2005) and natural aquatic environments (Dale and Gillespie 1976, Room and Kerr 1983). Growth rate and mat temperature of common duckweed (*Lemna minor* L.) growing in eutrophic ditches were found to increase as plant mats approached full surface coverage (Driever et al. 2005). Giant salvinia mats in open water were consistently warmer than surrounding air during both winter and summer, attributed to the air spaces within plant tissue that form an insulating layer to reduce heat loss at the water surface (Room and Kerr

1983). Compared to open water, floating aquatic plants reflect more solar radiation, increase water surface temperature, and increase temperature stratification in the water column (Dale and Gillespie 1976).

Crowding plants with containment booms is a common practice in salvinia weevil rearing ponds to promote mat-forming tertiary growth (Wahl et al. 2016). In Lake Kariba, Australia, tertiary growth-stage giant salvinia plants grew 1.6-times faster than secondary stage plants (Mitchell and Tur 1975). In nitrogen-limited water, self-crowding of giant salvinia results in reduced relative growth rate (Room and Thomas 1986). However, rearing ponds that supply sufficient nitrogen fertilizer may not experience such growth limitations. Salvinia weevils prefer tertiary growth for feeding and oviposition due to greater plant size and ability for larvae to tunnel within rhizomes (Tipping and Center 2005). Dense floating plant mats create warmer microhabitats (Dale and Gillespie 1976) and could buffer against adverse environmental conditions for overwintering salvinia weevils. In addition to conditions experienced at the water surface, root zone conditions are important for moderating biological processes and root zone temperatures are good indicators of water column stratification (Room and Kerr 1983).

An advantage of plant crowding as a temperature management strategy is that it can be adapted to naturally occurring infestations as well as intensively managed mass-rearing operations. The goal of this research was to explore the viability of increasing plant density to increase overwinter survival and establishment of salvinia weevils in a floating plant mat. I hypothesized that high plant density would increase mat temperatures by reducing heat loss through open water spaces, increase water column stratification, and provide greater thermal insulation below the plant mat. If sufficient temperature increases occurred, this could result in

reduced salvinia weevil mortality and increased overwinter establishment of salvinia weevil populations.

The specific objectives of this research were: (1) to determine how giant salvinia mat and water column temperature are affected by plant density, and (2) to evaluate how plant density affects overwinter survival and establishment of salvinia weevil populations.

3.2. Materials and Methods

3.2.1. Evaluation of plant density effects on air and water column temperatures

To determine the effect of giant salvinia plant density on air and water temperatures, an outdoor mesocosm experiment was conducted from August to September 2016 and during three cold fronts occurring in November 2016, December 2016, and January 2017 (US Climate Data 2017). Floating 0.10 m^2 polystyrene rings (Swimways Corporation, Virginia Beach, VA) were placed into white 1300 L tanks held outdoors at the Louisiana State University Research Greenhouse Facility in Baton Rouge, LA. Tanks were filled with 900 L reverse osmosis water and fertilized to 5 mg/L nitrogen using Miracle-Gro® Water Soluble Lawn Food (36-0-6, The Scotts Company LLC, Marysville, OH). An additional 30 L of water from a greenhouse giant salvinia colony was added to each tank at trial establishment to provide micronutrients and pH buffering. The bottom of each ring was fitted with black, 1.9-cm mesh polypropylene netting to allow water and nutrient exchange and prevent plant loss from the rings. Each ring was tethered with rope to a corner of the tank to limit movement. Giant salvinia was added to rings at one of three treatment densities: low (3.5 kg/m^2), medium (7.0 kg/m^2), and high (10.5 kg/m^2) (n = 8), in a completely randomized design. These three densities were determined from natural giant

salvinia infestations, where medium density represents the average plant density found in tertiary stage mats in midsummer in Louisiana (L. Moshman, unpublished data).

Water quality parameters pH, electrical conductivity, and dissolved oxygen were monitored weekly in each tank using handheld meters (HANNA® Instruments, Carrollton, TX). Percent coverage of the water surface (to nearest 10%) and mat thickness were measured weekly in each replicate. Temperature data were collected every 10 minutes using type K thermocouples (REED Instruments, Wilmington, NC). Four thermocouples were mounted onto a single wooden dowel and placed in the center of a ring to record temperature at four locations: 2 cm above the plant mat (air), and 0, 2, and 10 cm below the water surface (Figure 3.1). These locations were selected to match those used by Room and Kerr (1983). Three thermocouple units (one per treatment) were operated simultaneously. Each recording event lasted 24 hours, and after each 24 hour cycle thermocouple units were moved to a randomly selected ring from the same treatment until all rings had been recorded for one cycle (n = 8). Three 24-hour temperature recordings were made in the center of a randomly selected tank as an open-water control.

Figure 3.1. Measurement of air and water temperature at four vertical positions within the water column (+2 cm, 0 cm, -2 cm, and -10 cm) along the center of a giant salvinia mat. Four thermocouples were mounted to a wooden dowel and positioned in the center of rings at low, medium, and high giant salvinia densities.

After two weeks, each ring was harvested completely and plants were dried in an oven at 65°C for a minimum of 72 hours to determine final dry weight biomass. Daily growth rate of each treatment was calculated by estimating initial dry weight biomass from a wet weight-dry weight linear regression equation. Surface coverage, mat thickness, and biomass data were analyzed using one-way analysis of variance (ANOVA). Daily temperature curves were averaged for each of the crowding treatments plus the open water control. Average temperature at each vertical location along the water column (2 cm air, 0 cm water, 2 cm water, and 10 cm water) was analyzed using one-way ANOVA.

In winter 2016-17, floating rings and tanks were set up in an outdoor setting as previously described one or two days prior to a predicted cold front (n = 4 replicates per treatment). Three cold fronts were monitored during November 18 to 21 (63 hours), December 8 to 10 (50 hours), and January 5 to 9 (90 hours). Type K thermocouples were placed at (0 cm) and below (2 cm) the water surface in the giant salvinia root zone. Temperature data were collected every 10 minutes through the cold front. Temperature curves for each treatment were averaged and analyzed using one-way ANOVA. All statistical analyses were carried out in JMP® Pro 13.0.0 (SAS Institute Inc., Cary, NC, 2016).

3.2.2. Laboratory evaluation of effects of plant density on giant salvinia and salvinia weevil survival following acute cold exposure

To determine how density of giant salvinia affects plant tissue damage and weevil survival during acute cold exposure, a laboratory experiment was conducted in climate-controlled growth chambers (Thermo Fisher Scientific, Marietta, OH, USA). Opaque plastic containers (1.4 L, 22 cm x 22 cm x 7 cm) were filled with 1 L reverse osmosis water. A second, identical container holding 200 mL water served as a water bath to provide a layer of insulation

around the sides and bottom. Weevil-free, tertiary stage giant salvinia plants were obtained from an outdoor pond at the LSU AgCenter Iberia Research Station in Iberia Parish, LA, and placed into containers at three density treatments: low (3.5 kg/m^2), medium (7.0 kg/m^2), and high (10.5 kg/m^2). A fourth, open water treatment containing no giant salvinia was used as a negative temperature control (n = 4 replicates; 16 containers total).

Live adult salvinia weevils were obtained from an outdoor pond at the University of Louisiana at Lafayette in Lafayette Parish, LA and extracted in Berlese funnels using a method modified from Boland and Room (1983). Twenty adult weevils were placed in each replicate, with the exception of the four open water controls. All containers were acclimated for 72 hours at 13°C in a growth chamber set at 10:14h (L:D) to simulate winter conditions. After acclimation, containers were randomly positioned inside a second growth chamber held at -7°C in total darkness to simulate a winter cold front. One HOBO Pendant® data logger recorded water surface temperature every 15 minutes in a randomly selected replicate from each treatment. Additional data loggers were used to monitor the air temperature of each growth chamber to ensure that the chambers maintained constant temperatures. In addition, a hygrometer (VWR International, Radnor, PA) monitored relative humidity of the growth chambers during the experiment.

After 24 hours of exposure at -7°C, the temperature of the growth chamber was raised to 25°C over a period of 15 hours at 10:14h (L:D). Container position was randomized every other day to account for possible temperature variation within the chamber. After one week at 25°C, plant tissue damage resulting from cold exposure was assessed on a qualitative scale of 0 to 5. A score of 0 indicates healthy plants showing no signs of browning; 1 indicates mostly green plants with less than 10% of plants showing browning of leaf edges; 2 indicates mostly green plants

with 11 to 30% showing browning of leaf edges and some discoloration of outer leaves; 3 indicates 31 to 50% leaf browning and loss of integrity (limpness) of leaf edges with partial browning of terminal buds; 4 indicates over 51 to 99% leaf browning and loss of integrity in two or more leaves with partial browning of terminal buds; and 5 indicates dead plants with complete necrosis and loss of integrity in the leaves and buds (Figure 2.2).

