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Lauren W. Cozad
lacozad3@gmail.com

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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

A Thesis

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

in

The Department of Plant, Environmental and Soil Sciences

by
Lauren W Cozad
B.S., Northwestern State University, 2010
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To my best friend and husband, without your love and support throughout this journey, this dream could not have become a reality. To my three girls, Alexis, Aubrey, and Annabelle always believe in yourself, the path you seek may not always be the path that was destined for you. Dream big, I know you'll move mountains.

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ABSTRACT

Salvinia molesta is one of the world's worst aquatic weeds and has cost Louisiana nearly $7 million worth of damage and economic impact each year. While aquatic herbicides and the biological control agent, *Cyrtobagous salviniae*, are the most efficacious control methods, there are limitations with each technology. Therefore, studies were conducted to evaluate insect mortality at upper and lower lethal temperatures and investigate integrated pest management (IPM) with insects and herbicides. Three Louisiana populations of *C. salviniae* were tested to evaluate cold tolerance and found that at 0°C, the Bayou Nicholas population was 1.3- and 1.4-times more cold tolerant at LT_{50} and LT_{90} (lethal time to kill 50 and 90% of test population) compared to the Houma and Natchitoches populations, respectively. At -5°C, the Bayou Nicholas population was 1.1- to 1.3-times more cold tolerant than the other populations. There were no differences between populations at -9°C. These findings demonstrate *C. salviniae* can survive at lower temperatures than previously reported. Growth chamber trials investigated the tolerance of *C. salviniae* at 35 to 50°C to determine impact of high temperatures on mortality. At 35°C, the LT_{50} was 27.5 hours of exposure, while at 40°C, the LT_{50} was 14.8 hours. As expected, mortality occurs more rapidly at higher temperatures (45 and 50°C). Mesocosm trials were conducted to determine optimum timing for treating *S. molesta* with aquatic herbicides along with integrated management techniques compared with herbicides alone. An early season (April) herbicide application alone or in combination with *C. salviniae* was more efficacious than a late season application with or without *C. salviniae*. Glyphosate + diquat was efficacious against *S. molesta* at both application timings. Although *C. salviniae* alone was capable

of reducing plant biomass, higher efficacy was achieved when used in conjunction with herbicides. Insect densities were highest at 6 and 9 weeks after treatment (WAT) regardless of treatment; however, the penoxsulam + *C. salviniae* treatment produced the lowest insect density overall. In addition, flumioxazin was efficacious in both trials and offers an alternate mode of action to treat *S. molesta*.

CHAPTER 1. INTRODUCTION

1.1 Impact of Invasive Weeds and Giant Salvinia

Invasive plants have interfered with ecosystems in the United States, dating back to the 15th century (Bryson and DeFelice 2009) and pose serious threats to native plants. The impacts on native species include competing for resources, altering the natural environment, disrupting biodiversity, and causing long-term impacts on agricultural practices and societal interests (Bryson and DeFelice 2009; Reichard and White 2001; Westbrooks 1998). Invasive plants, also known as undesirable plants or weeds (Radosevich et al. 2007) are generally non-native, relatively new to an eco-region, and thus have no natural enemies to limit their ability to reproduce (Mashhandi and Radosevich 2004; Westbrooks 1998). Because of their adaptation, invasive weeds can successfully establish, develop self-sustaining populations, and spread without further assistance from humans (Randall 1997). Sources of introduction of these plants include the horticulture and landscaping industry, intentional plantings for erosion control, aquarium trade, crop seed contamination, and discharged water from ship's ballasts in cargo transportation (Baker 1986; Reichard and White 2001). However, with ongoing expansion in global travel and trade, changes in the environment, and increasing development of land for human use, invasive plant species are difficult to overlook (Kolar and Lodge 2001). Furthermore, they can be problematic by increasing operational costs in agricultural crops, provide harboring sites for disease carrying insects, degrade water quality, negatively impact transportation, decrease property value, and displace wildlife and fish habitats (Andersen et al. 2004; Holm et al. 1977; Radosevich et al 2007; Rockwell 2003; Ross and Lembi 1999). Moreover, invasive

aquatic species continue to spread due to the interconnected nature of many systems of the world's waterways and ecosystems, and prompting vegetation managers with more challenges to control invasive species at the local level, before there are regional ramifications (Reichard and White 2001, Zumerchik and Danver 2010).

Giant salvinia, *Salvinia molesta* Mitchell (Salviniales: Salviniaceae), is native to Brazil and is one of the world's worst aquatic weeds (Koutika and Rainey 2015). It is considered invasive in numerous countries including the U.S. (McFarland et al. 2004), Sri Lanka, Cuba, Caribbean Islands, Colombia, Fiji, New Zealand, Trinidad and Tobago (Holm et al. 1977), India (Cook 1971), Australia, Papua New Guinea (Mitchell 1979), New Zealand, Indonesia, Malaysia, Singapore (Nelson 2009), and more than 20 African countries (Cilliers 1991, Mitchell and Tur 1975). *Salvinia molesta* is the second most invasive species in the world based on environmental, economic, and human health impacts (Barrett 1989) and is the predominant species of the Salviniaceae family found in the U.S. (USDA-NRCS 2017). It was first discovered in 1995, in a small pond in South Carolina; however, it was successfully eradicated with herbicides and no further cases were reported (Chilton et al. 2002; Johnson 1995). In 1998, a new outbreak was reported on Toledo Bend, a natural reservoir bordering Louisiana and Texas (Chilton et al. 2002), which continues to thrive today. By the conclusion of 1999, *S. molesta* was reported in more than 50 waterbodies across the U.S. including Louisiana, Texas, Arizona, California, Mississippi, Alabama, Florida, Georgia, South Carolina, North Carolina and Virginia. (USDA-NRCS 2017). *Salvinia molesta* was listed on the Federal Noxious Weed list in 2010 by the U.S. Department of Agriculture (USDA 2010) and up

to $100 million per year are spent annually in the U.S. to manage this noxious species (OTA 1993).

Salvinia molesta is considered one of the world's worst weeds because of its high mobility, tolerance to environmental stress, exponential growth rate and level of difficulty to control (Nelson et al. 2001; Thomas and Room 1986; Tipping 2004). It is a sterile, free floating aquatic fern indigenous to south-east Brazil (Hennecke and Postle 2006, Loyal and Grewal 1966) and is found most abundant between latitude 25° and 30°S (Forno 1983). Individual plants are comprised of two floating fronds (leaves), which are lined with branched trichomes and fused at the distal end, similar to an "egg-beater", with a third modified submerged leaf that is greatly dissected, suspended in the water, and functions as a root (Croxdale 1978; Forno 1983; Room 1983). Sterile sporocarps are subsessile and attached by elongate chains among the submersed underwater leaves (Loyal and Grewal 1966). A phyllotactic unit of *S. molesta* is comprised of three sets of individual plants, including a lateral bud and interconnected by branching rhizomes, which forms colonies on the water surface (Croxdale 1978; Room 1983). The upper surface trichomes aid the plant in repelling liquid and creating air traps for flotation, while the submersed plant tissues provide resistance to the water and help to stabilize the plant (McFarland et al. 2004).

Salvinia molesta has an aggressive growth rate and three distinct growth stages, primary, secondary, and tertiary. During the primary growth stage, or the initial plant invasion stage, *S. molesta* produces smaller leaves (1.5 cm), has long internodes, and floating leaves are flat on the water's surface, whereas leaves in the secondary growth stage are larger in width (2cm), slightly cupped and partially in contact with the water's

surface (Mitchell and Tur 1975). The tertiary growth stage is the mat-forming stage and the only stage to bear sterile sporocarps (Mitchell and Tur 1975). The leaves are much larger in width (6 cm), intricately folded along shorter internodes, and sometimes do not come in contact with the water's surface (Mitchell and Tur 1975). As plants mature into tertiary growth stage, dense mats expand and restrict boat travel for commerce, recreational activities, and irrigation (Sullivan and Postle 2012; Thomas and Room 1986; Tipping 2004). Thick mats of *S. molesta* can also pose health risks to humans by providing ideal breeding habitats for mosquitoes, which are vectors for human pathogens (Lounibos et al. 1990; Room et al. 1989). In addition, thick surface mats of *S. molesta* alter the natural dynamics of the water column by preventing sunlight and oxygen from entering the waterbody (van Oosterhout 2006). Furthermore, natural plant mortality can lead to toxic algal blooms, loss of submerged aquatic vegetation, and shifts in food webs due to excessive nutrient load from plant degradation that exceeds the natural capacity for nutrient assimilation (Rabalais 2002). Overall, the excessive growth rate of *S. molesta* can degrade habitats for other aquatic plants, fish, invertebrates and wildlife (Barrett 1989; Madsen 2014).

1.2 Control Methods for Giant Salvinia

Management techniques to control *S. molesta* include physical and mechanical removal, lake drawdowns, aquatic herbicides and biological control agents (van Oosterhout 2006; Richardson 2008; Thomas and Room 1986). Physical and mechanical removal is useful for small infestations; however, this process is highly labor intensive and aquatic plants generally consist of more than 90% water, making disposal of plant material problematic (Madsen 2000). Even small infestations of floating plants

can be expensive and time consuming for mechanical harvesting equipment (Haller 2014). Other problems associated with floating plants are their high mobility, which create an unpredictable plant harvest during unfavorable windy conditions. In addition, some plant harvesters are unable to maneuver in shallow water as well as among trees, rocks and stumps where *S. molesta* quietly inhabits (Haller 2014). Water drawdowns in lakes, by usage of drainage structures, have been effective by exposing the vegetation to lethal freezing or drying conditions (Bellaud 2014); however, downstream water body infestations are inevitable. Aquatic weed booms can be placed for prevention and isolation of new infestations downstream, but requires integration of chemical control and intensive monitoring for potential breaches in the equipment (Mike Boydstun, Red River Waterway Commission (RRWC), personal communication, 2017).

Chemical control is the most frequently and successfully used aquatic plant control method in the U.S., especially for *S. molesta* management (Netherland 2014; Ross and Lembi 1999). Currently, there are fourteen active ingredients registered for use in and around aquatic habitats by the U.S. Environmental Protection Agency (USEPA) (Netherland and Jones 2012; University of Florida 2014). Ten of those herbicides are efficacious against *S. molesta* including bispyribac-sodium, diquat dibromide, flumioxazin (Masser et al. 2013), carfentrazone-ethyl (Glomski and Getsinger 2006), copper, glyphosate, endothall (Nelson et al. 2001), topramezone (Mudge 2016), fluridone and penoxsulam (Mudge et al. 2012); all of which have varying degrees of activity. Large scale, repeated applications of herbicides are made annually in Louisiana and Texas, but yearly estimates of *S. molesta* have been increasing since 2011 (Alexander Perret, Louisiana Department of Wildlife and Fisheries (LDWF),

personal communication, 2017) and total annual costs for herbicide treatments are increasing [Mike Boydstun RRWC, Thomas Decker Texas Parks and Wildlife Department (TPWD), personal communication, 2017]. While herbicides are the most widely used form of aquatic weed management, Thayer and Haller (1985) indicated that small floating aquatic plants, including the salvinia species, can be difficult to treat chemically due in part to proximity to water and growth habits. Additionally, costs associated with chemicals and mechanical removal have led researchers to investigate biological control options, which have been shown to significantly reduce management costs of *S. molesta* over time (Cilliers 1991; Cuda 2014; Room et al. 1981, 1989).

Classical biological control of *S. molesta* involves a small weevil (*Cyrtobagous salviniae* Calder and Sands Coleoptera: Curculionidae) that is native to the same region in Brazil as *S. molesta*, and is highly host specific to the genus salvinia (Forno et al. 1983). Adult *C. salviniae* are approximately 2 to 3 mm in length and primarily feed on the new buds of the *S. molesta* (Sullivan et al. 2011). Females can lay over 300 eggs that are deposited singly in feeding scars in the lower leaf tissue (Hangay and Zbrowski 2010). Eggs hatch in approximately 10 days at optimal temperatures, then larvae complete three instars (growth stages) in 23 days (Cilliers 1991). Larva are white and grub like in appearance and feed on new buds before completing development within the rhizome (stem) of the plant (Sullivan and Postle 2012). Pupation occurs in approximately 10 to 15 days and cocoons are spun below the water surface in the root mass (Cilliers 1991). Newly emerged adults are brown, turn black in approximately 5 days, and immediately begin feeding on host plants (Sullivan and Postle 2012). Both adults and larvae damage *S. molesta*; however, larvae are thought to be most

destructive due to tunneling into the rhizome, disrupting nutrient transfer from the modified root mass to the growing points of the plant (Sands et al. 1983).

1.3 Challenges with Control Methods

While chemical control is the most widely used form of management to control the exponential growth of *S. molesta* in public and private waterbodies throughout the U.S. (Netherland 2014), there are associated risks with such management strategies. Unlike aquatic plant management, agronomic crops have been plagued since 1970 with weed resistance reports and have accelerated dramatically (Heap 2017, Ryan 1970). Although plant mechanisms involved in developing resistance are still unclear, it is widely believed that resistance is due to 1) repeated use of herbicides over multiple growing seasons, or 2) the repeated use of herbicides that have the same mode of action within the plant (i.e. amino acid inhibitors) (Netherland 2014). Recent reports of the first major aquatic weed resistance in *Hydrilla verticillata*, another non-native species (Michel et al. 2004), has increased sensitivity to the issue among aquatic plant managers (Netherland 2014). It is believed that in the absence of sexual reproduction, the evolution of herbicide resistance is unlikely (Powles and Holtum 1994); however, *H. verticillata* and *S. molesta* both reproduce asexually, by vegetative propagation. Although there is no history of herbicide resistance with regard to *S. molesta* in Texas and Louisiana, the same herbicides (i.e. glyphosate and diquat) have been used repeatedly for nearly a decade (Mike Boydstun RRWC, Thomas Decker TPWD, Alex Perret LDWF, personal communication 2017). Although, recent research has shown that alternative herbicide mixes in the growing season provided similar control (Mudge et al. 2016), natural resource agencies have not utilized herbicides with alternate modes

of action on an operation scale. Nonetheless, implementation and use of biological control remains important.

Several tropical and subtropical countries including Australia, India, Sri Lanka, Papua New Guinea, and South Africa have reported complete control with the *C. salviniae* (Center et al. 2002; Cilliers 1991; Room et al. 1981, 1989; Sullivan et al. 2011). Unfortunately, winter mortality of *C. salviniae* has been reported in temperate regions of Australia (Julien et al. 2009), suggesting that the range of the insect's survivability during winter months is limited. Natural resource agencies are often required to reintroduce *C. salviniae* to previously infested release sites, particularly after a severe winter where water temperatures remain at or below freezing for more than twelve hours. The first U.S. release of *C. salviniae* was conducted in Texas and Louisiana in 1999 on the Toledo Bend Reservoir (Tipping and Center 2005) and mass releases of weevils have subsequently followed since 2001 (Johnson et al. 2010). Although Tipping and Center (2003) reported that both *S. molesta* and *C. salviniae* overwintered in Louisiana and Texas, the most northern recovery point was south of the 31°N latitude (northern Toledo Bend Reservoir), which is not inclusive to the northern distribution of *S. molesta* (34°N) in Louisiana (Thayer et al. 2017).