After assessing plant damage, all plants were transferred into Berlese funnels. Live weevils were extracted into 95% ethanol over a period of 24 hours and counted. Percent mortality was calculated by subtracting the number of recovered weevils from the initial 20 that were placed in each replicate and dividing this number by 20. Plant damage rating and weevil mortality were analyzed for the three plant density treatments using one-way ANOVA followed by Tukey HSD at $\alpha = 0.05$. Average water temperature over the 24-hour acute cold exposure period was analyzed for all four treatments using one-way ANOVA.

To measure tissue damage that occurred during a less severe cold front (0°C) under the same four treatments (low, medium, and high plant density plus open water control), the experiment was replicated using weevil-free giant salvinia plants. Containers with plants and water were prepared as described above, without the addition of salvinia weevils, as previous experiments found low weevil mortality at this temperature (Cozad 2017, Russell 2017). Containers were randomly assigned to one of four treatments ($n = 4$) and placed in a growth chamber set to 0°C for 24 hours. After exposure, plants were returned to 25°C over a period of 12 hours and evaluated after one week using the plant damage scale previously described. Plant damage ratings were analyzed using one-way ANOVA with post-hoc Tukey HSD at $\alpha = 0.05$. Each experiment (with and without weevils) was replicated twice and results were pooled for statistical analysis.

3.2.3. Effect of plant density on water temperature during acute cold exposure

To determine the effect of three plant densities on giant salvinia root zone temperature under a simulated overnight cold exposure, a laboratory experiment was conducted in climate-controlled growth chambers (Thermo Fisher Scientific, Marietta, OH, USA). Opaque plastic containers (1.4 L, 22 cm x 22 cm x 7 cm) were filled with 1 L reverse osmosis water, and nested within a second container to serve as a water bath, as previously described. Weevil-free, tertiary stage giant salvinia plants were obtained from the Louisiana State University Research Greenhouse Facility in Baton Rouge, LA and placed into containers at three density treatments: low (3.5 kg/m^2), medium (7.0 kg/m^2), and high (10.5 kg/m^2). A fourth, open water treatment containing no giant salvinia was used as a negative temperature control. All containers were acclimated for 72 hours at 13°C in a growth chamber at 10:14h (L:D). After acclimation, containers were placed four at a time into a second growth chamber held at 0°C in total darkness. A type K thermocouple (REED Instruments, Wilmington, NC) was placed 4 cm beneath the water surface of each container. For each trial consisting of three different plant densities plus open water control, temperature curves were generated by logging water temperature every 5 minutes for a minimum of 14 hours. A HOBO Pendant® data logger was placed in each growth chamber and checked to ensure that the chambers maintained constant temperature. At the conclusion of ten trials, temperature was averaged over the 14 hour exposure period. Temperature averages for each treatment were analyzed using one-way ANOVA, followed by Dunnett's method for comparison to a single control (Dunnett 1955).

3.2.4. Field assessment of three levels of giant salvinia crowding in outdoor ponds

To determine the effect of plant density on giant salvinia plant quality and salvinia weevil overwintering, a field experiment was conducted in floating giant salvinia nurseries. Three ponds in north, central, and south Louisiana were selected as field sites: Eddie D. Jones Park, Keithville, in Caddo Parish, LA (32.26°N, -93.93°W), the Red River Waterway Commission, Lena, in Natchitoches Parish, LA (31.52°N, -92.73°W), and a private crawfish pond in Paradis, St. Charles Parish, LA (29.89°N, -90.45°W). Keithville (hereafter north LA) and Lena (hereafter central LA) are located in USDA plant hardiness zone 8b, with average annual minimum winter temperature of -9.4 to -6.7°C. Paradis (hereafter south LA) is located in zone 9a, with average annual minimum winter temperature of -6.7 to -3.9°C (USDA PHZM 2012). These sites were selected to show how winter climatic differences may influence management strategies. Ten floating nurseries were constructed at each site from 1.2-m diameter plastic wading pools (General Foam Plastics Corporation, Norfolk, VA) with bottoms removed to allow water and nutrient exchange. Each pool was held afloat by two 1.4-m polystyrene pool noodles. Weevil-infested tertiary stage giant salvinia obtained from an outdoor pond in Natchitoches Parish, LA (31.52°N, -92.73°W) was placed in the pools at three treatment densities: low (3.5 kg/m^2), medium (7.0 kg/m^2), and high (10.5 kg/m^2) (fresh weight). Initial adult weevil density was determined by Berlese extraction of ten 0.5-kg samples from the original plant source. All nurseries were covered with a nylon organza (1-mm mesh) to prevent plant or weevil escape, and floating booms were used to contain the nurseries to a sectioned area of each pond. Plants were sprayed monthly with the insecticide *Bt* var. *kurstaki* (Javelin, 0.3 grams/liter) to control feeding damage by *Samea multiplicalis* Guenée (Lepidoptera: Crambidae) (Wang et al. 2016).

Nurseries were established from November to December 2016 and were sampled once monthly from January through April 2017. Water quality was monitored monthly by measuring pH, electrical conductivity, and dissolved oxygen using handheld meters (HANNA® Instruments, Carrollton, TX). Water nitrate samples were analyzed in the laboratory using a nitrate ion electrode (Vernier Software & Technology, Beaverton, OR).

Each month, a 0.5 kg sample of giant salvinia was randomly collected from each pool and placed into Berlese funnels to extract salvinia weevil adults and larvae for determination of weevil population density. During each month of the experiment, percent coverage of the water surface was estimated to the nearest 10% using a 0.10 m^2 PVC quadrat and mat thickness was measured to the nearest centimeter. Five plants per nursery were selected haphazardly and observed for terminal bud feeding damage and number of adult weevils present. Feeding damage was determined by presence or absence of characteristic tissue scarring on the terminal bud of each plant. Entire plants were inspected for presence of adult weevils and the total number of weevils per five plants was counted. New plants were selected for each sampling event. On the final sampling date in April 2017, percent green was visually estimated to the nearest 10% for each replicate and plant tissue samples were sent to the LSU AgCenter Soil Testing and Plant Analysis Laboratory for carbon and nitrogen analysis. Adult and larval weevil density, percent surface coverage, mat thickness, number of damaged buds, and number of weevils observed per five plants were analyzed using a two-way ANOVA to compare the main effects of date and treatment, and the interaction effect of date*treatment on each dependent variable. Variables found to be significant at $P < 0.05$ were further analyzed using a post-hoc LSMeans Tukey test. Percent green and C:N ratio were analyzed after the final sampling event using one-way ANOVA.

Temperature data were recorded at 30 minute intervals at each site. Four HOBO Pendant® data loggers were deployed in the center of the plant mat at the water surface in each treatment. One data logger was deployed at each site to record air temperature data. Temperature curves generated for each treatment were averaged and compared within sites using one-way ANOVA. The total number of exposures at or below 0°C, mean air temperature during exposures, average duration of exposures, and cumulative duration of exposure were analyzed by filtering air temperature data in JMP® Pro 13.0.0.

3.3. Results

3.3.1. Evaluation of plant density effects on air and water column temperatures

Giant salvinia was placed into floating rings at low (3.5 kg/m^2), medium (7.0 kg/m^2), and high (10.5 kg/m^2) densities in an outdoor mesocosm experiment. In summer 2016, temperatures were recorded every 10 minutes at 2 cm above the plant mat, at the water surface (0 cm), and at 2 and 10 cm below the surface. Water pH averaged 8.5 ± 0.0 (mean ± SE), electrical conductivity 11.5 ± 0.9 µS/cm, and dissolved oxygen 8.3 ± 0.1 mg/L. Percent surface coverage and mat thickness were significantly greater in medium and high density treatments than the low density treatment, and daily growth rate did not differ significantly among treatments (Table A.2).

Air temperature 2 cm above the plant mat averaged 29.2 ± 0.1°C throughout a 24 hour period and did not differ among treatments (F = 0.6; df = 3, 572; P = 0.6201) (Figure 3.2a). At the water surface (0 cm), giant salvinia mats were on average 0.6 to 1.0°C warmer than open water (F = 6.1; df = 3, 572; P = 0.0004) (Figure 3.2b). At 2 cm below the mat surface, medium and high density treatments collectively were 0.6°C and 1.5°C warmer than low density or open water treatments, respectively (F = 22.6; df = 3, 572; P < 0.0001) (Figure 3.2c). At 10 cm below

the mat surface, average water temperature was highest in the high density treatment (31.5 ± 0.1°C) followed by medium density (31.0 ± 0.1°C), low density (30.5 ± 0.1°C), and open water treatments (29.6 ± 0.1°C) (F = 48.7; df = 3, 572; P < 0.0001) (Figure 3.2d).