More research is needed to locate a cold-tolerant ecotype of *C. salviniae* for use in northern Louisiana, otherwise biological control will be subject to winter kill on an annual basis (Micinski and Fitzpatrick 2016). Although thermal tolerance of *C. salviniae* has been investigated, research is lacking on the overall effect of cold temperatures on adult performance and reproduction (Allen et al. 2012, 2014). *Cyrtobagous salviniae* are reported to begin oviposition (Mukherjee et al. 2014) and eggs are reported to begin

hatching above 19°C (Forno et al. 1983). Mukherjee et al. (2014) also measured cold tolerance by recording the recovery time of four *C. salviniae* ecotypes when exposed to partially frozen water and found that an Australian ecotype possessed 1.4-times greater tolerance at 0°C for 36 hours than the ecotypes previously released in the U.S. Recent attempts to locate a foreign ecotype that will withstand northern Louisiana winters, proved to be successful based on chill coma recovery time, supercooling point, and survival at 0°C from a South American population (Russell et al. 2017). While this research is important for future management, the recovered population from South America will have to be quarantined and screened to meet federal guidelines before being deployed in the natural environment (Schultz 2016). Research by Mukherjee et al. (2014) provides a foundation for comparing U.S. populations that have recently overwintered in the northern range. Focus should be on testing cold tolerance of these surviving populations from the most recent winters in northern Louisiana, with potential to mass rear and release insects to infested areas in northern Louisiana, especially due to the difficulty to import the Australian ecotype and time restrictions on the South American ecotype.

Mass rearing of *C. salviniae* occurs in outdoor earthen ponds or in temperature controlled greenhouses to maximize production for release into field locations (Knutson and Nachtrieb 2012; Wahl et al. 2016). Currently in the U.S., these facilities harvest and release the entire plant, containing all life stages of the insect and transfer infested *S. molesta* to the desired location (Sanders et al. 2011; Wahl et al 2016). Sanders et al. (2011) recommended to relocate the infested plant material within 24 to 36 hours of harvest, to ensure minimal insect mortality; however, literature describing *C. salviniae*

mortality was unavailable. Furthermore, Wahl et al. (2016) recommended that transportation totes should be kept in shaded areas if harvested weevils are not released on the same day. Sanders et al. (2011) also recommended releasing infested material in the spring and/or early fall to avoid summer and winter months that can cause stress to both the plant and insect. Unfortunately, a literature review for these recommendations was also unavailable. Sullivan and Postle (2012) noted that releasing all life stages in the spring maximizes the chances for weevil populations to increase before winter; however, if weevil populations in greenhouses increase to desirable release rates, then infested material should be comprised primarily of adults, since they will overwinter the best and begin egg laying the following spring. Australian researchers also recommend that infested material should be transported in plastic containers with minimal water and containers kept in a cool shaded area (Sullivan and Postle 2012). Although researchers claim cooler months are the most efficient time to establish *C. salviniae* populations (Sullivan and Postle 2012; Sullivan et al. 2011), no literature exists on the ideal temperatures for releasing *C. salviniae*. Along with the potential benefits of a more suitable insect in its most northern range in Louisiana and Texas, additional research is required to identify the optimal conditions for the transportation of *C. salviniae* infested *S. molesta*, including evaluating the survivability of adults within transportation containers for specific time/temperature intervals.

Under ideal growth conditions, *S. molesta* can exceed the effectiveness of mechanical, herbicide or biological treatments alone. Consequently, individual management techniques will only provide temporary relief and require continuous management (Johnson 2005; Thomas and Room 1986; van Oosterhout 2006). Utilizing

an integrated pest management (IPM) approach by combing management techniques that are environmentally compatible can reduce long term costs, ultimately reducing pest populations to tolerable levels (USDA-NIFA 2017). Watson and Wymore (1989) explain that maintaining a detrimental environment to weed populations, by using a variety of methods, is the key to a successful management system. Haseler (1980) emphasizes that in addition to biological control, some level of alternative control method is necessary to achieve satisfactory reduction in target weeds. Natural resource managers in Louisiana and Texas are challenged with new infestations annually and the increasing costs associated with the chemical management of *S. molesta* (Mudge and Harms 2012). As suggested by Mudge and Harms (2012), a more prudent approach to managing *S. molesta* would be to combine technologies instead of an herbicide or biological control alone focused program, potentially providing more effective and long-term management of *S. molesta*. In addition to implementing an IPM program and searching for a cold tolerant U.S. ecotype of the *C. salviniae*, focus should be toward improving and understanding the complexities of current methods of mass rearing and releasing weevil populations.

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CHAPTER 2. COLD TOLERANCE OF THREE GIANT SALVINIA WEEVIL POPULATIONS FROM LOUISIANA

2.1 Introduction

Salvinia molesta Mitchell (Salviniales: Salviniaceae), giant salvinia, is one of the world's most invasive weeds (Koutika and Rainey 2015) due to its rapid growth, potential to spread, and competition with native plant species (Holm et al. 1977; Mitchell 1979). *Salvinia molesta* also causes degradation of water quality due to excessive siltation and disruption of nutrient cycling, ultimately affecting wetland functions (Masser 2007). Control of *S. molesta* includes physical, mechanical, and chemical methods (Thomas and Room 1986); however, surviving plants or fragments that persist after these control methods often recolonize rapidly, and can lead to economically impractical and indefinite applications (Tipping et al. 2008). Biological control is a self-sustaining, environmentally safe alternative for management of *S. molesta* (Van Driesche et al. 2010).

Classical biological control involves the introduction of host-specific natural enemies, found in the native range of the pest, and reunites the natural enemy with the invasive pest to restore the ecological balance of a habitat (Culliney 2005; Manrique et al. 2011). *Cyrtobagous salviniae* Calder and Sands (giant salvinia weevil, Coleoptera: Curculionidae) is highly host-specific to the genus *Salvinia* (Forno et al. 1983), measures approximately 2.5 mm in length (Sullivan et al. 2011), and lives gregariously among *S. molesta* mats in its native range (Cilliers 1991). Larvae and adults feed on all parts of the plant with preference to apical buds due to high nitrogen content (Cilliers 1991; Sands et al. 1983). Females oviposit eggs singly in feeding scars either in stem cavities or suspended in the root mass, larva tunnel into the rhizome to complete

development, and pupation occurs in a cocoon, usually in the root mass (Cilliers 1991). While adult feeding on terminal nodes restricts plant growth, plant mortality is primarily due to tunneling of rhizomes by the larvae (Sullivan and Postle 2010).

Worldwide success has been reported with *C. salviniae*, particularly in tropical and subtropical climates similar to Brazil (Mitchell 1979; Room et al. 1981; Thomas and Room 1986), yet limited success has been reported in temperate climates similar to northern Louisiana and Texas (Mukherjee et al. 2014). In Louisiana, success has been limited to the southern regions (Bogren 2016), particularly in the USDA Plant Hardiness Zone 9a (Figure 2.1) where the average extreme minimum temperatures range from -6.7 to -3.9°C. Efforts to establish *C. salviniae* in more northern Louisiana sites have been limited and if successful, unreported. These zones include 8b and 8a where the average extreme minimum temperatures range from -9.4 to -6.7°C and -12.2 to -9.4°C, respectively. In northern Louisiana and Texas, severe winters of 2009-2010 and 2010-2011 decimated weevil populations (Sanders 2011, Mukherjee et al. 2014). Sanders (2011) suggested that biological control agents will be subject to winter kill, and more research is needed to find a cold-tolerant ecotype of *C. salviniae* for use in northern Louisiana. In 2015, overwintered populations of *C. salviniae* were observed in northern Louisiana, specifically in plant hardiness zone 8b and 8a (L. Cozad, unpublished data).

Previous research demonstrated that ecotypes of *C. salviniae* from Australia (Mukherjee et al. 2014), Argentina, and Uruguay (Russell et al. 2017) had greater cold tolerance to 0°C when compared the southern Louisiana *C. salviniae*. Although researchers are seeking overseas *C. salviniae* populations for potential management of *S. molesta* in northern Louisiana and Texas, these ecotypes are not available for

immediate release, and they must be quarantined until federal guidelines are met before mass production and distribution (Schultz 2016). An expedited approach would be to compare the cold tolerance of established U.S. populations from the northern range of Louisiana, and if cold tolerance is exhibited, these populations can immediately be mass reared and released, without federal restrictions.

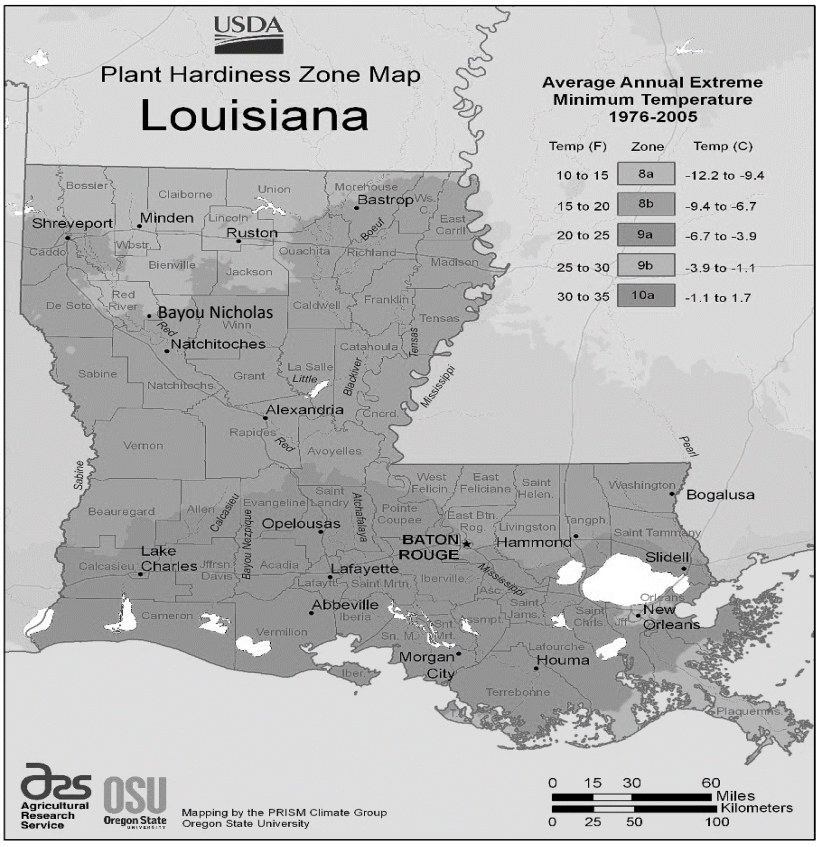


Figure 2.1. Plant Hardiness Zone Map of Louisiana (USDA.gov).

Because weevil populations have been released since 2012, I hypothesized that overwintered weevils in northern locales of Louisiana will have greater cold tolerance compared to those from southern locations. To test this hypothesis, I obtained weevil populations from two northern Louisiana sites, Bayou Nicholas with average extreme minimum temperatures of -12.2 to -9.4°C, and Natchitoches with average extreme

minimum temperatures of -9.4 to -5.7°C. Additionally, weevils were obtained from a southern site in Houma, Louisiana with average extreme minimum temperatures of -6.7 to -3.9°C. The specific objective of this study was to compare cold tolerance and adult survival of these weevil populations at 0, -5, and -9°C with varying lengths of exposure using climate controlled growth chambers.

2.2 Materials and Methods

Weevil populations evaluated in this study were from Houma, LA (29.56°, -90.77°), the originating population established in 2007 (Sanders et al. 2012); Natchitoches, LA (31.77°, -93.06°), established in 2013; and Bayou Nicholas (31.96°, -93.30°), established in 2012. Populations hereafter will be referred to as Houma, Natchitoches, and Bayou Nicholas populations, respectively. All trials were conducted at Red River Waterway Commission's Aquatic Research Facility located in Lena, LA (31.52°, -92.73°). Adult *C. salviniae* were collected from three field sites in Louisiana in January and February of 2016 for trial 1. Insects from Houma and Natchitoches were isolated and overwintered outside the laboratory between trials, while the Bayou Nicholas population was collected from the original field site for trial 2, completed in February and March of 2017. Adults were extracted from *S. molesta* host plants using Berlese funnels (Boland and Room 1983) and held at laboratory temperatures (24°C) for a maximum of 96 hours. Adult *C. salviniae* were acclimated in an environmental growth chamber (Percival I-36VLC8) on *S. molesta* plants for 72 hours at 13°C prior to exposure treatments (Hennecke and Postle 2006; Russell et al. 2017). Acclimation and experimental exposures were conducted in complete darkness to ensure consistent temperatures (Russell et al. 2017).

Survival of adult *C. salviniae* was measured by placing groups of 10 adults on 2 complete phyllotactic units of fresh *S. molesta* plants (Croxdale 1978) in glass jars containing 400 mL of rainwater (pH 6.7). Groups of 10 adults encompassed a replicate and each treatment was replicated four times (n = 40). Immediately following acclimation, treatment groups were simultaneously exposed to either 0, -5, or -9°C in separate environmental growth chambers, and optimum length of exposure for each temperature was determined from preliminary experiments (Table 2.1). Following exposure treatments, experimental groups were removed from environmental growth chambers and allowed to recover at laboratory temperatures (24°C) for 24 hours (Mukherjee et al. 2014). Survival was assessed by removing adult *C. salviniae* from plant material and placing them in Petri dishes for two hours to record movement. Adults were placed in the dorsal position and considered deceased if they could not right themselves into walking position (Mukherjee et al. 2014). Survivability data was analyzed using logistic regression analysis with condition after exposure as the dependent binary value (0 for dead, 1 for alive) plotted against the exposure time for coinciding temperatures (JMP®, Version 13). Inverse prediction was used to calculate the lethal time at which 50 and 90% of each population was deceased (LT_{50} and LT_{90}, respectively), with 95% confidence intervals (Bean et al. 2007).

Table 2.1. Temperatures and length of exposures that three Louisiana populations of adult *Cyrtobagous salviniae* were exposed to after a 13°C acclimation period for 72 hours.