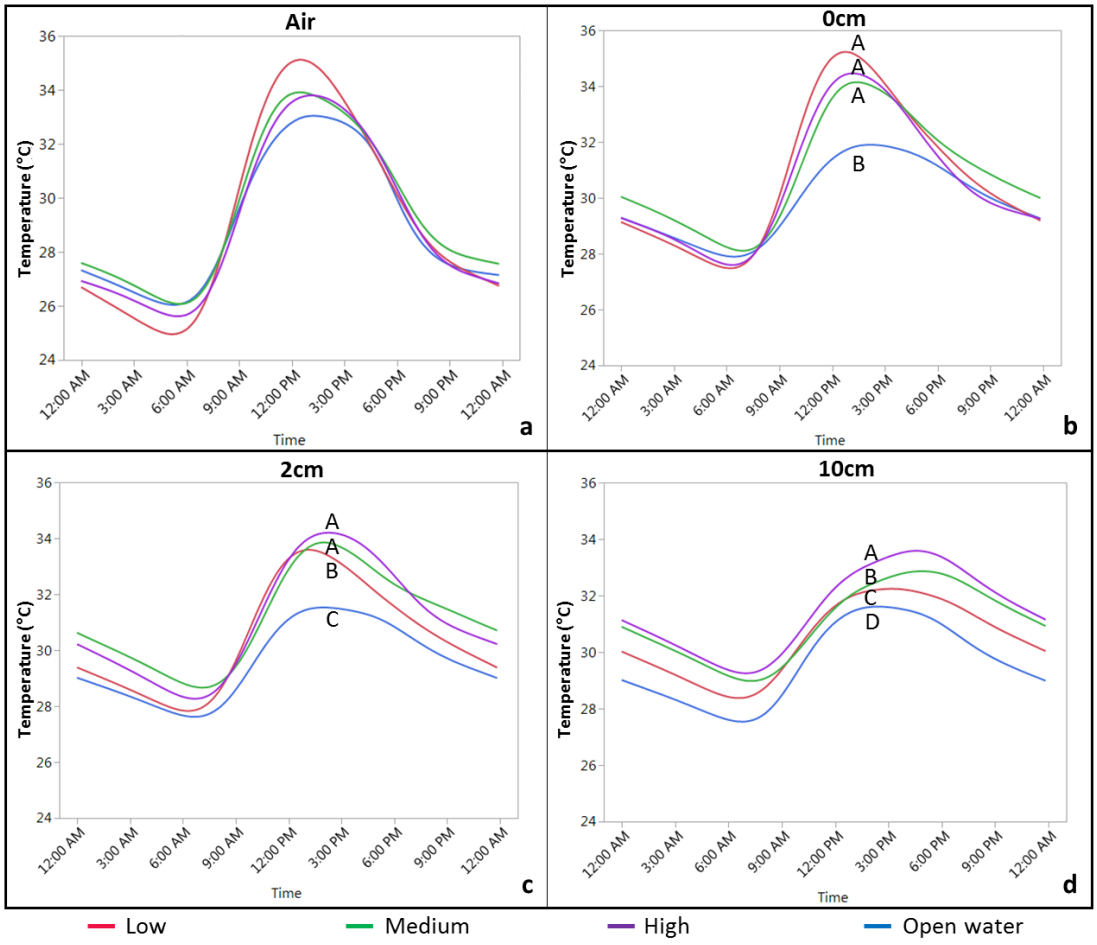

Figure 3.2. Average hourly temperature of low (3.5 kg/m^2), medium (7.0 kg/m^2), and high (10.5 kg/m^2) density giant salvinia mats plus open water control cultured in an outdoor setting over a two-week period in summer 2016. (a) 2 cm above water, (b) water surface (0 cm), (c) 2 cm below water, (d) 10 cm below water. Different letters denote significant difference among treatments according to Tukey HSD at $\alpha < 0.05$.

During winter of 2016-17, three cold fronts lasting from 50 to 90 hours were measured in the months of November, December, and January. Temperatures were recorded every 10

minutes at the water surface (0 cm) and at 2 cm below the plant mat. Air temperature during the cold fronts averaged 12.0 ± 0.3°C (November), 6.4 ± 0.2°C (December), and 3.7 ± 0.3°C (January). Ice formation occurred only in January. Water temperature at 0 cm and 2 cm below the mat surface did not follow a consistent trend in the three density treatments (Table 3.1).

Table 3.1. Average water temperature (mean ± SE, °C) of low (3.5 kg/m^2), medium (7.0 kg/m^2), and high (10.5 kg/m^2) density giant salvinia mats measured in an outdoor setting at the water surface (0 cm) and 2 cm below water during three cold fronts in winter 2016-17.

	November	**December**	**January**
0cm			
Low	14.8 ± 0.2**a**	8.9 ± 0.2**a**	4.3 ± 0.2
Medium	14.0 ± 0.2**b**	7.7 ± 0.2**b**	4.2 ± 0.2
High	13.1 ± 0.2**c**	7.3 ± 0.2**b**	4.1 ± 0.2
2cm			
Low	17.3 ± 0.2	10.0 ± 0.1**B**	6.8 ± 0.2**B**
Medium	17.0 ± 0.2	10.8 ± 0.1**A**	7.5 ± 0.2**A**
High	17.5 ± 0.2	10.4 ± 0.1**A**	6.7 ± 0.2**B**

Means with different letters within a column and within a given plant density denote significant differences between treatments according to Tukey HSD at $\alpha < 0.05$.

At 0 cm in November, low density plant mats were 0.8°C and 1.7°C warmer than medium or high density plant mats, respectively ($F = 14.6$; df = 2, 1134; $P < 0.0001$). At 0 cm in December, low density plant mats were 1.2 to 1.6°C warmer than medium or high density plant mats ($F = 15.1$; df = 2, 900; $P < 0.0001$). Mat temperature at 0 cm did not differ among treatments in January ($F = 0.2$; df = 2, 1620; $P = 0.7874$). At 2 cm in November, water

temperature did not differ among treatments (F = 1.9; df = 2, 1134; P = 0.1444). At 2 cm in December, water temperature was 0.4 to 0.8°C warmer in medium and high density plant mats than in low density plant mats (F = 15.1; df = 2, 900; P < 0.0001). At 2 cm in January, medium density plant mats were 0.7 to 0.8°C warmer than low or high density plant mats (F = 6.1; df = 2, 1620; P = 0.0023).

3.3.2. Laboratory evaluation of effects of plant density on giant salvinia and salvinia weevil survival following acute cold exposure

Plastic containers filled with water and giant salvinia at low (3.5 kg/m^2), medium (7.0 kg/m^2), and high (10.5 kg/m^2) densities, plus an open water control, were exposed to simulated cold fronts at -7°C and 0°C for periods of 24 hours. Prior to the -7°C exposure, twenty adult salvinia weevils were added to each replicate. One week after exposure, plants were evaluated for tissue damage using a qualitative scale and weevil mortality was determined.

One week following exposure at -7°C, all three treatments showed extensive tissue necrosis in the outermost layer of the plant mat. Plant damage rating was 1.3-times greater in the low density treatment (4.3 ± 0.2) than in the high density treatment (3.3 ± 0.2). Damage rating in the medium density treatment (3.8 ± 0.2) was intermediate between the low and high density treatments (F = 9.3; df = 2, 21; P = 0.0013) (Figure 3.3a).

One week following exposure at 0°C, plant damage rating was 1.3-times greater in the high density treatment (3.1 ± 0.1) than in the low density treatment (2.5 ± 0.2). Damage rating in the medium density treatment (2.8 ± 0.2) was intermediate between the high and low density treatments (F = 3.8; df = 2, 21; P = 0.0390) (Figure 3.3b). Water surface temperature over the 24-hour exposure period did not differ significantly among treatments (F = 2.4; df = 3, 384; P = 0.0654).

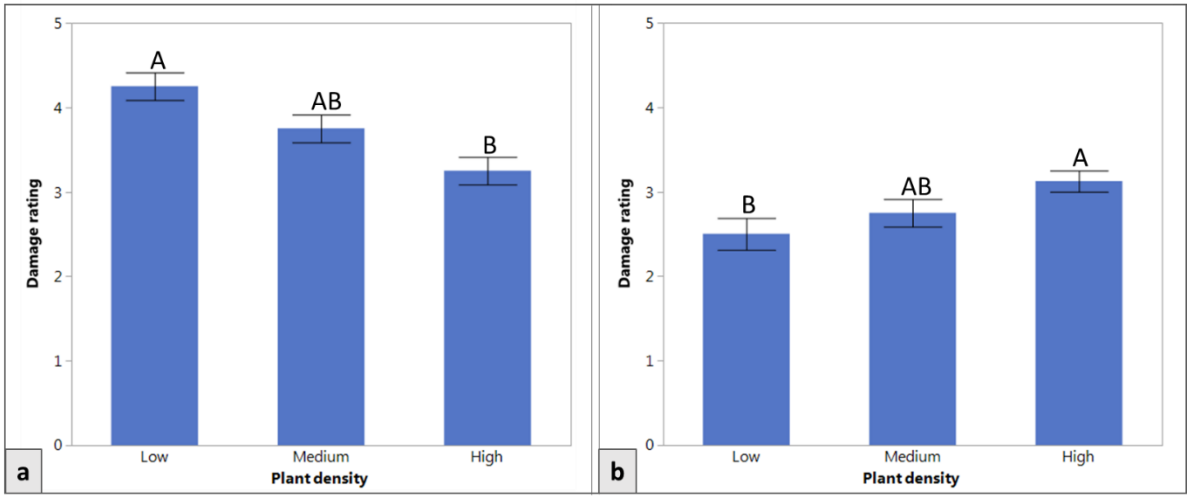

Figure 3.3. Plant damage rating one week after a 24-hour exposure at (a) -7°C and (b) 0°C in low, medium, and high density giant salvinia mats. Ratings of 0 indicate healthy plants and ratings of 5 indicate complete necrosis and loss of leaf integrity. Bars with different letters are statistically significant according to Tukey HSD at $\alpha < 0.05$.

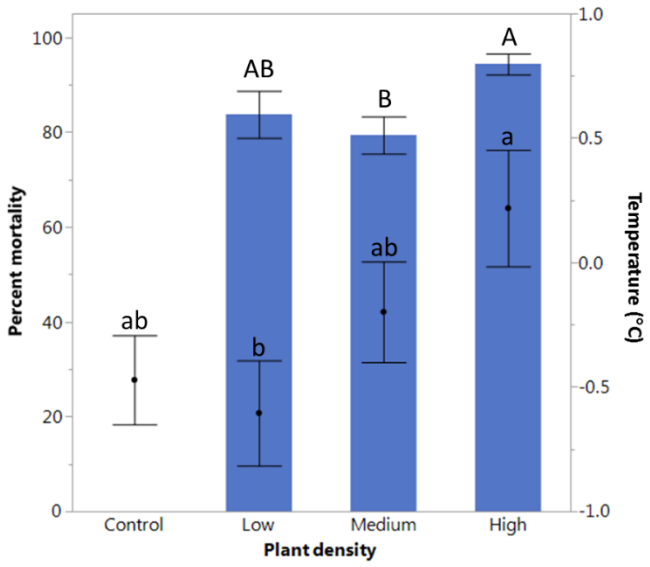

Figure 3.4. Adult salvinia weevil mortality (blue bars) and water surface temperature (black dots) in low, medium, and high density giant salvinia mats plus open water control one week after a 24-hour cold exposure at -7°C. Bars with different letters within a given parameter (mortality or temperature) are statistically significant according to Tukey HSD at $\alpha < 0.05$.

After 24 hours at -7°C, salvinia weevil mortality was 15% greater in the high density treatment (94.4 ± 2.2%) than in the medium density treatment (79.4 ± 3.9%), and mortality in the

low density treatment (83.8 ± 5.0%) was intermediate between the medium and high density treatments (F = 3.9; df = 2, 21; P = 0.0350) (Figure 3.4). Water surface temperature was 0.8°C warmer in the high density treatment (0.2 ± 0.2°C) than the low density treatment (-0.6 ± 0.2°C), whereas the medium density treatment (-0.2 ± 0.2°C) and open water control (-0.5 ± 0.2°C) were intermediate between these values (F = 3.1; df = 3, 384; P = 0.0282).

3.3.3. Effect of plant density on water temperature during acute cold exposure

Plastic containers filled with water and giant salvinia at low (3.5 kg/m^2), medium (7.0 kg/m^2), and high (10.5 kg/m^2) densities, plus an open water control, were exposed to simulated cold fronts at 0°C for periods of 14 hours in a laboratory growth chamber. Thermocouples placed 4 cm below the water surface recorded temperature every five minutes. Average water temperature was 1.4-, 1.6-, and 1.8-times greater in low, medium, and high density treatments compared to the open water control, respectively (F = 9.6; df = 3, 672; P < 0.0001) (Table 3.2).

Table 3.2. Average water temperature (mean ± SE) experienced 4 cm below giant salvinia mats during a 14-hour simulated cold front at 0°C.

Treatment	**Temperature (°C)**	***P* (Dunnett's test)**
Low	2.8 ± 0.2	0.0382*
Medium	3.2 ± 0.2	0.0005*
High	3.6 ± 0.2	< 0.0001*
Control (open water)	2.0 ± 0.2	1.0000

*Treatment differs significantly from control according to Dunnett's test at $\alpha < 0.05$.

3.3.4. Field assessment of three levels of giant salvinia crowding in outdoor ponds

Floating nurseries were established in outdoor ponds in north, central, and south Louisiana from November through December 2016. At each pond site, nurseries were filled with weevil-infested giant salvinia at low (3.5 kg/m^2), medium (7.0 kg/m^2), and high (10.5 kg/m^2) densities (n = 5). Each nursery was sampled monthly from January through April 2017 to determine changes in weevil population density and plant quality over time.

In north and south LA, initial salvinia weevil density was determined to be 54.9 ± 5.7 adults/kg (mean ± SE) and 3.0 ± 1.7 larvae/kg giant salvinia. In central LA, initial densities were 34.3 ± 4.8 adults/kg and 4.4 ± 1.1 larvae/kg giant salvinia. Average water quality parameters for each site are reported in Table A.3.

In north LA, adult and larval weevil population density differed significantly by date, but not by treatment (adult density, $F = 12.2$; df = 3, 48; $P < 0.0001$; larval density, $F = 21.3$; df = 3, 48; $P < 0.0001$). From the time of plant establishment in December until the final sampling in April, average adult weevil density decreased by 92% and larval weevil density increased by 684% (Figure 3.5a, d). Mat surface coverage and mat thickness increased significantly with increasing plant density; high density mats covered 1.1- and 1.4-times the area of medium and low density mats, respectively ($F = 153.5$; df = 2, 48; $P < 0.0001$), and were 1.2- to 1.4-times thicker than medium and low density mats ($F = 15.7$; df = 2, 48; $P < 0.0001$). The number of damaged buds did not differ by date or by treatment (average 1.3 ± 0.1, out of 5) ($F = 1.9$; df = 14, 60; $P = 0.0507$). The number of weevils observed per five plants did not differ by date or by treatment (average 0.5 ± 0.1) ($F = 0.8$; df = 14, 60; $P = 0.6624$) (Table 3.3). Percent green did not differ among treatments (average 58.7 ± 2.7%) ($F = 3.4$; df = 2, 12; $P = 0.0659$). The C:N

ratio was 15% greater in medium (23.3 ± 0.6) than low density plants (20.3 ± 0.5), with high density plants intermediate in value (21.8 ± 0.4) (F = 8.4; df = 2, 12; P = 0.0051).