Temperature	Length of Exposure (hours)
0°C	0, 24, 48, 72, 96, 120, 144, 168, 192, 216, 240
-5°C	0, 3, 6, 9, 12, 15, 18, 20, 24, 26
-9°C	0, 3, 5, 7, 9, 11, 15, 18

2.3 Results and Discussion

Cyrtobagous salviniae from the Bayou Nicholas population exposed to 0°C was 1.3- and 1.4-times more cold tolerant in 2016 at both LT_{50} and LT_{90} values compared to the Houma and Natchitoches populations, respectively (Table 202). In 2017, there were no significant differences between populations; however, there was a year effect ($p<0.05$), and an increase in cold tolerance by 34.6 hours for the Houma population at LT_{90}, and an increase in cold tolerance for the Natchitoches population by 26.6 and 41.1 hours at LT_{50} and LT_{90} values, respectively (Table 2.2). The higher level of cold tolerance exhibited by the Bayou Nicholas population in 2016 is likely due to phenotypic plasticity as a result of differential environmental conditions that existed since field establishment in 2012. Biotic and abiotic factors are involved in an insect's ability to adapt to different environments and the induced changes within a single genotype can facilitate evolutionary effects, thus resulting in the plasticity of the insect and an altered phenotype (Whitman and Agrawal 2009). For example, the average minimum winter temperature (November to January) was 1.7°C for Bayou Nicholas compared to 6.4°C for Houma and 2.2°C for Natchitoches (US Climate Data 2017). Although all insects populations are from the same Brazilian genotype (Eisenberg and Johnson 2012), environmental conditions (i.e. minimum winter temperatures) may have altered the Bayou Nicholas population's plasticity, or the ability to withstand the winter in the specific region (Bradshaw 1965) prior to this study.

Table 2.2. Survival of *Cyrtobagous salviniae* populations from Houma, Natchitoches, and Bayou Nicholas, LA, acclimated at 13°C for 72 hours then exposed to 0°C in 2016 and 2017.

Year	Population	n	Slope ± SE	LT_{50}[a] (95% CI)[b]	LT_{90}[a] (95% CI)[b]	χ^{2}, [c]
2016	Houma	400	-0.04 (±0.004)	73.7 (65.6-81.6)	129.6 (118.3-144.9)	103.42
	Natchitoches	400	-0.05 (±0.005)	70.0 (62.9-76.0)	114.3 (104.7-127.6)	91.74
	Bayou Nicholas	400	-0.03 (±0.003)	98.9 (90.3-107.6)	164.1 (151.3-181.5)	113.29
2017	Houma	400	-0.03 (±0.003)	88.1 (78.4-97.6)	164.2 (149.4-184.8)	101.74
	Natchitoches	400	-0.04 (±0.003)	96.6 (88.2-105.0)	158.4 (145.9-175.6)	106.43
	Bayou Nicholas	400	-0.03 (±0.003)	106.4 (97.0-116.1)	182.4 (166.9-204.1)	101.60

[a] LT_{50} and LT_{90} values represent the time in hours to kill 50 and 90% of the populations.
[b] Non-overlapping CI (confidence intervals) indicate differences in populations.
[c] χ^{2} is the Wald test for the hypothesis that the slope parameter is zero.

Similar to the 0°C trial, *C. salviniae* exposed to -5°C showed a year effect and increased cold tolerance from 2016 to 2017 in the Houma and Natchitoches populations. At -5°C, the Bayou Nicholas population was 1.1- and 1.3-times more cold tolerant in 2016 than the Houma and Natchitoches populations, respectively (Table 2.3). Insect phenotypic plasticity based on geographic location is also likely the explanation for results at this temperature exposure. In 2017, there were no differences in cold tolerance between populations at the LT_{50} or LT_{90}, presumably due to the increase in cold tolerance of the Natchitoches and Houma populations from 2016 to 2017.

Table 2.3. Survival of *Cyrtobagous salviniae* populations from Houma, Natchitoches, and Bayou Nicholas, LA, acclimated at 13°C for 72 hours then exposed to -5°C in 2016 and 2017.

Year	Population	n	Slope ± SE	LT_{50}[a] (95% CI)[b]	LT_{90}[a] (95% CI)[b]	χ^{2}, [c]
2016	Houma	400	-0.47 (±0.05)	14.1 (13.3-14.9)	18.9 (17.8-20.3)	94.10
	Natchitoches	400	-0.49 (±0.05)	12.3 (11.6-13.1)	16.9 (15.8-18.3)	90.49
	Bayou Nicholas	400	-0.35 (±0.03)	15.5 (14.6-16.4)	21.7 (20.4-23.5)	107.42
2017	Houma	320	-0.26 (±0.03)	15.5 (14.3-16.8)	24.0 (22.2-26.7)	89.91
	Natchitoches	360	-0.52 (±0.06)	15.2 (14.4-16.0)	19.4 (18.4-20.9)	83.27
	Bayou Nicholas	320	-0.35 (±0.04)	16.4 (15.3-17.4)	22.6 (21.2-24.6)	75.84

[a] LT50 and LT_{90} values represent the time in hours to kill 50 and 90% of the populations.
[b] Non-overlapping CI (confidence intervals) indicate differences in populations.
[c] χ^{2} is the Wald test for the hypothesis that the slope parameter is zero.

At -9°C exposures, there were no significant differences between weevil populations or trial years (Table 2.4); however, these data demonstrate survival of *C. salviniae* at lower temperatures than previously reported. Allen et al. (2012) reported 50% mortality of *C. salviniae* at -7.2°C for a one hour exposure period, whereas the Bayou Nicholas LT_{50} was 8.6-times higher in 2016 and 2017, at a colder temperature (-9°C). The notable differences in survival could be resultant of the insects utilized in the studies. Allen et al. (2012) utilized *C. salviniae* from an outdoor colony in Cato Ridge, South Africa (-29.74°, -30.59°) that were exposed to natural conditions prior to experiments; however, the average low

temperature during the coldest month in this region was 13.6°C (WWO 2017). The U.S. populations used in the current research were exposed to much lower temperatures throughout the winters after establishment (US Climate Data 2017). The results from this study, where all Louisiana populations survived at lower temperatures for longer periods of time compared to Allen et al. (2012), is indicative that this particular insect is capable of adapting to localized conditions (phenotypic plasticity) among a single genotype of *C. salviniae*.

Table 2.4 Survival of *Cyrtobagous salviniae* populations from Houma, Natchitoches, and Bayou Nicholas, LA, acclimated at 13°C for 72 hours then exposed to -9°C in 2016 and 2017.

Population	n	Slope ± SE	LT_{50}[a] (95% CI)[b]	LT_{90}[a] (95% CI)[b]	χ^{2}, [c]
Houma	600	-0.46 (0.04)	7.3 (6.9-7.8)	12.1 (11.3-13.2)	156.70
Natchitoches	640	-0.68 (0.05)	7.9 (7.5-8.3)	11.1 (10.6-11.8)	158.32
Bayou Nicholas	440	-0.49 (0.05)	8.6 (8.0-9.3)	13.1 (12.2-14.5)	111.43

[a] LT_{50} and LT_{90} values represent the time in hours to kill 50 and 90% of the populations.
[b] Non-overlapping CI (confidence intervals) indicate differences in populations.
[c] χ^2 is the Wald test for the hypothesis that the slope parameter is zero.

Overall, the results from these trials show *C. salviniae* capable of overwintering and naturally developing cold tolerance in northern Louisiana. The Natchitoches and Bayou Nicholas populations were directly harvested from Houma and relocated to northern Louisiana 4 and 5 years prior to the completion of this study, respectively. Although the Natchitoches and Bayou Nicholas populations are close in proximity (36.4 km), field sites differed with respect to water depth, size, and canopy coverage. The waterbody where the Natchitoches population was collected is shallower and smaller in size compared to the Bayou Nicholas location, but leaf litter from deciduous trees may have aided in the long-term overwintering success of the insects. Visual assessments of

these sites showed less canopy coverage for the Bayou Nicholas site and ultimately less leaf litter during winter months (L. Cozad personal observations), thus subjecting the insects to colder and more natural air temperatures, which may have selected more cold tolerant weevils. While the results from this experiment indicate the Natchitoches population to be the least cold tolerant in 2016 and 2017 at -5° and -9°C, respectively, the overwintering conditions in the field (i.e. leaf litter refugia) may have allowed this population to persist naturally, but thrive with less influence from harsher winters.

Many factors contribute to an insect's ability to tolerate cold temperatures, including specific developmental stage and the direct nutritional status of the insect as a result of host plant nutrition (Bentz and Mullins 1999; Morey et al. 2016). For instance, *C. salviniae* prefer high nitrogen host plants (Forno and Semple 1987), and Awmack and Leather (2002) reported that high nitrogen content in host plants increased growth rate in *Samea mulitplicalis* (Lepidoptera: Pyralidae), a phytophagous insect that also feeds on *S. molesta*. Similarly, high host plant nutrition (i.e. nitrogen) aided *Dendroctonus ponderosae* (Coleoptera: Curculionidae) larvae to accumulate nutritive reserves (i.e. weight) before winter which increased pupation rates following winter (Goodsman et al. 2012). The overwintering conditions and plant nutrition (i.e. nitrogen) for the Natchitoches and Houma populations prior to the 2017 trial may explain the increased insects' fitness and nutritional status.

Other factors contributing to insect's overwintering fitness include behavioral avoidance, physiological and biochemical factors (Bale and Hayward 2010; Lee 1989), as well as fluctuating thermal regimes (FTR) in natural habitats (Koštál et al. 2007). For example, when the adult tropical beetle, *Alphitobius diaperinus* (Coleoptera:

Tenebrionidae), was exposed to FTRs of 0°C (12H/20°C (12H), survival was considerably improved, compared to a constant 0°C temperature (Lalouette et al. 2007). Additionally, cold survival was enhanced in *Drosophila melanogaster* (Diptera: Drosophilidae) when exposed to an acclimation period followed by FTRs compared to adult flies exposed to constant low temperatures (Marion et al. 2016). Increased field survival of *C. salviniae* may have been due to large variability of FTR's during winter, where northern Louisiana winter temperatures fluctuate from 15 to -8°C, and southern Louisiana winter temperatures fluctuate from 19 to 8°C (JRTC-POLK 2017). The difference in FTR's within the state may be a plausible explanation for a significantly more cold tolerant Bayou Nicholas population compared to the Houma population in the south.

Survival of adult *C. salviniae* in the introduced range is vital to the spread and growth of insect populations (Allen et al. 2012), and the Bayou Nicholas population could improve management strategies in the northern range of Louisiana and Texas. The importance of this cold tolerant, phenotypically plastic ecotype is vital for sustaining insect populations in the short term. In the long term, researchers should continue to focus on implementation of the foreign ecotype of *C. salviniae* from the Lower Paraná-Uruguay Delta, which had an LT_{50} of 175 hours at 0°C (Russell et al. 2017), compared to Bayou Nicholas population with an LT_{50} of 99 hours at the same temperature exposure in 2016. Also, the importance of artificial or natural winter substrate creating a refugia may provide microclimates for insect survivability and aid in long term management of *S. molesta* (Moshman 2016). Furthermore, efforts should focus on

FTR's and the necessity of these regimes prior to exposure temperatures in future research.

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CHAPTER 3. HEAT TOLERANCE OF THE GIANT SALVINIA WEEVIL

3.1 Introduction

The effects of non-native invasive species on ecosystems include reduction of biodiversity, decline in native species (Gilbert and Levine 2013), changes in ecosystem function (Vilà et al. 2011), and negative economic impacts (Simberloff et al. 2005). *Salvinia molesta* Mitchell (Salviniales: Salviniaceae), giant salvinia, is one of the world's worst invasive weeds (Koutika and Rainey 2015), and is responsible for nearly $7 million in damage to the state of Louisiana (LSU 2015). The rapid growth rate of *S. molesta* can degrade habitats for other aquatic plants, fish, invertebrates and wildlife (Barrett 1989; Madsen 2014), and can alter dynamics of the water column by preventing sunlight and oxygen from entering the waterbody (van Oosterhout 2006). Furthermore, mats of *S. molesta* can provide ideal breeding habitats for mosquitoes, which are vectors for human pathogens (Lounibos et al. 1990; Room et al. 1989).

Management efforts include physical and mechanical removal, lake drawdowns, aquatic herbicides and biological control agents (van Oosterhout 2006; Richardson 2008; Thomas and Room 1986). While these methods are circumstantially efficacious, recent attention has been directed toward biological control for long-term, cost effective control. The biological control agent utilized in the U.S. for *S. molesta* is *Cyrtobagous salviniae* Calder and Sands (Coleoptera: Curculionidae), a small weevil that is native to the same region as *S. molesta* (Brazil) and is highly host specific (Forno et al. 1983). The first U.S. release of *C. salviniae* was conducted in Texas and Louisiana in 1999 (Tipping and Center 2005), and in 2001, mass releases of *C. salviniae* began

throughout the adjoining states (Johnson et al. 2010) and continue throughout Texas and Louisiana.

Mass rearing of *C. salviniae* occurs in outdoor earthen ponds or in temperature controlled greenhouses to maximize production for release into field locations (Knutson and Nachtrieb 2012; Sullivan et al. 2011; Wahl et al. 2016). In the U.S., rearing facilities harvest and release *S. molesta* that contains all life stages (egg, larva, pupa and adult) of *C. salviniae* when densities reach more than 30 to 50 adults per kilogram of fresh *S. molesta* (Wahl et al. 2017) then transported to the desired location (Knutson and Nachtrieb 2012; Wahl et al 2016). Natural resource managers in Louisiana and Texas typically utilize light colored plastic storage totes (68 to 76 L) to transport *S. molesta* infested with *C. salviniae* from the source (i.e. greenhouse or pond) to a new field location (Wahl et al. 2017). These totes are modified with ten or more holes drilled in the bottom and sides to allow for drainage of excess water, covered with a fastened lid, and secured with zip ties. The containers are transported in truck beds, utility trailers and/or in metal boats (towed behind a truck) to the new location up to 500 kilometers from origination site. Despite the importance to a *S. molesta* biological control program, the heat tolerance and survival of *C. salviniae* during transport conditions have not been studied for local Louisiana populations. Although previous studies in south Africa determined that the upper lethal temperature to kill 50% of a *C. salviniae* population was 43.7±0.2°C for one hour of exposure in laboratory conditions (Allen et al. 2014), this current research sought to define relationships between air temperatures and exposure temperatures utilizing a U.S. population of *C. salviniae*.

Summer temperatures in Louisiana and Texas could reach the upper thresholds for insect survival. The average high temperatures in spring and fall in Louisiana are 25°C, while the summer months have an average high of 33°C (NCDC 2017); however, temperatures above 35°C are commonplace (JRTC-POLK 2017). *Cyrtobagous salviniae* is commonly released in the spring or fall to minimize the impact of the extreme heat during the summer months (Sanders et al. 2011). Although previous researchers claim cooler months are the most efficient time to establish *C. salviniae* populations (Sullivan and Postle 2012; Sullivan et al. 2011), no literature exists on specific heat temperatures U.S. insect populations are exposed to during transport. Additional research is required to identify the impact of heat stress on *C. salviniae* infested *S. molesta*, including evaluating the survivability of adults from Louisiana at upper lethal time/temperature intervals. Therefore, the objectives of this research were 1) to define temperatures *C. salviniae* are exposed to in summer months during biological control operations, and 2) to determine the upper temperature threshold for adult weevil survival.