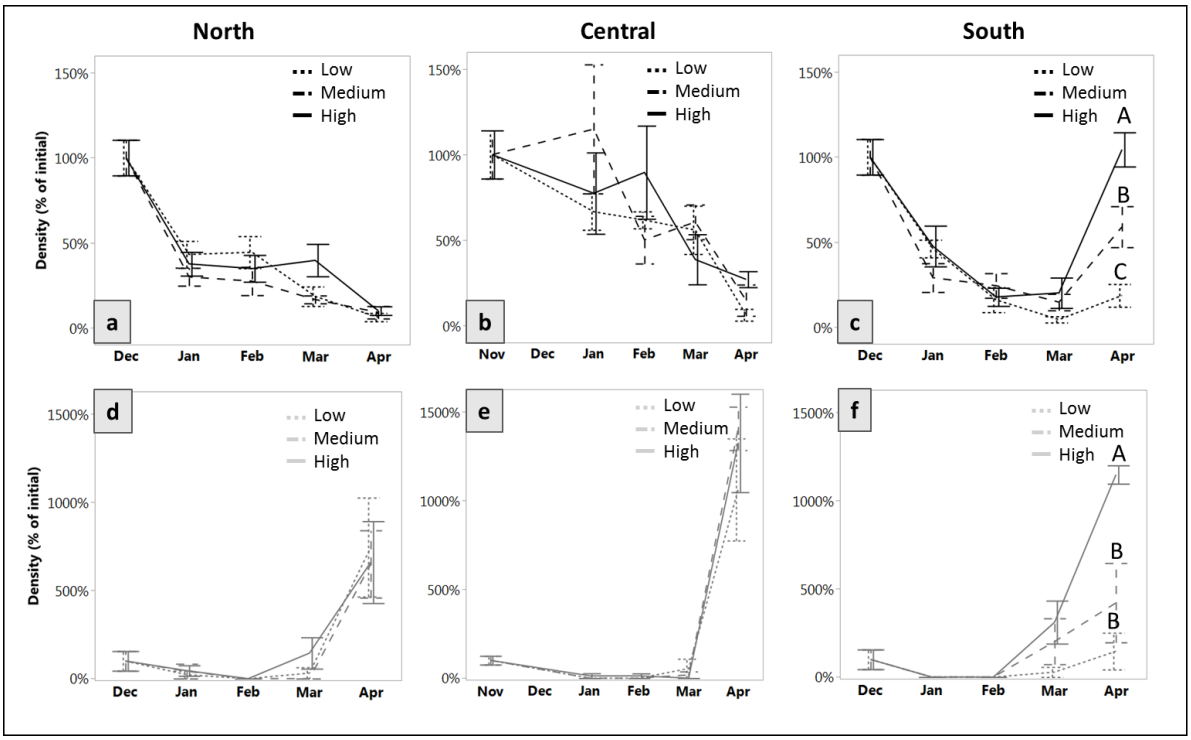

Figure 3.5. Change in salvinia weevil population density of low, medium, and high density giant salvinia mats from November 2016 through April 2017. Adult densities: (a) north, (b) central, and (c) south LA (top row). Larval densities: (d) north, (e) central, and (f) south LA (bottom row). Lines with different letters denote significant difference between treatments according to Tukey LSMeans at $\alpha < 0.05$.

In central LA, adult and larval weevil population density differed significantly by date but not by treatment (adult density, F = 8.6; df = 3, 48; $P < 0.0001$; larval density, F = 79.4; df = 3, 48; $P < 0.0001$). From the time of establishment in November until the final sampling in April, average adult weevil density decreased by 84% and larval weevil density increased by 1263% (Figure 3.5b, e). Mat surface coverage and mat thickness increased significantly with increasing plant density; high density mats covered 1.1- and 1.7-times the area of medium and low density mats, respectively (F = 155.4; df = 2, 60; $P < 0.0001$), and were 1.1- and 1.7-times

thicker than medium and low density mats, respectively (F = 48.4; df = 2, 60; P < 0.0001). The number of damaged buds differed by date, but not by treatment, with the greatest bud damage (1.7 to 2.3 buds damaged, out of 5 buds inspected) occurring in February through March (F = 11.6; df = 4, 60; P < 0.0001). The number of weevils observed per five plants likewise differed by date, but not treatment, with the most weevils (1.6 ± 0.6) occurring in February (F = 4.0; df = 4, 60; P = 0.0064) (Table 3.3). Percent green did not differ among treatments (average 55.3 ± 2.6%) (F = 0.1; df = 2, 12; P = 0.9431). The C:N ratio did not differ among treatments (average 26.9 ± 0.8) (F = 3.2; df = 2, 12; P = 0.0791).

In south LA, adult and larval weevil population densities differed significantly by date and by treatment (adult density, F = 11.7; df = 11, 48; P < 0.0001; larval density, F = 13.9; df = 11, 48; P < 0.0001). At the final sampling in April, adult weevil population density was 1.5- to 2.2-times greater in high density plant mats (57.3 ± 5.5 weevils/kg, mean ± SE) than in medium (32.3 ± 6.6 weevils/kg) and low density (10.2 ± 3.7 weevils/kg) plant mats, respectively. Larval population density was 2.3- to 8.3-times greater in high density plant mats (33.9 ± 1.5 weevils/kg) compared to the medium (12.4 ± 6.6 weevils/kg) and low density (4.3 ± 3.1 weevils/kg) plant mats, respectively (Figure 3.5c, f). Mat surface coverage and mat thickness increased significantly with increasing plant density; high density mats covered 1.1- and 1.6-times the area of medium and low density mats, respectively (F = 100.2; df = 2, 60; P < 0.0001), and were 1.2- and 1.4-times thicker than medium and low density mats, respectively (F = 13.6; df = 2, 60; P < 0.0001). The number of damaged buds differed by date, but not by treatment, with the greatest number of terminal buds damaged (2.1 ± 0.4, out of 5) occurring in February (F = 2.6; df = 4, 60; P = 0.0426). The number of weevils observed per five plants likewise differed by date, but not treatment, with the most weevils (1.3 ± 0.3) occurring in January (F = 3.6; df =

4, 60; $P = 0.0111$) (Table 3.3). Percent green in low density plant mats (100.0 ± 0.0) was 16% and 42% greater than medium density (84.0 ± 5.1) and high density (58.0 ± 4.9) plant mats, respectively ($F = 27.0$; df = 2, 12; $P < 0.0001$). The C:N ratio did not differ among treatments (average 27.0 ± 0.6) ($F = 1.6$; df = 2, 12; $P = 0.2519$).

Table 3.3. Giant salvinia mat quality and plant inspection parameters (mean ± SE) measured from five haphazardly collected plants at field sites in north, central, and south Louisiana from November 2016 through April 2017.

	Coverage (%)	**Mat thickness (cm)**	**# Damaged buds**	**# Weevils**
North LA				
Low	71.0 ± 5.2**c**	3.5 ± 0.3**b**	1.0 ± 0.2	0.5 ± 0.2
Medium	90.0 ± 2.3**b**	4.1 ± 0.4**b**	1.4 ± 0.2	0.2 ± 0.1
High	98.5 ± 0.8**a**	5.1 ± 0.3**a**	1.5 ± 0.2	0.6 ± 0.3
Central LA				
Low	56.8 ± 2.6**c**	3.3 ± 0.2**c**	1.3 ± 0.2	0.7 ± 0.2
Medium	86.0 ± 1.6**b**	5.1 ± 0.3**b**	1.5 ± 0.2	1.2 ± 0.4
High	95.2 ± 1.5**a**	5.7 ± 0.4**a**	1.5 ± 0.2	0.6 ± 0.2
South LA				
Low	56.4 ± 5.1**c**	4.2 ± 0.6**c**	1.1 ± 0.2	0.5 ± 0.1
Medium	84.4 ± 2.5**b**	4.9 ± 0.5**b**	1.4 ± 0.3	0.6 ± 0.2
High	92.8 ± 2.2**a**	5.7 ± 0.3**a**	1.7 ± 0.3	1.0 ± 0.2

Means with different letters within a column and within a given location (north, central, or south) denote significant differences between treatments according to Tukey LSMeans at $\alpha < 0.05$.

Water temperature in north LA did not differ among treatments (average 16.6 ± 0.0°C) (F = 0.1; df = 2, 18675; P = 0.9337). In central LA, low density plant mats (16.4 ± 0.1°C) were 0.3 to 0.5°C warmer than medium (16.1 ± 0.1°C) and high density (16.0 ± 0.1°C) plant mats, respectively (F = 15.5; df = 2, 20742; P < 0.0001). In south LA, low density plant mats (19.2 ± 0.1°C) were 0.2°C warmer than high density plant mats (18.9 ± 0.1°C), and medium density plant mats were intermediate in temperature (19.0 ± 0.1°C) (F = 4.1; df = 2, 18702; P = 0.0159). Air temperature analysis indicated 11 discrete periods of cold exposure below 0°C in north LA, 10 discrete periods in central LA, and two in south LA. Total duration below 0°C was 97 hours in north LA, 62 hours in central LA, and 11.5 hours in south LA (Table 3.4).

Table 3.4. Air temperature analysis from north, central, and south LA outdoor ponds. Data were filtered to show exposure temperature and duration during times when air temperature ≤ 0°C.