3.2 Materials and Methods

3.2.1 Temperature Conditions Inside Transportation Totes

Data were collected to determine temperatures experienced by *S. molesta* and *C. salviniae* inside plastic totes during transportation compared to air temperatures. All three experiments were conducted during field harvests from rearing ponds in Houma, Louisiana (29.56°, -90.77°) or Lena, Louisiana (31.52°, -92.73°) to various release sites throughout Louisiana from May through September of 2016. Although these months are

not recommended due to high air temperatures (Sanders et al. 2011), trials were conducted under worst-case conditions for transportation of *C. salviniae*.

In the first study, internal tote temperature data were collected to determine temperature differences within a single tote, particularly at the top of the tote from sunlight, in the middle of the tote, and at the bottom of the tote due to conductive heat from trucks, trailers, or boats. Data were collected on May 14 and 22, 2016 using two light colored plastic totes (Rubbermaid®, 68L, 41.9 x 60.7 x 40.4 cm) and 6 HOBO® Pendant® data loggers (Onset Computer Corp., Pocasset, MA) set to record temperature every 30 minutes. Data loggers were placed directly on the bottom of the tote below the plant material, in the middle of the plant material, and directly on top of the plant material. Two types of lids were used to secure the totes, one with a conventional lid and one with a modified lid containing 10 holes (1.3 cm). Temperature collection occurred on both harvest days from 0930 to 1930 (n=42 per lid type). Both types of lids were secured using zip ties, transported in a boat (towed by a truck) from the insect nursery, and released into a field site 10 hours after initial harvest from the rearing site. Potential temperature differences due to placement of data loggers within the totes fastened with two types of lids in May 2016 were subjected to a three-way analysis of variance (ANOVA) at $p \leq 0.05$ (SigmaPlot 11.0).

In a second study, HOBO® Pendant® data loggers were used to evaluate impacts of air circulation within the tote using conventional and modified lids with holes and compared to air temperatures. Data loggers were placed in the top 2.5 cm of plant material within the tote to record internal tote temperature, air temperatures were also collected. Initial data yielded abnormally high air temperatures when data loggers were

placed directly on the outside of the lid and exposed to direct sunlight. For instance, on June 8, 2015, an external temperature of 48.3°C was recorded; however, the maximum recorded temperature for that day was 32.8°C (NCEI 2017). As a result of these initial findings, subsequent trials included HOBO® Pendant® loggers placed in two separate M-RSA Solar Radiation Shields™ (Onset Computer Corp., Pocasset, MA) and mounted to transportation vehicles, instead of directly mounted to the totes. The Solar Radiation Shield is a multi-plate plastic housing to protect the pendant device from direct sunlight and function as a thermal insulator for the most accurate measurements compared to several other methods (Ribeiro da Cunha 2015). Air temperature, tote temperature with conventional lids, and tote temperate with modified lids were subjected to a one way ANOVA and post-hoc tests (Fisher's protected LSD) were used for pairwise comparisons ($p \leq 0.05$, n=80) (SigmaPlot 11.0). Bivariate analysis was also used to define the linear relationship between the air temperature and the internal temperature of two types of lids ($y = ß_0 + ß_1x_i$) (JMP®, Version 13). The results of these analyses were used to determine temperatures and exposure periods for the laboratory mortality experiments.

3.2.2 Adult *Cyrtobagous salviniae* Heat Mortality Under Laboratory Conditions

Insect populations tested in this study were from Natchitoches, Louisiana (31.77°, -93.06°), established in 2013 and originally collected from Houma, Louisiana (29.56°, -90.77°). Adult *C. salviniae* were extracted from *S. molesta* host plants collected from the field site in the summer of 2016 using Berlese funnels (Boland and Room 1983) and held at laboratory temperatures (24°C) for a maximum of 96 hours at the Red River Waterway Commission facility in Lena, Louisiana. Survival of adult *C.*

salviniae was measured by placing groups of 10 adults on 2 complete phyllotactic units of fresh *S. molesta* (Croxdale 1978) in covered sterile Petri dishes (100 x 15 mm) containing moistened qualitative filter paper (Ahlstrom 9 cm diameter). Filter paper was moistened with 1 mL of rain water (pH 6.7) for treatment groups less than 24 hours, and 2 mL of rain water for exposures ≥24 hours to prevent plant and insect mortality as a result of desiccation. Groups of 10 adults comprised a replicate, each treatment was replicated 4 times, and the entire trial was repeated within 1 month. To understand a worst-case scenario and mimic field conditions, insects were not provided an acclimation period and adult *C. salviniae* were immediately exposed to 35, 40, 45, or 50°C in separate environmental growth chambers (Percival I-36VLC8). Length of exposures for each temperature scenario are detailed in Table 3.1. Exposure temperatures were selected from temperatures experienced in transportation totes (35 to 50°) and exposures were conducted in complete darkness to mimic transport conditions.

Table 3.1. Temperatures and length of exposures that a Natchitoches, Louisiana, population (31.77°, -93.06°) of adult *Cyrtobagous salviniae* were exposed to in environmental growth chambers to determine heat mortality.

Temperature	Length of Exposure (units)
35°C	0, 5, 10, 15, 20, 24, 28, 32, 36, 40 (hours)
40°C	0, 1, 5, 10, 20, 22, 24, 26 (hours)
45°C	0, 20, 40, 60, 80, 100, 120, 140 (minutes)
50°C	0, 2, 5, 7, 10, 15 (minutes)

Immediately following exposure, groups were removed from the growth chambers and allowed to recover at laboratory temperatures (24°C) for 1 hour. Insect mortality was assessed by removing adult *C. salviniae* from plant material and placing

them in Petri dishes with moistened filter paper (1 mL rainwater) for an additional hour before recording survival. Adults were placed in the dorsal position and considered deceased if they could not right themselves into walking position (Mukherjee et al. 2014). Due to no significant differences between trials (p=0.992), data were pooled (n=80). Mortality data were analyzed using linear regression analysis ($y = ß_0 + ß_1x_i$, ± 95% confidence intervals) with temperature as the independent variable and percent survival as the dependent variable for the coinciding temperatures (JMP). Also, lethal time at which 50 and 90% mortality was experienced (LT_{50} and LT_{90}, respectively) was calculated, with 95% confidence intervals.

3.3 Results and Discussion

3.3.1 Temperature Conditions Inside Transportation Totes

There were significant temperature differences between collection dates in May 2016 (p<0.001) (Table 3.2). For example, on May 15 and 22, 2016 the recorded high temperature for both days was 29°C (NCEI 2017), but sunlight or cloud cover may have impacted temperatures, which resulted in the difference. Regardless of collection date, there was a significant difference in the lid type used (p=0.048) and the mean internal tote temperature for conventional lids was 2°C higher than modified lids (Table 3.2). In addition, there were no differences in placement of the temperature logger throughout the tote (i.e. top vs. middle vs. bottom) (p=0.729) (Table 3.2, Figure 3.1); therefore, loggers were placed in the top 2.5 cm of plant material for future studies where air temperature was collected using solar radiation shields.

Table 3.2. Three Way Analysis of Variance (p≤0.05) for date of temperature collection, placement of temperature logger within transportation tote, and type of lid used on transportation tote.

Source of Variation	DF	SS	MS	F	P
Date[a]	1	4756555	4756555	89.191	<0.001
Placement of logger[b]	2	33713	16857	0.316	0.729
Lid Type[c]	1	211201	211201	3.960	0.048
Date x Placement	2	189009	94504	1.772	0.172
Date x Lid type	1	53737	53737	1.008	0.316
Placement x Lid type	2	21301	10651	0.200	0.819
Date x Placement x Lid type	2	8348	4174	0.078	0.925
Residual	240	12799190	53330		
Total	251	18073054	72004		

[a] May 15 and May 22, 2016.
[b] Top, middle, and bottom of plant material within transportation tote.
[c] Conventional lids and lids modified with 10 (1.3cm) holes

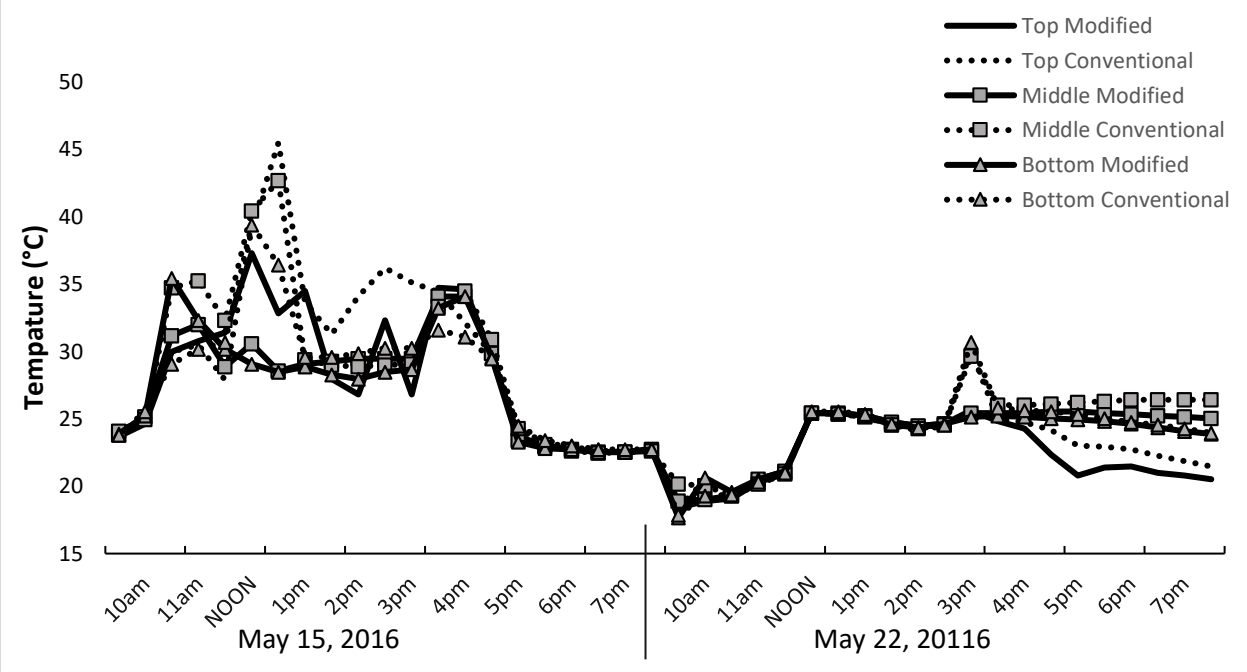


Figure 3.1. Temperature (°C) over time in transportation totes with two lid types. Placement of data loggers were at the top, middle, and bottom of plant material within the tote during two harvest days in May 2016.

Following previous results, temperatures were collected from separate totes secured with both types of lids and included air temperature to determine if a linear relationship existed. These data confirmed a difference in lid type used and there were significant differences in mean temperatures for all three components. For the collection periods in May 2016, the mean air temperature, conventional lid, and the modified lid were 37.8, 38.3, and 35.5°C, respectively, with an LSD of 1.3 ($p \leq 0.05$). The modified lid yielded temperature means that were 2.8°C cooler than conventional lids and 2.3°C cooler than air temperature. A bivariate analysis was conducted to aid plant managers in estimating internal tote temperature, regardless of lid type, based on air temperature (Figure 3.2 a, b) for estimating potential adult *C. salviniae* mortality. For example, when the average air temperature is 35°C, the predicted temperatures with conventional and modified lids would be 36.1 and 33.2°C (Figure 3.2 a, b). Alternately, if an air temperature reaches 40°C, internal temperatures would be 40.8°C for conventional lids while internal temperature of modified lids would be 36.9°C, and 3.9°C cooler than conventional lids. Although, these internal tote temperature differences are minimal, *C. salviniae* may benefit from cooler conditions by modifying lid types particularly at higher temperatures (≥40°C) during transport. Consequently, natural resource managers should consider lid modifications in the future and release infested *S. molesta* when air temperatures would yield the lowest internal tote temperatures (i.e. early morning), regardless of time of year.

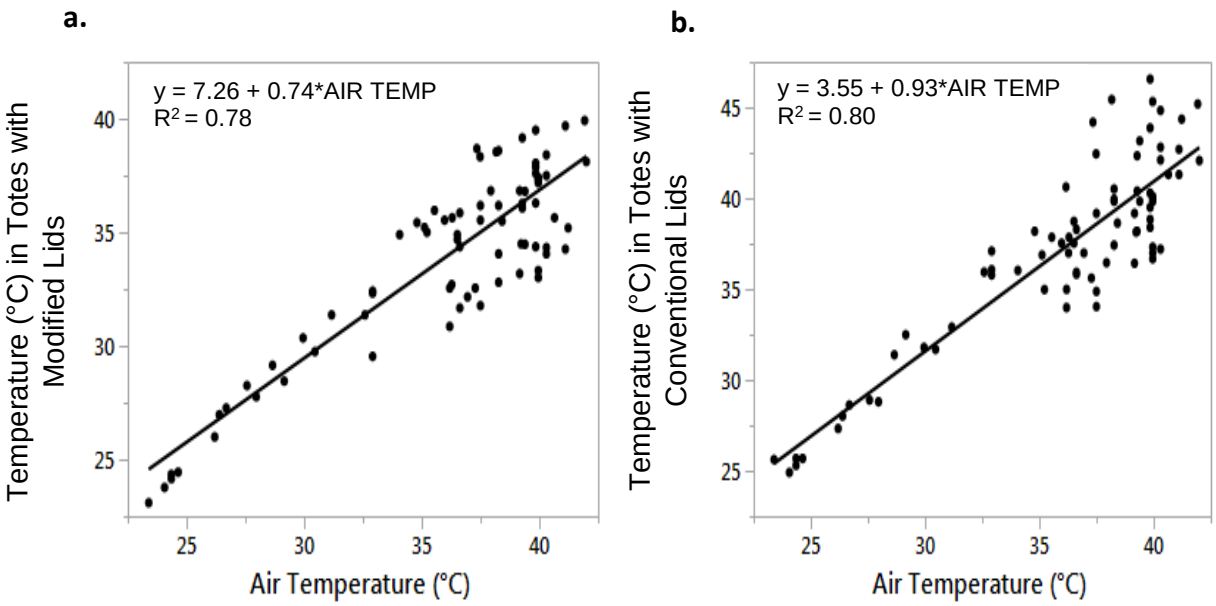


Figure 3.2. Bivariate analysis of air temperature and temperature experienced in transportation totes for *Cyrtobagous salviniae*. a) with circulation holes in fitted lids (n=80), b) without circulation holes in fitted lids ($p \leq 0.05$, n=80).