Parameter	**North**	**Central**	**South**
Air max. (°C)	30.4	31.0	29.8
Air min. (°C)	-9.1	-6.1	-1.6
Average air (°C)	14.1 ± 0.1	15.6 ± 0.1	17.9 ± 0.1
# times ≤ 0°C	11	10	2
Average exposure temp. (°C)	-3.2 ± 0.2	-2.3 ± 0.2	-0.9 ± 0.1
Avg. exposure duration (hours)	8.8 ± 1.9	6.2 ± 1.8	5.8 ± 0.8
Cumulative duration (hours)	97	62	11.5

3.4. Discussion

3.4.1. Effect of crowding on giant salvinia mat temperature and plant quality

Mesocosm studies in winter and summer demonstrated that the effect of increased plant density is most evident between 2 and 10 cm in the water column, producing temperature increases of as much as 2°C between high density plant mats and open water. This agrees with findings by Dale and Gillespie (1976) and Room and Kerr (1983) that floating plant mats increase temperature stratification in the water column.

In field studies in outdoor ponds, low density plant mats had the highest surface temperature compared to medium or high density plant mats. These results contradict my original hypothesis but are consistent with findings from the mesocosm study, indicating that factors other than plant density alone may influence mat temperature. When giant salvinia's dark root-like fronds are allowed to float to the water surface in low density plant mats, they may absorb more solar radiation than the light-colored leaves, producing higher water surface temperature. Differences in solar reflectivity of light versus dark organic matter has been shown to produce similar effects in shallow ponds (Dale and Gillespie 1977).

During severe cold exposures, plant quality may be conserved in high density plant mats as the upper plant layers insulate the lower layers of the mat. After laboratory-simulated cold fronts, high density plant mats suffered less visible tissue damage at -7°C, but more damage at 0°C compared to low and medium density mats. High density plant mats create a buffer against ice formation in lower layers and may contain greater biomass beneath the water surface, both of which can increase plant viability during freezes (Room and Kerr 1983, Whiteman and Room 1991). In the field experiments, plant quality measured by percent green of the plant mats did not differ among treatments in north and central LA, but in south LA, low density mats were 16

to 42% greener (i.e. healthier) than high density mats. Because the south LA site did not experience many cold fronts, this difference was attributed to salvinia weevil feeding activity rather than direct cold exposure. The plant mats in north and central LA were thicker with increasing plant density, so it is possible that higher density treatments contained more viable biomass than lower density treatments despite appearing superficially similar.

3.4.2. Effect of plant crowding on salvinia weevil survival and overwintering

During simulated cold fronts lasting 24 hours at -7°C, adult salvinia weevil mortality was highest (94%) in the high density treatment and lowest (79%) in the medium density treatment. This contradicted my hypothesis that high density plant mats should protect adult weevils from the effects of cold exposure. Interestingly, the high density plants in this experiment had the highest surface temperature (0.2 ± 0.2°C) and the lowest plant damage rating (3.3 out of 5) out of all treatments. Because weevil mortality was assessed one week following the simulated cold front, it is possible that weevils in the high density treatment were smothered by the top layer of dead plants that decayed rapidly following the cold exposure. Alternatively, the weevils may not have been able to burrow far enough into the plant mat to find protection from the cold air in this treatment. Future experiments may benefit from observing the location of weevils within each treatment following cold exposure to determine if mortality was a direct result of plant density or if other factors contributed to this trend. It is of interest to note the relatively low mortality of weevils in the medium density treatment, as this treatment represented the level of plant crowding observed in natural infestations.

Adult and larval weevil density did not differ among plant density treatments in north and central LA ponds. These ponds experienced similar winter conditions, with 11 and 10 discrete

exposures below 0°C (air temperature) and cumulative duration of 97 and 62 hours, respectively, in below-zero conditions. By contrast, the south LA pond experienced only two discrete exposures below 0°C for a cumulative duration of 11.5 hours. In the south LA pond, both adult and larval weevil densities were significantly greater in high density plant mats compared to medium or low density mats. These data indicate that plant crowding may be a viable strategy for increasing overwinter survival of adult salvinia weevils and establishment of F1 larvae in the field, providing winter conditions are not too severe. In areas affected by severe winter temperatures, plant crowding may not be sufficient on its own as a management strategy, and continued use of integrated techniques such as winter herbicide application (Mudge and Sartain 2018) and lake drawdowns (Houston et al. 2017) may be necessary.

3.4.3. Comparison of plant crowding with other overwintering methods

Compared with other methods for overwintering cold-sensitive aquatic species, manipulation of plant density does not require purchasing materials for electronically controlled geothermal heating (Hall et al. 2002) or construction of cold frames (Putegnat 2013), making it a cost-effective management strategy. Booms or a variety of alternative materials can be used to contain an overwintering plant mat. Size and shape of an enclosed area can be modified as needed to maintain a desired level of crowding throughout the winter. Booms are commonly used in salvinia weevil release sites to reduce disturbances and prevent downstream spread (Van Oosterhout 2006), therefore existing containment structures could be easily adapted to manage plant density. In contrast to greenhouse plastics, which primarily warm the air layer above the plant mat, plant crowding appears to have a greater effect on root zone temperature than on mat surface temperature.

3.4.4. Practical uses of plant crowding and management implications

These experiments have shown that increasing the plant density of giant salvinia mats can increase water column temperatures and increase plant viability during severe cold exposure. In the field, plant crowding appears to benefit overwintering salvinia weevil populations in southern ponds (below 30°N), but does not affect populations in areas that experience colder winter extremes. The relative benefit of plant crowding will depend on site-specific variables including location, time of year, size and severity of the infestation, and water body characteristics. A practical limitation of using booms to artificially crowd giant salvinia is that too much pressure from the growing plant mat could cause plants to escape or even damage the boom (Van Oosterhout 2006). Plant crowding is most likely to benefit overwintering salvinia weevils under conditions where sparse plant mats risk transitioning into a single layer of secondary growth, which would be more susceptible to temperature fluctuations and provide less refuge space for adult weevils. Salvinia weevils disperse slowly through plant mats, even in warm conditions (Room and Thomas 1985) and have limited flight activity during winter (Micinski et al. 2016), therefore crowding of plants into a smaller area concentrates weevil feeding activity and facilitates movement to new plants. Future research on plant crowding in varying water depth would be informative for predicting the effectiveness of this management strategy in deep versus shallow water bodies.

Biological control programs for plants with similar growth habit and geographic distribution, such as water hyacinth (Center et al. 2002) and water lettuce, *Pistia stratiotes* L. (Mitchell 1969), may likewise benefit from management of plant density during times of the year when the agent population is most vulnerable. In addition to temperature moderation, plant crowding could increase the availability of nearby food resources and increase mating success as

compared to sparsely arranged plant mats. This is of particular importance for agents which may have limited long-distance dispersal, such as the water hyacinth weevils *Neochetina* spp. (Center et al. 1999) and alligatorweed thrips, *Amynothrips andersoni* O'Neill (Thysanoptera: Phlaeothripidae) (Buckingham 2002). Although continued use of integrated strategies will be necessary in regions affected by severe winters, plant crowding may be a useful tool that can be added to the arsenal of available management strategies for free-floating invasive aquatic species.

3.5. References

Bale, J. S. 2005. Effects of temperature on the establishment of non-native biocontrol agents: the predictive power of laboratory data, pp. 593-602. *In* M. S. Hoddle (ed.), Second International Symposium on Biological Control of Arthropods (ISBCA), vol. II. Publication FHTET-2005-08. USDA Forest Service, Morgantown, WV.

Boland, N. P., and P. M. Room. 1983. Estimating population density of a *Cyrtobagous* sp. (Coleoptera: Curculionidae) on the floating weed salvinia using Berlese funnels. J. Aust. Entomol. Soc. 22: 353-354.

Buckingham, G. R. 2002. Alligatorweed, pp. 5-15. *In* R. Van Driesche, B. Blossey, M. Hoddle, S. Lyon, R. Reardon (eds.), Biological control of invasive plants in the eastern United States. USDA Forest Service Publ. FHTET-2002-04, Forest Health Technology Enterprise Team, Morgantown, WV.

Calder, A. A., and D.P.A. Sands. 1985. A new Brazilian *Cyrtobagous* Hustache (Coleoptera: Curculionidae) introduced into Australia to control *Salvinia*. Journal of the Australian Entomological Society 24: 57-64.

Center, T. D., F. A. Dray, Jr., G. P. Jubinsky, and A. J. Leslie. 1999. Waterhyacinth weevils (*Neochetina eichhorniae* and *N. bruchi*) inhibit waterhyacinth (*Eichhornia crassipes*) colony development. Biol. Control 15: 39-50.

Center, T. D., M. P. Hill, H. Cordo, and M. H. Julien. 2002. Waterhyacinth, pp. 41-64. *In* R. Van Driesche, B. Blossey, M. Hoddle, S. Lyon, R. Reardon (eds.), Biological control of invasive plants in the eastern United States. USDA Forest Service Publ. FHTET-2002-04, Forest Health Technology Enterprise Team, Morgantown, WV.

Cozad, L. W. 2017. Giant salvinia, *Salvinia molesta* (Salviniaceae): evaluation of sub-optimum temperatures on survival of the giant salvinia weevil, *Cyrtobagous salviniae* (Coleoptera: Curculionidae) and integration of management practices with aquatic herbicides. M.S. Thesis, Louisiana State University, Baton Rouge.

Dale, H. M., and T. Gillespie. 1976. The influence of floating vascular plants on the diurnal fluctuations of temperature near the water surface in early spring. Hydrobiologia 49: 245-256.

Dale, H. M., and T. Gillespie. 1977. Diurnal fluctuations of temperature near the bottom of shallow water bodies as affected by solar radiation, bottom color and water circulation. Hydrobiologia 55: 87-92.

Driever, S. M., E. H. van Nes, and R.M.M. Roijackers. 2005. Growth limitation of *Lemna minor* due to high plant density. Aquat. Bot. 81: 245-251.

Dunnett, C. W. 1955. A multiple comparison procedure for comparing several treatments with a control. J. Amer. Statist. Assoc. 50: 1096-1121.

EDDMapS. 2017. Early detection & distribution mapping system. The University of Georgia - Center for Invasive Species and Ecosystem Health. Available online at http://www.eddmaps.org/; last accessed September 3, 2017.

Forno, I. W., D.P.A. Sands, and W. Sexton. 1983. Distribution, biology and host specificity of *Cyrtobagous singularis* Hustache (Coleoptera: Curculionidae) for the biological control of *Salvinia molesta*. Bull. Entomol. Res. 73: 85-95.

Galam, D., J. Silva, D. Sanders, and J. H. Oard. 2015. Morphological and genetic survey of giant salvinia populations in Louisiana and Texas. Aquat. Bot. 127: 20-25.

Grevstad, F. S., and L. B. Coop. 2015. The consequences of photoperiodism for organisms in new climates. Ecol. Appl. 25: 1506-1517.

Hall, S. G., J. Finney, R. P. Lang, and T. R. Tiersch. 2002. Design and development of a geothermal temperature control system for broodstock management of channel catfish *Ictalurus punctatus*. Aquacult. Eng. 26: 277-289.

Houston, K., J. Seales, J. Sibley, and E. Thames. 2017. Lake Bistineau: lake history & management issues. Waterbody Management Plan Series Part VI-A. Louisiana Department of Wildlife and Fisheries, Office of Fisheries, Inland Fisheries Section, Baton Rouge, LA.

Jacono, C. C., T. R. Davern, and T. D. Center. 2001. The adventive status of *Salvinia minima* and *S. molesta* in the southern United States and the related distribution of the weevil *Cyrtobagous salviniae*. Castanea 66: 214-226.

Johnson, S., D. Sanders, L. Eisenberg, and K. Whitehead. 2010. Fighting the blob: efforts to control giant salvinia. Louisiana Agriculture 53: 6-9.

Manning, J. H. 1979. Establishment of waterhyacinth weevil populations in Louisiana. J. Aquat. Plant. Manage. 17: 39-41.

McClay, A. S. 1996. Biological control in a cold climate: temperature responses and climatic adaptation of weed biocontrol agents, pp. 377-383. *In* V. C. Moran and J. H. Hoffman (eds.), Proceedings of the IX international symposium on biological control of weeds, 19-26 January 1996. Stellenbosch, South Africa, University of Cape Town.

McFarland, D. G., L. S. Nelson, M. J. Grodowitz, R. M. Smart, and C. S. Owens. 2004. *Salvinia molesta* D. S. Mitchell (giant salvinia) in the United States: a review of species ecology and approaches to management. Environmental Lab. SR-04-2. US Army Corps of Engineers, Engineer Research and Development Center, Vicksburg, MS.

Micinski, S., B. J. Fitzpatrick, M. L. Ferro, S. J. Johnson, B. Johnson, and S. Williams. 2016. Flying activity of *Cyrtobagous salviniae* Calder and Sands in Louisiana. Southwest. Entomol. 41: 313-320.

Mitchell, D. S. 1969. The ecology of vascular hydrophytes on Lake Kariba. Hydrobiologia 34: 448-464.

Mitchell, D. S., and N. M. Tur. 1975. The rate of growth of *Salvinia molesta* (*S. auriculata* auct.) in laboratory and natural conditions. J. Appl. Ecol. 12: 213-225.

Miller, I. L., and C. G. Wilson. 1989. Management of salvinia in the Northern Territory. J. Aquat. Plant Manage. 27: 40-46.

Mudge, C. R., and B. T. Sartain. 2018. Influence of winter on herbicide efficacy for control of giant salvinia (*Salvinia molesta*). J. Aquat. Plant Manage. *In Press*.

Nachtrieb, J. G. 2013. Mass-rearing *Cyrtobagous salviniae* for biological control of giant salvinia: field release implications. J. Aquat. Plant Manage. 51: 122-126.

Obeysekara, P. T., A. Knutson, A. Mukherjee, and K. M. Heinz. 2015. Repeated cold exposure effects on mortality and feeding activity of the salvinia weevil, *Cyrtobagous salviniae* (Coleoptera: Curculionidae). Environ. Entomol. 44: 1590-1598.

Parys, K. A., and S. J. Johnson. 2013. Biological control of common salvinia (*Salvinia minima*) in Louisiana using *Cyrtobagous salviniae* (Coleoptera: Curculionidae). Fla. Entomol. 96: 10-18.

Putegnat, J. W. 2013. Design and field-test of solar-passive thermal refugia for overwintering Mozambique tilapia (*Oreochromis mossambicus*) in central Texas. M.S. Thesis, Texas A&M University, College Station.

Room, P. M., and J. D. Kerr. 1983. Temperatures experienced by the floating weed *Salvinia molesta* Mitchell and their prediction from meteorological data. Aquat. Bot. 16: 91-103.

Room, P. M., and P. A. Thomas. 1985. Nitrogen and establishment of a beetle for biological control of the floating weed *Salvinia* in Papua New Guinea. J. Appl. Ecol. 22: 139-156.

Room, P. M., and P. A. Thomas. 1986. Population growth of the floating weed *Salvinia molesta*: field observation and a global model based on temperature and nitrogen. J. Appl. Ecol. 23: 1013-1028.

Room, P. M., K.L.S. Harley, I. W. Forno, and D.P.A. Sands. 1981. Successful biological control of the floating weed salvinia. Nature 294: 78-80.

Russell, A. D. 2017. Cold tolerance and overwintering physiology of the salvinia weevil (*Cyrtobagous salviniae*): improving the biological control of giant salvinia in temperate Louisiana. M.S. Thesis, Louisiana State University, Baton Rouge.

Sands, D.P.A., M. Schotz, and A. S. Bourne. 1983. The feeding characteristics and development of larvae of a salvinia weevil *Cyrtobagous* sp. Entomol. Exp. Appl. 34: 291-296.

Schooler, S. S., B. Salau, M. H. Julien, and A. R. Ives. 2011. Alternative stable states explain unpredictable biological control of *Salvinia molesta* in Kakadu. Nature 470: 86-89.

Sullivan, P. R., L. A. Postle, and M. Julien. 2011. Biological control of *Salvinia molesta* by *Cyrtobagous salviniae* in temperate Australia. Biol. Control 57: 222-228.

Tipping, P. W., and T. D. Center. 2003. *Cyrtobagous salviniae* (Coleoptera: Curculionidae) successfully overwinters in Texas and Louisiana. Fla. Entomol. 86: 92-93.

Tipping, P. W., and T. D. Center. 2005. Influence of plant size and species on preference of *Cyrtobagous salviniae* adults from two populations. Biol. Control 32: 263-268.

Tipping, P. W., M. R. Martin, T. D. Center, and T. M. Davern. 2008. Suppression of *Salvinia molesta* Mitchell in Texas and Louisiana by *Cyrtobagous salviniae* Calder and Sands. Aquat. Bot. 88: 196-202.

U.S. Climate Data. 2017. Climate Baton Rouge – Louisiana. https://www.usclimatedata.com/climate/baton-rouge/louisiana/united-states/usla0033/2017/1 (Accessed 6 October 2017).

USDA PHZM, 2012. Agricultural Research Service, U.S. Department of Agriculture. http://planthardiness.ars.usda.gov. (Accessed 7 September 2017).