3.3.2 Adult *Cyrtobagous salviniae* Heat Mortality Under Laboratory Conditions

Laboratory experiments yielded a strong relationship between length of exposure and air temperature. At 35°C, the lethal time to kill 50 and 90% of the test population (LT_{50} and LT_{90}, respectively) was at 27.5 and 42.8 hours of exposure (Table 3.3). At 40°C, the LT_{50} and LT_{90} values were 15 and 25 hours, respectively. Although, lengthy exposures are unlikely, some plant managers travel 500 kilometers from harvest to release and may take up to 10 hours. When *C. salviniae* are exposed to higher temperatures, mortality occurs more rapidly in shorter periods of time. In the laboratory studies when *C. salviniae* were exposed to 45°C, the LT_{50} and LT_{90} values were 57 and 110 minutes, respectively (Table 3.3). At 50°C, the calculated LT_{50} and LT_{90} values are 5 and 11 minutes, respectively (Table 3.3). While these air temperatures are unlikely in

Louisiana and Texas, internal tote temperatures (with and without holes) reached 45 to 50°C for short periods of time (<30 minutes) during field trials. The temperatures evaluated in these experiments are outside of optimum range (19 to 30°C) for *C. salviniae* development and (13 to 33°C) for *S. molesta* growth (Room et al. 1984); and are therefore valuable in understanding survival under these extreme conditions. Establishment, reproduction, and persistence of *C. salviniae* is vital to the efficacy of this biological control agent (Allen et al. 2014), and while little is known about heat stress on other life stages, these extreme temperatures, even in short cycles, may likely affect future generations of *C. salviniae* and could impact establishment.

Table 3.3. Survival of a Natchitoches, Louisiana population of *Cyrtobagous salviniae* exposed to four temperature regimes.

Exposure (0°C)	n	Slope ±SE	LT_{50}[a] (95% CI)[b]	LT_{90}[a] (95% CI)[b]	r^2
35	400	-21.90 (4.7)	27.5 (22.8-32.2) h[c]	42.8 (38.1-47.5) h	0.83
40	320	-9.03 (2.6)	14.8 (12.2-17.4) h	24.8 (22.2-27.4) h	0.96
45	320	6.77 (4.3)	56.9 (52.6-61.2) m[c]	109.5 (105.2-113.8) m	0.88
50	240	17.7 (4.6)	5.0 (0.4-9.6) m	11.1 (6.5-15.7) m	0.86

[a] LT_{50} and LT_{90} values represent the time in hours to kill 50 and 90% of the populations, respectively.
[b] Non- overlapping CI (confidence intervals) indicate significant differences.
[c] Abbreviations: h = hours of exposure, m = minutes of exposure.

Using the bivariate formulas to predict internal tote temperatures based on air temperatures, plant managers can then use formulas derived from laboratory experiments to predict *C. salviniae* mortality based on length of exposure. If the predicted internal tote temperature is 35°C and the travel time is 10 hours, plant managers can expect 4.2% insect mortality (Figure 3.3a). As expected, warmer temperatures within the totes results in increased mortality. At 40°C for 10 hours of exposure (transport), plant managers can expect 31% mortality (Figure 3.3b) and

should estimate lower insect densities at the time of release to reflect this loss. Although the data presented in this research is a worst-case scenario where *C. salviniae* would be exposed to a specific temperature (i.e. 40°C) continuously for several hours, the totes will have an opportunity to cool down if abiotic factors such as cloud cover and rain occur. These conditions will likely increase weevil survival.

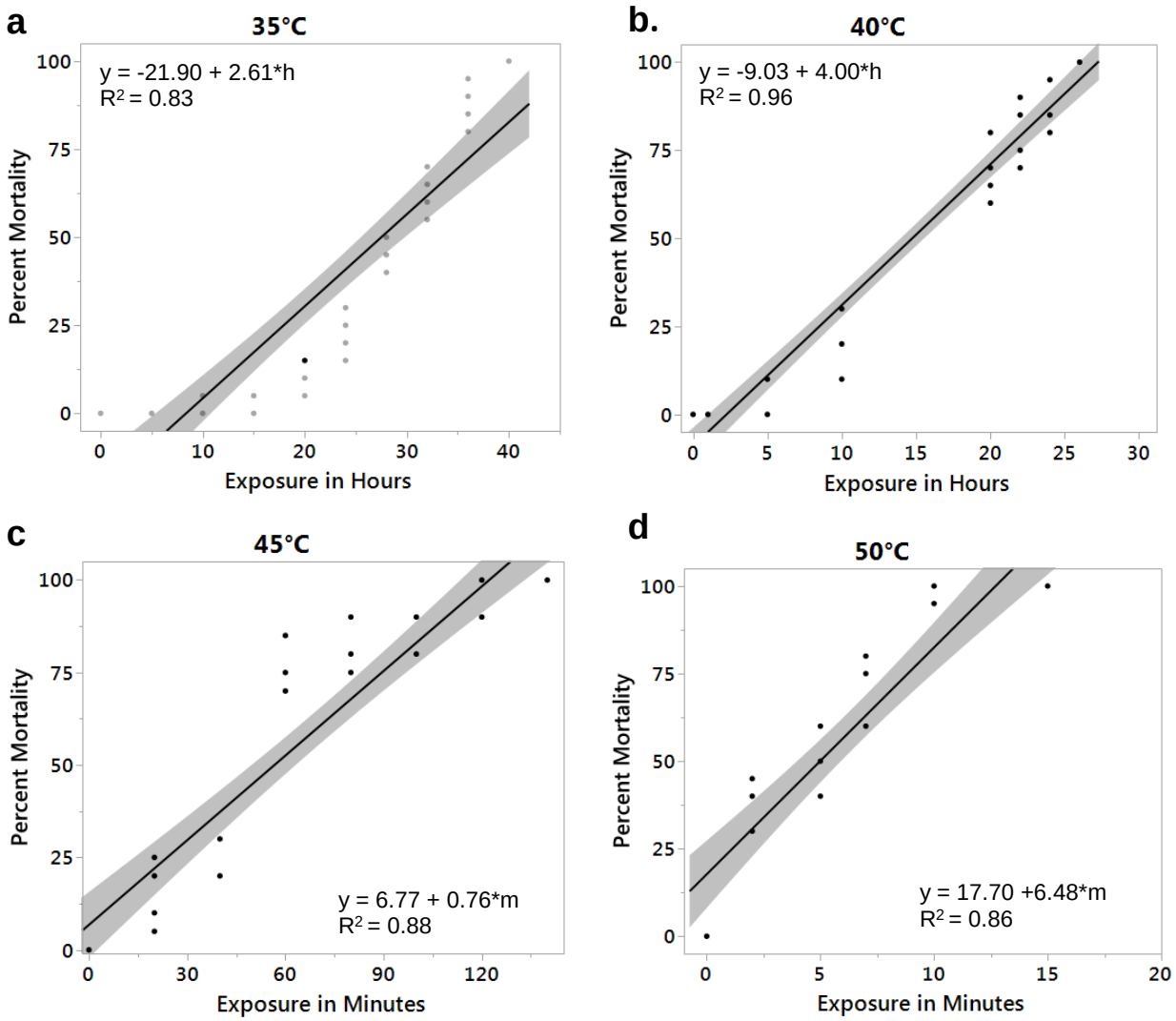


Figure 3.3. Percent mortality of *Cyrtobagous salviniae* exposed to four temperatures in environmental growth chambers: a) 35°C, b) 40°C, c) 45°C, and d) 50°C.

Until recently, low *C. salviniae* densities after release were assumed to be due to insect dispersal; however, these findings offer alternate insight to low densities as a result of unfavorable heat exposures. This research will provide plant managers with information to correct insect release estimates and explain lower insect densities in the future depending on air temperature and length of travel. Furthermore, plant managers can confidently release *C. salviniae* during summer months if the anticipated air temperatures are lower than data presented here.

Weather conditions and heat events, particularly in the hottest part of the day, directly affect the behavior and development of insects (Cui et al. 2011), and the impacts of heat stress on different life stages have been studied on a limited number of species (Zani et al. 2005). This research supports previous findings when *C. salviniae* were exposed to lethal temperatures for 1 hour and a LT_{50} of 43.7°C was estimated (Allen et al. 2014). In the present research, a 1 hour exposure at 45°C yielded 52% *C. salviniae* mortality. Similarly, *S. molesta* heat mortality was investigated in a laboratory setting and results by Whitman and Room (1991) indicated buds were killed at temperatures >43°C for exposures of 2 to 3 hours. Although the current study focused on *C. salviniae* mortality, temperatures were greater for shorter periods of time, and likely would have impacted plant survival under these extreme conditions. Since natural resource agencies release the entire plant along with all life stages of the insect, future research should investigate mortality of eggs, pupae, and larvae. Additionally, plant managers should modify current practices, and utilize lids with holes for optimum transportation and survival. Although previous recommendations, were to release in *C. salviniae* in the spring or fall when temperatures are cooler (Sanders et al. 2011;

Sullivan and Postle 2012; Sullivan et al. 2011), this foundational data defines the temperature conditions and associated mortality so that releases can be made throughout the *S. molesta* growing season. For instance, when *C. salviniae* in rearing facilities reach optimum densities, particularly outside of the spring or fall, this data provides modifications to alleviate extreme temperatures within transportation totes, as well as foundational methods to estimate *C. salviniae* mortality at specific air temperatures.

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CHAPTER 4. USING AQUATIC HERBICIDES AND THE GIANT SALVINIA WEEVIL (*CYRTOBAGOUS SALVINIAE*) FOR INTEGRATED MANAGEMENT OF GIANT SALVINIA, (*SALVINIA MOLESTA*)

4.1 Introduction

Salvinia molesta Mitchell (Salviniales: Salviniaceae), giant salvinia, is considered one of the world's worst weeds because of its high mobility, tolerance to environmental stress (Tipping 2004), exponential growth rate, and level of difficulty to control (Nelson et al. 2001; Tipping 2004). These characteristics allow *S. molesta* to rapidly invade aquatic environments (Tipping 2004). Under optimal greenhouse conditions, Cary and Weerts (1983) reported that *S. molesta* can double surface coverage in as little as 53 hours, whereas Johnson et al. (2010) demonstrated exponential growth with plants doubling surface coverage in as little as 36 hours under controlled environmental conditions. This explosive growth rate enables *S. molesta* to form dense mats of floating vegetation which has been reported up to one meter thick (Thomas and Room 1986), sometimes causing waterbodies to be mistaken for land (Johnson et al. 2010; Sullivan and Postle 2012). Initially, *S. molesta* outcompetes native and desired vegetation for resources such as nutrients, light, and surface area (Mitchell and Tur 1975), and it can quickly form a monoculture, thus inhibiting the growth of other plant species (Sullivan and Postle 2012). As plants mature into its tertiary (final) growth stage, dense mats expand and restrict boat travel for commerce, impede recreational activities (fishing, swimming and waterfowl hunting), and impair irrigation for agronomic purposes (Sullivan and Postle 2012; Thomas and Room 1986; Tipping 2004).

Aquatic plants provide benefits to an ecosystem; however, the aggressiveness of an invasive plant species such as *S. molesta* has increased the need for integrated

management (Richardson 2008) including physical and mechanical removal, lake drawdowns, aquatic herbicides and biological control agents (van Oosterhout 2006). While, aquatic herbicides are the most common and efficacious method used to control *S. molesta* (Netherland 2014; Ross and Lembi 1999), small floating aquatic plants can be difficult to treat chemically due to proximity to water and growth habits (Thayer and Haller 1985). Conversely, biological control agents are environmentally friendly and can reduce costs of *S. molesta* management over time. (Cuda 2014; Zachariades et al. 2017). Researchers who have studied both *S. molesta* and the biological control agent, *Cyrtobagous salviniae* suggest that instead of a single method for control, a more prudent approach would integrate multiple management techniques to provide more effective long-term management of *S. molesta* (Mudge et al. 2013).

Integrated Pest Management (IPM) is defined as an environmentally sensitive approach to manage pests by using a combination of common sense management practices (USEPA 2014). There are advantages and disadvantages with each management technique (Richardson 2008). For example, the most commonly used and efficacious treatment in Louisiana and Texas, are aquatic herbicides; however, there are difficulties associated with accessing some infested sites. The primary habitats of *S. molesta* are slow flowing streams and rivers, lakes, ponds, marshes, rice fields, and backwater swamps (Horner 2002). Specifically, applicators are tasked with maneuvering the herbicide application equipment (i.e. boats or aircraft) and spraying the target species through tree infested swamps, particularly bald-cypress (*Taxodium distichum* (L.) Rich.) stands. Furthermore, these swamps provide hiding places for unnoticed weeds during droughts and when re-flooded, *S. molesta* can re-infest the

connected body of water. Alternately, releasing biological control agents in these areas and allowing *C. salviniae* to disperse naturally would be a benefit in the management of *S. molesta* in addition to an overall reduction of annual expenditures (i.e. chemical, fuel, labor, etc.) associated with spray treatments. Biological control can be limited by environmental constraints including nutrient availability, water quality, length of time for insect establishment and plant reduction (Sullivan and Postle 2012), appropriate stocking densities (Room and Thomas 1985; Tipping and Center 2005) and unfavorable winter conditions, particularly in the northern range of the plant (Mukherjee et al. 2014; Sullivan and Postle 2012; Tipping et al. 2008).

Integrated pest management is a relatively new concept for aquatic natural resource managers in Louisiana and Texas. For more than a decade, Texas managers used an herbicide mixture for the management of *S. molesta* until 2014 (Thomas Decker, Texas Parks and Wildlife Department (TPWD), personal communication), while Louisiana managers still heavily rely on the same mixture (Alexander Perret, Louisiana Department of Wildlife and Fisheries (LDWF). This mixture consists of a glyphosate (N-(phosphonomethyl)glycine), diquat (6,7-dihydrodipyrido[1,2-a:29,19-c]pyrazinediium ion), and 1 or 2 aquatic surfactants (Mudge et al. 2014; Mudge et al. 2016). By using a single tank mix repeatedly, plant managers may face challenges with *S. molesta* management, particularly because glyphosate-resistant weeds are now directing research advances (Heap 2014). Numerous factors can contribute to plants developing resistance and not all are completely understood; however, continued long-term use of herbicides with the same modes of action has resulted in herbicide resistant weeds (Anderson 2007). Despite the theory that asexually reproducing plants are not capable

of developing herbicide resistance (Powles and Holtum 1994), *Hydrilla verticillata* ((L.f.) Royle) was the first aquatic weed with documented herbicide resistance (Michel et al. 2004). Because *H. verticillata* and *S. molesta* reproduce asexually by vegetative propagation, there is increased sensitivity to the issue of resistance among plant managers (Netherland 2014). Also, as herbicide resistance issues become more abundant, researchers are focusing on herbicides with alternate modes of action to combat *S. molesta* (Mudge 2016) in addition to biological control programs.

Biological control is being utilized more as an alternative for *S. molesta* management; however, there is limited research on the interaction of aquatic herbicides and *C. salviniae*. Previous research has addressed integrated control of water hyacinth [*Eichhornia crassipes* (Mart.) Solms] using water hyacinth weevils (*Neochetina spp*.) and aquatic herbicides (Harley 1990; Pellessier 1988). Interactions of *C. salviniae* and the aquatic herbicides penoxsulam (2-(2,2-difluoroethoxy)-N-(5,8-dimethoxy[1,2,4]triazolo[1,5-c]pyrimidin-2-yl)-6-(trifluoromethyl)benzenesulfonamide) and flumioxazin (2-[7-fluoro-3,4-dihydro-3-oxo-4-(2-propynyl)-2H-1,4-benzoxazin-6-yl]-4,5,6,7-tetrahydro-1H-isoindole-1,3(2H)-dione) have been explored (Mudge et al. 2013). The previous research (Mudge et al. 2013) that was conducted with *S. molesta* and *C. salviniae* was on a small scale and did not include a long growing season (6 wk). Therefore, the objectives of this research to address control of *S. molesta* were 1) to determine optimum timing for herbicide and combination treatments, and 2) to evaluate integrated management treatments (aquatic herbicides and *C. salviniae*).

4.2 Materials and Methods

S. molesta that was not infested with *C. salviniae* was collected from a local waterbody, and *C. salviniae*-infested *S. molesta* was collected from the rearing facility at the Red River Waterway Commission's Aquatic Plant Research Center in Lena, Louisiana (31.518, -92.732). Two studies were conducted, one in the early plant growing season (April), and in the late (August) plant growing season in 2016 to evaluate the integrated control of *S. molesta* using aquatic herbicides and *C. salviniae*. Twenty-four 2160 L round tanks (2.4 m diameter x 45.7 cm depth) tanks received a 1:1 ratio of rain water and local municipal water source with an average pH of 5.6 initially. Collected rain water was utilized to alleviate the high pH of the municipal water. In addition, water was amended initially and every other week for the duration of the experiment with fertilizer (Scott's Southern Turf Builder Lawn Fertilizer®) to provide 2 mg L^{-1} nitrogen in the water column (Glomski and Mudge 2013). Twelve total control and herbicide alone treated tanks were then infested with 10 kg of *C. salviniae* free, mature *S. molesta*. Twelve integrated (herbicide + *C. salviniae*) tanks received 5 kg of uninfested *S. molesta* and 5 kg of infested *S. molesta* that was evenly distributed throughout the tanks, with approximately 50 adults and larvae per kg of fresh weight biomass (w/kg^{-1}). After initial inoculation, all plant material was treated with *Bacillus thuringiensis* (Bt) for suppression of the S*. molesta* stem borer moth (*Samea multiplicalis* Guenne' (Lepidoptera: Pyralidae)) (Parys and Johnson 2013) and repeated every other week for the duration of the experiment. All tanks were allowed 1 week to acclimate prior to herbicide treatments.

The aquatic herbicide treatments were applied to 100% of the plant foliage in the herbicide only treatments, whereas the integrated tanks received 50% coverage from the herbicide application in two separate non-adjacent quadrants, leaving non-herbicide treated plant material for insect foraging. The integrated tanks were divided into quadrants (1 to 4) with opposite quadrants paired (i.e. 1 and 3, 2 and 4) and the herbicide treatments were randomly assigned to a quadrant pair, prior to herbicide application. Herbicides used in this trial included glyphosate (Roundup Custom™), diquat (Tribune™), flumioxazin (Clipper™), penoxsulam (Galleon®) and adjuvants including a nonionic surfactant and buffering agent blend (Aqua-King Plus®), a nonionic organosilicon surfactant (AirCover™), and a methylated vegetable oil and organosilicon blend (Turbulence™). Specific herbicide combinations and rates can be found in Table 4.1. Herbicide treatments were applied to the foliage of *S. molesta* using a forced air CO_2-powered sprayer at an equivalent rate of 935 L ha^{-1} diluent delivered through a single TeeJet® 80-0067 nozzle at 20 psi. A spray shield was placed over the non-treated quadrants to prevent herbicidal drift and cross-contamination to neighboring quadrants or tanks. The experimental design was completely randomized and all treatments were replicated three times. A non-treated control was also included to monitor plant growth in the absence of herbicides and/or *C. salviniae*.

Qualitative estimates of percent surface coverage were assessed pre-treatment, at 5 days after treatment (DAT), and at 6, 9, and 12 weeks after treatment (WAT). Percent surface coverage of *S. molesta* was determined on a scale of 0 to 100%, where 0=open water and 100=complete plant coverage of surface area (Flores and Carlson 2006). Quantitative measurements included fresh weight biomass, dry weight biomass,

and *C. salviniae* density based on number of insects per fresh weight biomass (CS per kg^{-1}) (Forno 1987). Quantitative measurements were collected pre-treatment and at 3, 6, 9, and 12 WAT. At the appropriate time, 1 sub-sample (1/32 m^2) per quadrant, per tank was randomly collected to measure *S. molesta* biomass (kg), then dried to a constant weight (g) using Berlese funnels (Boland and Room 1983) to achieve *C. salviniae* density. Corresponding quadrants from an individual tank were compiled to accommodate the numerous samples. At the conclusion of the study (12 WAT), all viable *S. molesta* biomass in each tank was harvested to collect final fresh weight, dry weight, and *C. salviniae* density.

Table 4.1. Treatment and application rates for integrated pest management of *Salvinia molesta* and *Cyrtobagous salviniae.*

Treatment[a]	Rate (g a.i. ha^{-1})	% Herbicide Coverage
Control	0	0
G + D + NIOS + NISBA	3364.1 + 560.1 + 0.25% v/v + 0.094% v/v	100
P + MVO	70.1 + 0.25% v/v	100
F + MVO	214.5 + 0.25% v/v	100
G + D + NIOS + NISBA + CS[b]	G + D + + 0.25% v/v + 0.094% v/v + 50 per kg	50[c]
P + MVO + CS	70.1 + 0.25% v/v + 50 per kg	50
F + MVO + CS	214.5 + 0.25% v/v + 50 per kg	50
CS	50 per kg	0

[a] Abbreviations: G, glyphosate; D, diquat; F, flumioxazin; P, penoxsulam; CS, *Cyrtobagous salviniae*.
[b] *Cyrtobagous salviniae* (CS) were evenly distributed throughout the *Salvinia molesta*.
[c] 50% of the *Salvinia molesta* was treated with herbicides in 2 separate quadrants.

Both qualitative and quantitative data were analyzed to detect differences in paired quadrants, and no significant differences were detected; therefore, paired quadrants were pooled for the analyses. Sub-sample quantitative data were subjected to a three-way analysis of variance (ANOVA) at $p \leq 0.05$ (SigmaPlot 11.0, Systat Software) (Nelson et al. 2001). There were no significant differences in *C. salviniae* densities between trials ($p=0.184$); therefore, data were pooled and a two-way ANOVA was performed. After a three way ANOVA, there was a three-way interaction between trial, treatment and timing after treatment, therefore least square means (LSM) are reported for fresh weight data. Dry weights and *C. salviniae* densities were pooled and subjected to a two-way ANOVA due to no significance in the timing of trials for sub-samples ($P=0.055$, $P=0.093$, respectively). Post-hoc tests (Fisher's Protected LSD) were used for all pairwise comparisons at $p \leq 0.05$ for dry weights. Whole tank data were subjected to a two-way ANOVA and Fisher's Protected LSD was used for all pairwise comparisons at $p \leq 0.05$. As a result of no differences between trials for *C. salviniae* densities in whole tank collections, data were subjected to a one-way ANOVA. Qualitative data failed to pass normality and equal variance tests; therefore, treatment means ±95% confidence intervals (CI) of the mean are reported.

4.3 Results and Discussion

Aquatic plants, particularly *S. molesta,* are comprised of more than 90% water (Haller 2014), and dry weight is a reliable and consistent indicator of biomass reduction (Grodowitz et al. 2014). In this experiment, all plant dry weight biomass was equivalent, regardless of treatment prior to application of herbicides (Table 4.2). In the herbicide only treatments, at 3 WAT, glyphosate + diquat and flumioxazin reduced plant biomass

56 to 67% compared to the control, and there was no significant reduction in biomass with penoxsulam. At 3 WAT, all integrated treatments reduced plant biomass 32 to 37% compared to the control. Conversely, the *C. salviniae* treatment failed to reduce biomass at 3 WAT. When comparing herbicide only treatments to the respective integrated treatments at 3 WAT, glyphosate + diquat reduced biomass 47% more compared to herbicides integrated with *C. salviniae*, where penoxsulam and flumioxazin dry weight biomasses were equivalent to the respective integrated treatments.

Table 4.2. Mean dry weight sub-sample biomass (g per 0.125 m^{-1}) of *Salvinia molesta* in response to herbicide, *Cyrtobagous salviniae*, and combination treatments applied on April 7, 2016 (Early) and August 4, 2016 (Late)[a] (n=6).

	WAT[2]				
Treatment	0	3	6	9	12
	---------- g 0.125 m^{-1} ----------				
C	16.7	24.9	31.8	32.2	32.8
G + D	16.3	8.3	1.0	1.5	1.6
P	17.0	18.7	16.3	11.6	6.6
F	17.1	11.0	5.6	4.3	5.4
G + D + CS	20.1	15.6	19.7	19.4	16.2
P + CS	19.4	16.4	33.9	9.7	4.6
F + CS	19.1	16.9	40.0	6.1	5.5
CS	19.0	22.5	28.1	23.6	22.6
LSD (0.05)	---------- 6.8 ----------				

[a] Ten kilograms of *S. molesta* was introduced in 4.7 m^2 tanks 1 week prior to pre-treatment data.
[b] Abbreviations: WAT, weeks after treatment; C, control; CS, *Cyrtobagous salviniae*; F, flumioxazin; P, penoxsulam; G, glyphosate; D, diquat.

At 6 WAT, in the herbicide only treatments, glyphosate + diquat, penoxsulam, and flumioxazin reduced *S. molesta* dry weight 97, 46, and 82%, respectively (Table 4.2). In the integrated treatments, glyphosate + diquat + *C. salviniae* decreased biomass 38% compared to the control, which was 30% less biomass than *C. salviniae* alone. Plants treated with penoxsulam + *C. salviniae* or flumioxazin + *C. salviniae*

increased in biomass from the onset of the trial and were equivalent to the control at 6 WAT. Comparing herbicide only treatments, glyphosate + diquat, and flumioxazin provided similar efficacy (>81%) by 6 WAT compared to the control, while penoxsulam only provided 49% control. With regard to the integrated treatments during the same evaluation period, biomass was significantly higher in the integrated treatments. Plants treated with glyphosate + diquat + *C. salviniae*, penoxsulam + *C. salviniae*, and flumioxazin + *C. salviniae* had 95, 52, and 86% more biomass than their respective herbicide only counterparts at 6 WAT; however, significant dry weight biomass reductions were observed in all treatments at 9 WAT.

In the herbicide only treatments, plants treated with glyphosate + diquat, penoxsulam, and flumioxazin were significantly lower in biomass by 95, 64, and 87%, respectively, compared to the control at 9 WAT (Table 4.2). At 9 WAT, all integrated treatments were efficacious; however, penoxsulam + *C. salviniae*, and flumioxazin + *C. salviniae* reduced biomass 70 to 81%, while glyphosate + diquat + *C. salviniae* provided only 40% control. Insects alone reduced plant biomass by 27% at 9 WAT. Although dry weight was significantly reduced in the glyphosate + diquat + *C. salviniae* and the *C. salviniae* only treatment, plant managers generally consider >85% control a successful treatment in field evaluations. When comparing herbicide only treatments, glyphosate + diquat and flumioxazin were similar in efficacy, but penoxsulam provided 63 to 87% less control. When comparing integrated treatments to their respective herbicide only treatments at 9 WAT, glyphosate + diquat provided significantly less control than when integrated with *C. salviniae*, while penoxsulam and flumioxazin alone were equivalent to the corresponding integrated treatments, although only 50% of plant material was

sprayed initially. The severe reduction in biomass between 6 and 9 WAT for the integrated treatments can likely be attributed to the hatching of a second generation of *C. salviniae*.

At the conclusion of the experiment (12 WAT), the herbicide only treatments were similar and reduced biomass 84 to 95%, and the integrated treatments with penoxsulam + *C. salviniae* and flumioxazin + *C. salviniae* provided 86 and 83%, respectively (Table 4.2). All of these treatments would be considered successful, but the glyphosate + diquat + *C. salviniae* treatment failed to provide sufficient efficacy. *Cyrtobagous salviniae* alone failed to provide substantial biomass reductions (31%), but was more efficacious when integrated with aquatic herbicides in a 12 week evaluation. In addition, penoxsulam + *C. salviniae* and flumioxazin + *C. salviniae* were equivalent to herbicide only treatments, but glyphosate + diquat + *C. salviniae* had 90% more biomass (i.e. 10% control) than the corresponding herbicide only treatment.

Over the length of the study, the control plants increased in dry weight biomass by 47% at 6 WAT and remained similar through 12 WAT (Table 4.2). The glyphosate + diquat treatment decreased biomass by 50% at 3 WAT and 90% by 12 WAT compared to pre-treatment. The penoxsulam treatment failed to reduce dry weight until 12 WAT (61%) compared to all other subsamples, whereas the flumioxazin treatment significantly reduced biomass similarly (67%) as early as 6 WAT. These results are disparate compared to previous research where flumioxazin was applied to 100% of the plant material and dry weight was reduced by 98% of the non-treated control at 6 WAT (Mudge et al. 2013). The difference in efficacy between the experiments could be attributed to the amount of initial plant material at herbicide application, where Mudge et

al. (2013) inoculated 120 g of fresh weight plant material and the current study used 10 kg of fresh weight plant material at inoculation.