Van Oosterhout, E. 2006. Salvinia control manual: management and control options for salvinia (*Salvinia molesta*) in Australia. New South Wales Department of Primary Industries, NSW, Australia. http://www.dpi.nsw.gov.au/__data/assets/pdf_file/0004/89554/salvinia-control-manual.pdf.

Wahl, C., L. Moshman, and R. Diaz. 2016. How to rear giant salvinia weevils in outdoor ponds. LSU AgCenter Pub. 3551. Louisiana State University Agricultural Center, Baton Rouge, LA.

Wang, Y., S. Brown, and R. Diaz. 2016. Water lettuce moth. LSU AgCenter Pub. 3538. Louisiana State University Agricultural Center, Baton Rouge, LA.

Whiteman, J. B., and P. M. Room. 1991. Temperatures lethal to *Salvinia molesta* Mitchell. Aquat. Bot. 40: 27-35.

Chapter 4: Conclusions and Recommendations

Winter management of giant salvinia is important to ensure that salvinia weevils maintain sufficient population density for continued control of infestations. In areas threatened by severe winters, active monitoring of plant mats can increase the likelihood of successful weevil establishment. In my research, I have demonstrated the potential utility of spunbonded polypropylene (SBP) fabrics and management of plant density in creating winter refugia for salvinia weevils.

There is strong evidence that SBP fabrics (row covers) maintain plant quality by reducing visible frost damage to giant salvinia mats. SBP fabrics increase plant mat surface temperature by insulating the boundary layer above the plant mat and reducing heat conduction to the air. Row covers are easily acquired at low cost and can be customized to fit areas of varied shape and size. Since the fabric requires a support structure, it is best suited to semi-permanent areas that can be regularly monitored to correct issues such as sagging and debris accumulation. Mass-rearing ponds or small corners of lakefront properties would likely benefit most from these artificial refugia.

In field experiments, SBP fabric increased adult weevil survival in south Louisiana but not in north Louisiana, where annual winter temperatures reach lower extremes. During severe winters, a single layer of SBP fabric may not be sufficient to protect overwintering salvinia weevil populations, so multiple fabric layers, heavier fabrics, or alternative materials such as greenhouse plastics may be required in these scenarios.

Crowding plants to increase the density of giant salvinia mats raises root zone temperature by increasing stratification of the water column, and ultimately reducing heat loss by convection. Containment booms are practical means to raise plant density and unlike fabric

covers, do not require a rigid support structure. Because booms are commonly deployed at salvinia weevil release sites and in mass-rearing operations, land managers are likely to have these at their disposal. Booms tend to be more expensive than SBP fabrics, but are more durable and can be used for many seasons. Alternative materials such as plastic wading pools or PVC pipes can also be used to create floating nurseries for managing plant density on a smaller scale, and these materials are easily accessible to homeowners or members of the public who wish to contribute to biological control efforts.

Field experiments demonstrated that high density (10.5 kg/m^2) plant mats did not raise mat surface temperature, but did increase adult and larval salvinia weevil population density in south Louisiana compared to medium and low density plant mats. In north and central Louisiana, plant crowding at the levels tested in this study may not create sufficient refuge to protect overwintering salvinia weevils during severe winters. However, crowding of secondary stage giant salvinia may be advantageous for salvinia weevil populations because it increases mat thickness from a single plant layer to multiple plant layers and reduces spaces of open water, thereby decreasing heat loss from evaporation.

Giant salvinia infestations in the southeastern United States will continue to be managed, but not eradicated. During mild winters, salvinia weevil populations can successfully overwinter in plant mats and rebound in the spring. However, effective control will still rely on annual spring releases to supplement winter losses. More research is needed to determine the impact of winter management strategies on salvinia weevil populations during severe winters. This research adds to existing evidence (Room and Thomas 1986, Miller and Wilson 1989, Flores and Carlson 2006, Sullivan et al. 2011) that giant salvinia management should be site-specific to address geographic and climatic idiosyncrasies of salvinia weevil release sites. Effective winter

management strategies for north Louisiana and Texas may allow greater access to mass-reared salvinia weevils for releasing onto problematic infestations. Mass-rearing weevils in northern regions may allow populations to adapt to cold climates faster (Cozad 2017). In general, more frequent releases will increase the likelihood of successful long-term establishment.

Future studies should examine whether integrating plant crowding and fabric insulation can provide greater temperature regulation of plant mats and increased rates of insect survival than either method alone. Experimental evidence suggests that SBP fabrics provide better temperature regulation of the mat surface, whereas plant crowding provides better temperature regulation of the water column root zone. Together, these two methods may work synergistically to further decrease heat loss from giant salvinia mats and possibly decrease weevil mortality. However, continued use of mechanical and chemical control methods will be necessary to complement biological control programs in areas affected by severe winter cold.

4.1. References

Cozad, L. W. 2017. Giant salvinia, *Salvinia molesta* (Salviniaceae): evaluation of sub-optimum temperatures on survival of the giant salvinia weevil, *Cyrtobagous salviniae* (Coleoptera: Curculionidae) and integration of management practices with aquatic herbicides. M.S. Thesis, Louisiana State University, Baton Rouge.

Flores, D., and J. W. Carlson. 2006. Biological control of giant salvinia in East Texas waterways and the impact on dissolved oxygen levels. J. Aquat. Plant Manage. 44: 115-121.

Miller, I. L., and C. G. Wilson. 1989. Management of salvinia in the Northern Territory. J. Aquat. Plant Manage. 27: 40-46.

Room, P. M., and P. A. Thomas. 1986. Nitrogen, phosphorus and potassium in *Salvinia molesta* Mitchell in the field: effects of weather, insect damage, fertilizers and age. Aquat. Bot. 24: 213-232.

Sullivan, P. R., L. A. Postle, and M. Julien. 2011. Biological control of *Salvinia molesta* by *Cyrtobagous salviniae* in temperate Australia. Biol. Control 57: 222-228.

Appendix: Supplemental Tables

Table A.1. Water quality parameters (mean ± SE) of outdoor ponds sampled monthly from November through April in north and south LA.

Parameter	North LA	South LA
Depth (m)	0.9 ± 0.1	0.5 ± 0.1
pH	6.7 ± 0.1	7.0 ± 0.2
Electrical conductivity (mS/cm)	0.3 ± 0.0	0.2 ± 0.1
Dissolved oxygen (mg/L)	6.7 ± 0.6	7.2 ± 0.7
Nitrate (mg/L)	2.5 ± 0.5	1.6 ± 0.4

Table A.2. Plant mat parameters (mean ± SE) of giant salvinia at low, medium, or high plant densities in 0.1m^2 floating rings.

Treatment[a]	Coverage (%)	Mat thickness (cm)	Growth rate[b] (g/day)
Low	61.3 ± 2.7**b**	3.4 ± 0.2**c**	0.3 ± 0.0
Medium	95.8 ± 1.0**a**	5.0 ± 0.2**b**	0.4 ± 0.0
High	100.0 ± 0.0**a**	6.4 ± 0.2**a**	0.4 ± 0.0

[a]Low, 3.5 kg/m^2; medium, 7.0 kg/m^2; high, 10.5 kg/m^2.
[b]Dry weight.
Means with different letters denote significant difference among treatments at according to Tukey LSMeans at $\alpha < 0.05$.

Table A.3. Water quality parameters (mean ± SE) of outdoor ponds sampled monthly from November 2016 through April 2017 in north, central, and south LA.

Parameter	North	Central	South
Depth (m)	0.9 ± 0.1	1.0 ± 0.1	0.8 ± 0.0
pH	6.7 ± 0.1	7.0 ± 0.2	7.2 ± 0.2
Electrical conductivity (mS/cm)	0.3 ± 0.0	0.3 ± 0.0	0.2 ± 0.0
Dissolved oxygen (mg/L)	6.7 ± 0.6	4.7 ± 0.9	1.6 ± 0.3
Nitrate (mg/L)	2.5 ± 0.5	1.3 ± 0.4	2.1 ± 0.5

Vita

Lori Moshman grew up in Brooklyn, New York with dreams of becoming an urban dentist. She attended Cornell University and received her Bachelor's Degree in Biological Sciences, Plant Sciences, and Entomology in 2012. Following her graduation she moved to Bluffton, Georgia, where she fell in love with the south's slow pace of life while working as entomologist and greenhouse manager for a diversified organic farm. Lori began her master's study at LSU in 2015 to feed her love of entomology and learn more about biological control of invasive species. She anticipates graduating in December 2017 with an M.S. in Entomology. Lori plans to remain an active proponent of biological control and extension teaching, while returning to her agricultural "roots" to pursue a life of self-sufficiency.