The integrated treatment of glyphosate + diquat + *C. salviniae* treatment did not reduce dry weight biomass throughout the experiment. Penoxsulam + *C. salviniae*, and flumioxazin + *C. salviniae* treatments, were not significantly different at 3 WAT compared to the pre-treatment; however, a significant increase in biomass occurred at 6 WAT. Plant weight increased for both treatments by more than 50% from 3 to 6 WAT; however, dry weight was significantly reduced at 9 WAT by 71 and 85%, for penoxsulam + *C. salviniae* and flumioxazin + *C. salviniae*, respectively. The plants exposed to the *C. salviniae* treatment increased in biomass at 6 WAT by 32%, which was similar to control, but the insects were able to overcome this increase in plant material and reduce biomass by 31% at 12 WAT compared to the control. *Cyrtobagous salviniae* at optimal temperatures require at least 6 weeks to complete a second generation (Cilliers 1991; Sullivan and Postle 2012). Although, this study was not conducted at optimal temperatures, the reduction in biomass between 6 and 9 weeks, can likely be attributed to the hatching of adults from the second generation. Despite biomass not being reduced by the *C. salviniae* treatment as originally anticipated within the 12 week trial, a longer study may have yielded greater reductions in biomass if the third weevil generation was allowed to complete its life cycle. The goal of this study was to understand the effects of herbicides and insects, alone and when integrated, during a three month period; however, longer studies should be considered.

Although dry weight is more reliable indicator for biomass reduction, fresh weight is often used as a “quick and dirty” assessment in field situations and is an important

factor in determining insect density. In this experiment, all herbicides and integrated treatments (herbicides + *C. salviniae*) were more efficacious when applied early (April) in the growing season than late (August), and all herbicide treatments alone or in combination with *C. salviniae* were efficacious at reducing fresh weight biomass regardless of timing compared to the control or *C. salviniae* alone (Table 4.3). At 3 WAT, glyphosate + diquat reduced biomass more than any other treatment and had 61 and 80% less biomass than the control during the early and late trials, respectively. Penoxsulam resulted in 37 to 40% less biomass compared to the control at 3 WAT, and flumioxazin reduced fresh weight biomass 51 to 63% for both trials. For the integrated trials, glyphosate + diquat + *C. salviniae* treated plants had 21% less biomass at 3 WAT in the early trail and 48% in late trial compared to *C. salviniae* alone. Penoxsulam + *C. salviniae* only reduced biomass by 9% for the early trial, but 37% for the late trial at 3 WAT compared to insects alone, while flumioxazin + *C. salviniae* reduced biomass by 20 and 39%, respectively, for the same evaluation periods. Although all integrated treatments were effective at reducing biomass, acceptable reductions were not observed at 3 WAT.

At 6 WAT, in the herbicide only treatments, the glyphosate + diquat treatment provided >97% control in both trials (Table 4.3). Penoxsulam compared to the control at 6 WAT reduced biomass 62 to 63% in both trials, while flumioxazin reduced biomass 84 to 87%. The integrated treatments compared to *C. salviniae* alone reduced biomass for both early and late trials. Glyphosate + diquat + *C. salviniae* had 41% less biomass in the early trial but only 9% less biomass in the late trial compared to *C. salviniae* at 6 WAT. This data indicates, early application versus late application of *C. salviniae* can

Table 4.3. Mean fresh weight subsample biomass (g per 0.125 m^{-1}) of *Salvinia molesta* in response to herbicide, CS (*Cyrtobagous salviniae*), and integrated treatments applied on April 7, 2016 (Early) or August 4, 2016 (Late).[a]

	Pre-treatment		3 WAT[b]		6 WAT		9 WAT		12 WAT	
Treatment	Early	Late	Early	Late	Early	Late	Early	Late	Early	Late
	g 0.125 m^{-1}									
C	279	344	461	505	473	727	473	767	534	664
G + D	244	355	182	101	11	25	4	40	5	48
P	267	355	290	302	176	279	81	231	14	151
F	285	378	227	188	61	118	12	128	21	140
G + D + CS	284	325	336	222	226	449	129	457	52	464
P + CS	233	346	385	269	280	265	111	184	11	126
F + CS	269	323	340	258	213	240	17	163	14	150
CS	273	315	423	426	383	494	272	486	244	438
LSM ($P \leq 0.05$)	34									

[a] Ten kilograms of *S. molesta* were introduced in 4.7 m^2 tanks 1 week prior to pre-treatment data collection. Herbicide tanks received 10 kg of *C. salviniae* free *S. molesta*; integrated tanks received 5 kg of *C. salviniae* free *S. molesta* and 5 kg of *S. molesta* infested with *C. salviniae*.

[b] Abbreviations: WAT, weeks after treatment; C, control; CS, *Cyrtobagous salviniae*; F, flumioxazin; P, penoxsulam; G, glyphosate; D, diquat.

have an impact on biomass reductions. Penoxsulam + *C. salviniae* reduced biomass by 27% for the early trial and 46% for the late trial at 6 WAT compared to insects only, and flumioxazin + *C. salviniae* reduced biomass by 44 and 51%, respectively, for the same evaluation period. Although more efficacious when integrated, *C. salviniae* were capable of reducing biomass at 6 WAT compared to the control by 19 and 32% in the early and late trials, respectively. While some of these reductions are not generally acceptable (90%) and many factors contribute to a particular level of control (Netherland and Schardt 2009), a meta-analysis of classical biological control agents, particularly in the U.S. from the

Curculionid family, reported that a 37 ±4% reduction in plant mass by classical biological control agents were considered successful (Clewley et al. 2012).

Glyphosate + diquat continued to be efficacious at 9 WAT compared to the control and reduced biomass by ≥95% in both trials (Table 4.3). Penoxsulam compared to the control at 9 WAT, reduced biomass by 70 to 83%, while flumioxazin reduced biomass by 83 to 97% during both trials. Glyphosate + diquat + *C. salviniae* provided 53% more control than *C. salviniae* alone in the early trial and had no fresh weight reduction in the late trial. Penoxsulam + *C. salviniae* reduced biomass 60 and 62% in the early and late trials, respectively, while flumioxazin + *C. salviniae* reduced biomass by 94 and 66% for the early and late trials, respectively.

By the conclusion of the experiment (12 WAT), fresh weight biomass were reduced 93 to 99%, 77 to 97%, and 79 to 96% by the glyphosate + diquat, penoxsulam, and flumioxazin treatments (Table 4.3). In the integrated treatments, glyphosate + diquat + *C. salviniae* provided 79% more control than *C. salviniae* alone at 12 WAT in the early trial and had no significant impact on biomass reduction in the late trial. Penoxsulam + *C. salviniae* reduced biomass by 95 and 71% in the early and late trials, respectively, compared to the control and flumioxazin + *C. salviniae* reduced biomass by 94 and 66% during the same trials. While *C. salviniae* alone were capable of reducing biomass 34 to 54% compared to the control, there was greater control observed when integrated with aquatic herbicides, particularly when applied during the early growing season (April). The insect's life cycle must be considered when making management decisions and longer exposure times may be needed to achieve acceptable efficacy in a mesocosm setting. In field settings, *S. molesta* control by C.

salviniae has been reported to clear a 400 hectare mat weighing 50,000 tons in fresh weight in as little as 15 months in tropical climates; however, in temperate zones, like Louisiana, success was reported between 1-5 years (Julien et al. 2012). Although, this study (12 weeks) was ample time to achieve herbicide efficacy, longer evaluation periods, may have portrayed better success for the biological control agent.

The glyphosate + diquat treatment was efficacious against *S. molesta* during the early and late trials at 3 WAT by decreasing biomass 72% for late trial. Further biomass reductions were observed at 6 WAT and biomass were reduced by 94% and 75% in the early and late trials, respectively, compared to 3 WAT, and remained similar through 12 WAT. There were no differences between pre-treatment and 3 WAT fresh weight biomass for penoxsulam and acceptable reductions were not observed at 6 WAT. Biomass reductions of 54% were observed in the early penoxsulam treatment at 9 WAT, but there were no significant differences in the late trial. By 12 WAT, penoxsulam reduced biomass of 83 (early) and 35% (late). Flumioxazin only reduced plant biomass by 50% in the late trial at 3 WAT; however, by 6 WAT, plant biomass was reduced during the early and late trials by 79 and 69%, respectively, compared to pre-treatment.

Plants exposed to the integrated treatments of glyphosate + diquat + *C. salviniae* increased in biomass by 15% at 3 WAT during the early trial of, but late trial biomass was reduced by 32%. At 6 WAT, biomasses were 33% lower in the early trial and 51% higher in the late trial compared to 3 WAT for glyphosate + diquat + *C. salviniae*. In this same treatment at 9 WAT, the early trial continued to decrease biomass at 6 and 12 WAT, while no significant reductions were observed in the late trial. Significant and acceptable biomass reductions were not observed from plants treated with penoxsulam

+ *C. salviniae* in either trial until 9 WAT. Foliar applications of flumioxazin + *C. salviniae* only reduced biomass by 20% in the late trial at 3 WAT. Flumioxazin + *C. salviniae* reduced biomass by 92 and 32% in the early and late trials, respectively, at 9 WAT compared to 6 WAT. At the conclusion of the experiment (12 WAT), there were no significant differences between 9 and 12 WAT biomasses in the flumioxazin + *C. salviniae* treatment. Plants exposed to *Cyrtobagous salviniae* increased in biomass at 3 WAT compared to pre-treatment by 35 and 26% in the early and late trials, respectively. By 9 WAT, there were no significant differences in the late trial, but the early trial reduced biomass by 29% compared to 6 WAT with insects only. By the conclusion of the experiment (12 WAT), there were no significant differences compared to 9 WAT with *C. salviniae* in the early trial; however, in the late trial biomass was reduced by 10%. The reduction in fresh weight biomass of the *C. salviniae* treatment at 6 WAT in the early trial may indicate that temperatures were more optimal for insect reproduction, and in the late trial, temperatures were outside of the optimum range until 12 WAT.

Densities of *C. salviniae* are based on fresh weight of *S. molesta* and used to estimate populations in rearing facilities and in the field (Grodowitz et al. 2014; Nachtrieb 2014). When *C. salviniae* densities were compared throughout the experiment, there was no significant difference at 3 WAT compared to pre-treatment; however, there was a 100% increase in insect density at 6 WAT, regardless of herbicide timing application (Figure 4.1 a). At 9 WAT, insect densities in all treatments were similar to 6 WAT, but by 12 WAT, decreased by 37% and were equal to pre-treatment levels. Similar to previous IPM research (Mudge et al. 2013), these data exhibited an increase in insect density for the *C. salviniae* alone treatment at 6 WAT. These data

from the present study, offer insight to the timing in which *C. salviniae* densities peak regardless of late or early season infestation. Alternatively, when all IPM treatments were compared across the length of the study, the density in the penoxsulam + *C. salviniae* treatments was 48 to 62% lower than other treatments (Figure 4.1 b). This indicates that this combination, although suitable, may not be the most viable option when using an integrated pest management approach as the result of to low insect densities at 12 WAT. Throughout the study, the *C. salviniae*, flumioxazin + *C. salviniae*, and glyphosate + diquat + *C. salviniae* treatments negatively impacted the target species and therefore would all be viable management options.

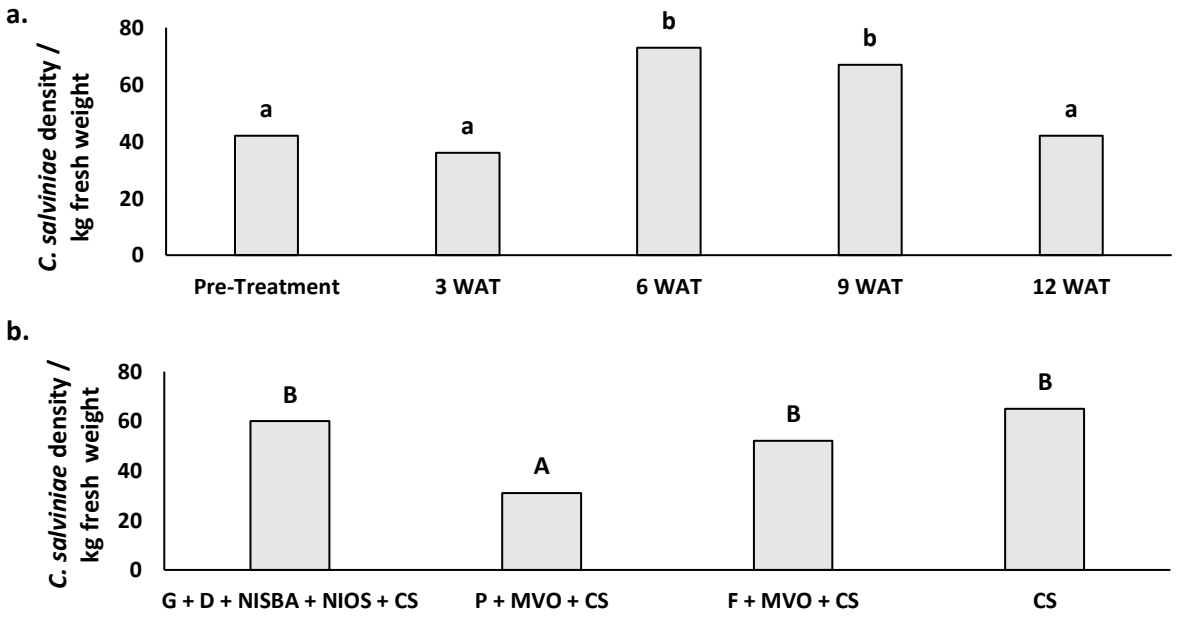


Figure 4.1. (a) Mean densities of *Cyrtobagous salviniae* for the early (April 7, 2016) and late (August 4, 2016) trials at pre-Treatment, 3, 6, 9, and 12 weeks after treatment (WAT), and (b) response to herbicide treatments plus surfactants. Means with different letters denote statistical differences according to Fisher's Protected LSD method at $P \leq 0.05$, n=6. Abbreviations: G, glyphosate; D, diquat; NISBA, nonionic surfactant buffering agent blend; NIOS, nonionic organosilicon surfactant; CS, *Cyrtobagous salviniae*; P, penoxsulam; MVO, methylated vegetable oil; F, flumioxazin.

Qualitative data is often used as a field assessment for aquatic vegetation managers to better understand the level of infestation in a particular waterbody. During the early season application, penoxsulam and glyphosate + diquat treatments reduced plant coverage more than all other treatments at 6 WAT. By 12 WAT, all herbicide and IPM treatments reduced *S. molesta* coverage (Table 4.4). In the late season trial, glyphosate + diquat was the only effective treatment at reducing plant coverage. The integrated treatments failed to reduce percent coverage in the late treatment trial, and by the conclusion of the experiment, the glyphosate + diquat + *C. salviniae* treatment was equivalent to the control and *C. salviniae* alone treatments. These qualitative data confirm fresh and dry weight data and can be utilized for estimating efficacy in the field.

Table 4.4. Percent coverage[a] of *Salvinia molesta* in response to herbicide, *Cyrtobagous salviniae*, and combination treatments, April 7, 2016 (Early) and August 4, 2016 (Late)[b]

	5 DAT[c]		6 WAT		9 WAT		12 WAT	
Treatment	Early	Late	Early	Late	Early	Late	Early	Late
	%							
C	88 (±11)	100 (±0)	92 (±2)	100 (±0)	88 (±2)	92 (±5)	84 (±5)	92 (±4)
G + D	67 (±4)	88 (±4)	4 (±0)	16 (±0)	12 (±4)	8 (±2)	4 (±0)	12 (±4)
P	57 (±8)	92 (±2)	12 (±13)	68 (±21)	12 (±2)	40 (±26)	6 (±1)	36 (±32)
F	60 (±2)	100 (±2)	64 (±8)	72 (±2)	24 (±2)	68 (±12)	12 (±1)	56 (±11)
P + CS	68 (±1)	98 (±2)	48 (±9)	76 (±7)	20 (±6)	76 (±17)	4 (±2)	76 (±21)
F + CS	75 (±7)	98 (±1)	66 (±4)	72 (±7)	64 (±5)	76 (±31)	6 (±1)	72 (±31)
G + D + CS	84 (±3)	96 (±0)	69 (±8)	92 (±3)	29 (±11)	100 (±0)	8 (±5)	100 (±2)
CS	85 (±6)	100 (±2)	84 (±6)	96 (±12)	60 (±4)	100 (±2)	60 (±7)	96 (±2)

[a] Percentage of coverage was on a scale of 0-100 with 0=no plants and 100=total coverage.
[b] Estimates were taken from 2160 L round tanks (2.4 m diameter x 45.7 cm depth)
[c] Abbreviations: DAT, days after treatment; WAT, weeks after treatment; C, control; CS, *Cyrtobagous salviniae*; F, flumioxazin; P, penoxsulam; G, glyphosate; D, diquat.

At the conclusion of each experiment, when all remaining *S. molesta* were collected from the tanks, fresh and dry weights in general, confirmed that treating *S. molesta* early in the growing season is more efficacious than late season herbicide applications and/or insect releases (Figure 4.2 a, b). In the early trial (April 2016), all treatments containing herbicides (alone or in combination with *C. salviniae*) had significantly less fresh and dry weight biomass than the *C. salviniae* only or control treatments. Conversely, all treatments, except glyphosate + diquat + *C. salviniae*, were efficacious in the late trial (August 2016). This data suggests that while glyphosate + diquat is efficacious at controlling *S. molesta* during both early and late season applications, but when integrated with *C. salviniae*, biomass is not significantly reduced. Additionally, it has been documented that *C. salviniae* are capable of flight dispersal (Tipping and Center 2005), and little is known on the factors influencing flight activity, duration and distance (Micinski et al. 2016).

Overall, these data demonstrate that an early application timing, either herbicide alone or in combination with *C. salviniae*, are more efficacious than late season applications for controlling *S. molesta* (plant weights and percent coverage). In these studies, all tanks were infested with 10 kg of *S. molesta* and allowed 1 week of acclimation prior to herbicide treatments; however, fresh weight was 30% more in the late (August) trial compared to the early (April) trial at herbicide application, indicating environmental conditions were more optimal for plant growth in August. All treatments were less efficacious in the late season, presumably from *S. molesta* forming more than 1 layer on the water's surface at the time of herbicide application. Although herbicides were applied at the same rate and time in both trials, complete chemical coverage was

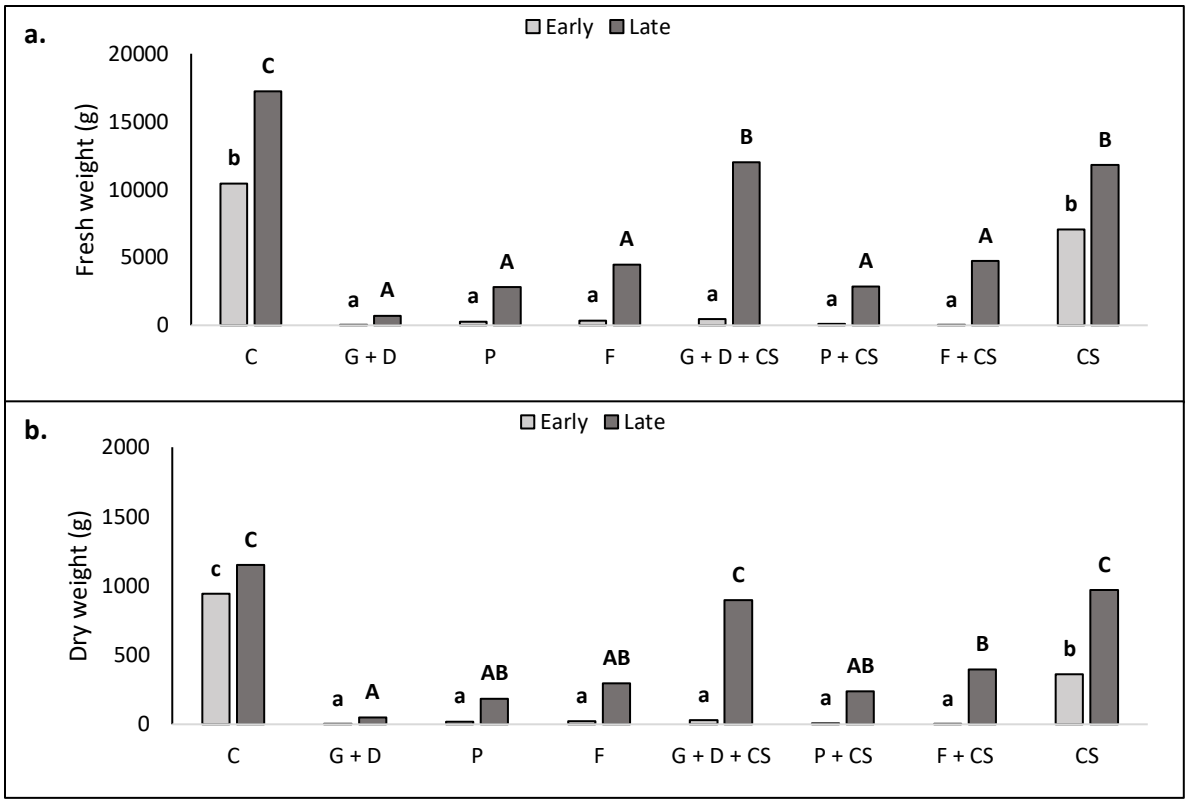


Figure 4.2. Giant salvinia (a) fresh weight and (b) dry weight biomass in response to herbicide, *Cyrtobagous salviniae*, and integrated treatments. Data is representative of entire tank biomass 12 weeks after treatment. Means with the same letter within dry or fresh weight at the early (April 7, 2016) and late (August 4, 2016) application timings trials are not significant according to Fisher's Protected LSD test at $P \leq 0.05$; n=3. Abbreviations: G, glyphosate; D, diquat; NISBA, nonionic surfactant buffering agent blend; NIOS, nonionic organosilicon surfactant; CS, *Cyrtobagous salviniae*; P, penoxsulam; MVO, methylated vegetable oil; F, flumioxazin.

not achieved of the second layer of plant material in the second trial and consequently there was more fresh weight biomass in all treatments at 12 WAT. Additionally, *C. salviniae* densities in the IPM treatments were highest at 6 and 9 WAT, possibly indicating the hatching of a second generation of insects. Longer studies may have observed the brood from a third generation and further biomass reductions or higher insect densities. Natural resource managers typically monitor and reapply herbicides to areas when acceptable reductions are not met within ample timing. Peak insect

densities (6 and 9 WAT) in this study may offer insight for these herbicide reapplications. These data provide alternatives to current methods used late in the growing season for management of *S. molesta* and explores integrated practices for use during early and late season plant management.

While the current herbicide mixture in Louisiana (glyphosate + diquat) is efficacious at reducing plant biomass during early and late season applications (Mudge et al. 2016), plant managers are also relying on *C. salvinia* as part of their management plan (Bonin 2016). While specific sites may be designated for *C. salviniae*, herbicide applications within the same waterbody may overlap insect infestations, and the impact of the combined technologies has not been documented. Based on previously published IPM mesocosm research, where 100% of the plant material was sprayed in integrated treatments, researchers reported penoxsulam + a nonionic and buffering blend surfactant (identical mixture used in the current study) had the lowest *C. salviniae* density compared to flumioxazin + the same surfactant 6 WAT (Mudge et al. 2013). In the Mudge et al. 2013 research, the significant decrease in weevil densities at 2 WAT in the penoxsulam treatment was unknown; however, the current data confirms the low insect density in this treatment throughout the study. This study attempted to avoid direct toxicity to insects in the IPM treatments by spraying only 50% of plant material, and leaving untreated plant material for foraging and harborage. While the low insect densities could be resultant of flight, indirect toxicity, herbicidal activity in the water column, or lack and degradation of food source, these findings should be taken into consideration when natural resource managers are selecting alternate modes of action.

Qualitative data, particularly using visual estimates for percent coverage are a subjective measure. Dethier et al. (1993) found that these estimates to be more accurate and have less variation for an experienced single observer than with other methods studied. Based on these results, experienced plant managers can utilize percent coverage as an estimate for herbicide and IPM efficacy. These IPM techniques coupled with research advances, could benefit stakeholders in Louisiana and Texas by reducing herbicide use and costs over time. In addition to reducing re-occurring expenditures, plant managers would be making a conscience effort to reduce the risks of herbicide resistance. Although herbicide resistance cases have been historically linked to agronomic crops, there are two herbicide resistant weeds in aquatics (Koschnick et al. 2006; Netherland and Jones 2015). In the future, researchers should also consider insect flight activity to evaluate the dispersal activity and linkage to plant nutrition, ambient temperature, and distance of travel after herbicide applications. Lastly, using these methods as a foundation, researchers should experiment with other efficacious herbicides (i.e. bispyribac sodium, fluridone, carfentrazone, and tompramezone) against *S. molesta* to understand the interactions with *C. salviniae*, along with cost estimates for practical aquatic vegetation management.

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CHAPTER 5. SUMMARY AND CONCLUSION

A series of growth chamber trials were conducted in Lena, Louisiana, during 2016 and 2017 to compare cold tolerance and adult survival of 1 southern (Houma) and 2 northern (Natchitoches and Bayou Nicholas) Louisiana populations of *Cyrtobagous salviniae*. After extraction from *Salvinia molesta* and a brief acclimation period (13°C for 72 hours), *C. salviniae* were exposed to cold temperatures of 0, -5, or -9°C. The results from cold exposures trials indicate *C. salviniae* is capable of overwintering and naturally developing cold tolerance in northern Louisiana. In the 2016 trial, the Bayou Nicholas population was 1.3- and 1.4-times more cold tolerant at LT_{50} and LT_{90} values when exposed to 0°C compared to the Houma and Natchitoches populations, respectively. Similarly, in 2016, the Bayou Nicholas population was 1.1- and 1.3-times more cold tolerant than the Houma and Natchitoches populations at -5°C exposures for LT_{50} and LT_{90} values, respectively. Laboratory studies indicated that the Bayou Nicholas population established in northern Louisiana, demonstrated phenotypic plasticity and was the most cold tolerant in 2016 compared to other established populations (Natchitoches and Houma). Trials conducted in 2017 showed an improvement in cold tolerance for the Natchitoches and Houma populations, but cold tolerance within the Bayou Nicholas population remained the same. Although the -9°C exposure data showed no significant differences between populations, these findings demonstrate the survival of *C. salviniae* at lower temperatures and longer intervals than previously reported.

Additional growth chamber and field trials were conducted in Lena during 2016 to determine the upper temperature threshold for adult *C. salviniae* survival, particularly

when exposed to unfavorable conditions during transport from the rearing facility to the release site. Comparisons were made between two types of lids and the placement of temperature loggers within secured transportation totes that are utilized in mass rearing and release operations in Louisiana and Texas. Results indicated that there were no significant differences ($p=0.729$) in the placement of the temperature logger (top, middle, bottom) within the totes and the conventional lids were 2°C higher than those with modified lids. Air temperatures were then compared to temperatures experienced in totes with both lid types to define the linear relationship. Laboratory experiments defined LT_{50} and LT_{90} values for heat exposure temperatures at 35, 40, 45, and 50°C. The LT_{50} values were 27.5 and 14.8 hours of exposure at 35 and 40°C, respectively, and 56.9 and 5.0 minutes for 45 and 50°C, respectively. Furthermore, predictions can be made by using the bivariate analysis conducted to define the linear relationship between predicted internal tote temperature and expected mortality for *C. salviniae* at these temperatures. Natural resource managers should consider modifying transportation tote lids with holes to reduce mortality during transport and can utilize these linear equations to correct estimates of insect densities released after transportation from nursery site.

Outdoor mesocosm trials were conducted in April and August 2016 to determine if integrating *C*. *salviniae* and aquatic herbicides are more efficacious than the herbicides alone. The results of these trials provided a better understanding of integrated pest management techniques and confirmed that the most widely used herbicide treatment in Louisiana, glyphosate + diquat + surfactants, is efficacious against *S. molesta* when used alone*,* regardless of application timing. The current

herbicide mixture of glyphosate + diquat was effective at reducing plant fresh weight, dry weight and percent coverage at 6 weeks after treatment (WAT). Herbicides with alternate modes of action (penoxsulam and flumioxazin) reduced plant biomass similar to glyphosate + diquat, but control varied depending on timing of application.

All integrated treatments, where 50% of *S. molesta* was treated with herbicides, reduced fresh weight biomass similarly to herbicide only treatments, where 100% of plant material was sprayed, but only when treated early in the growing season. Dry weight biomass was reduced similarly for all three herbicide mixtures. The late season herbicide application data suggest that the mixture of glyphosate and diquat is less efficacious than penoxsulam or flumioxazin when used as an IPM approach. Although *C. salviniae* alone were effective against *S. molesta*, this research suggests that incorporating herbicides and insects into a *S. molesta* management program is more beneficial than biological control alone. Insect densities were highest at 6 and 9 WAT, regardless of type of treatment. Conversely, densities were the lowest in the penoxsulam plus surfactant treatment, regardless of herbicide timing. Plant managers should consider treating *S. molesta* with herbicides early in the growing season, either coupled with *C. salviniae* or alone.

VITA

Lauren W. Cozad, a native of Slidell, Louisiana, moved to Natchitoches, Louisiana to pursue a Bachelor of Science degree from Northwestern State University, which she successfully completed in 2010. With a lifelong passion for the outdoors, especially aquatic recreational activities, she was inspired to start a career in aquatic vegetation management. After marriage, three children, and working a full-time career, she was elated at the opportunity to work full time while pursuing master's degree part-time at Louisiana State University. She anticipates graduating in December 2017 and plans to continue working in the aquatic plant management community.