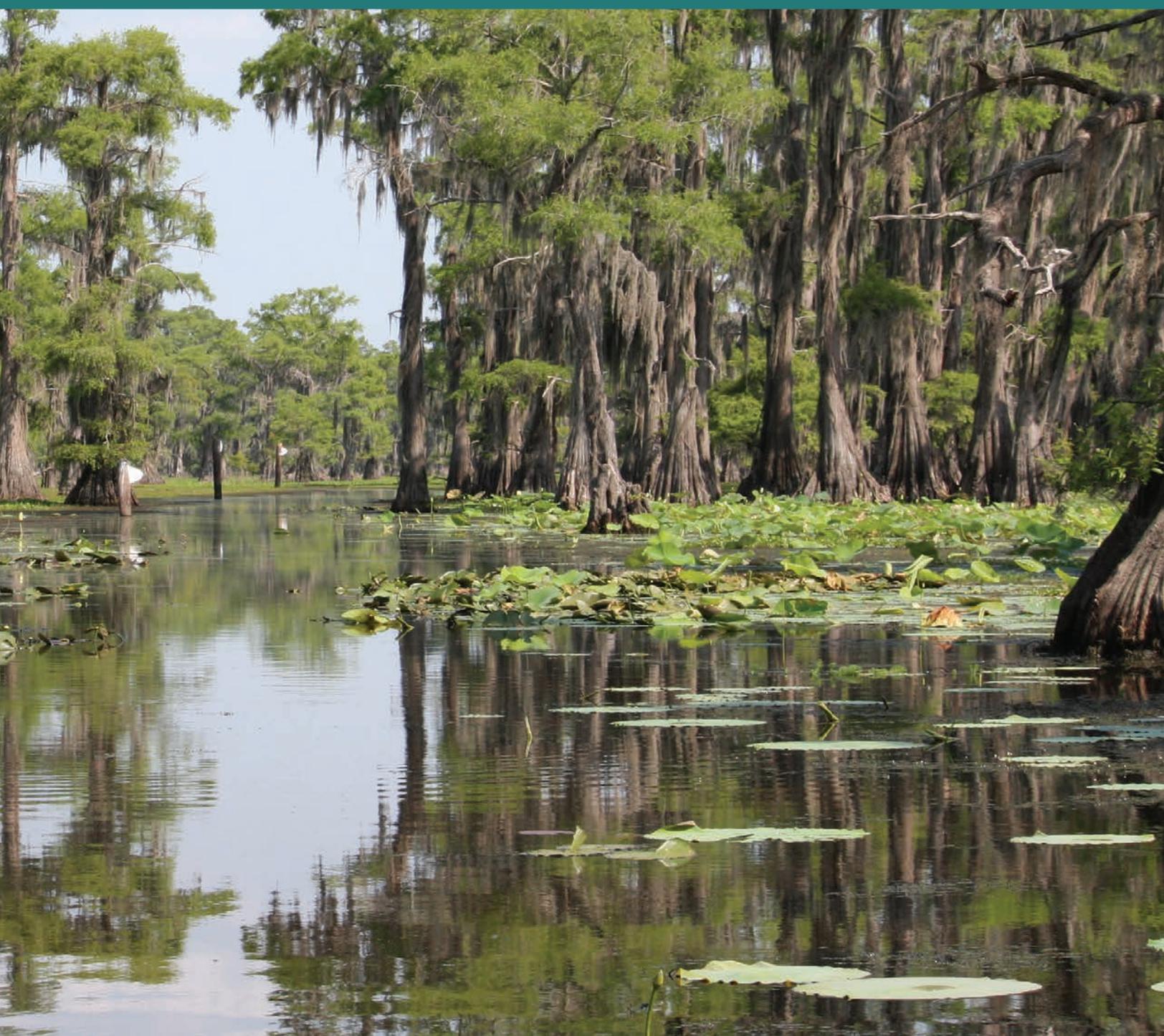


A Guide to Mass Rearing the Salvinia Weevil for Biological Control of Giant Salvinia



A Guide to Mass Rearing the Salvinia Weevil for Biological Control of Giant Salvinia

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Table of Contents

List of Figures	iv
List of Tables	v
List of Acronyms and Abbreviations	v
Preface	vi
A Note About Permits	vi
Disclaimer	vi
Chapter 1 Giant Salvinia, Salvinia Weevil, and Biological Control of Giant Salvinia.....	1
Giant Salvinia: Plant Structure, Growth, and Reproduction	2
The Salvinia Weevil: Life Cycle and Development	4
Biological Control of Giant Salvinia	6
References.....	7
Chapter 2 Water Quality Parameters Important to Growing Giant Salvinia	9
pH.....	10
Nitrogen.....	10
Conductivity and Salinity	11
Alkalinity.....	12
Iron and Other Micronutrients.....	12
Water Testing Laboratories.....	12
List of Suppliers.....	12
References.....	12
Chapter 3 Rearing the Salvinia Weevil for Biological Control of Giant Salvinia at the U.S. Army Corps of Engineers Lewisville Aquatic Ecosystem Research Facility.....	13
Introduction	14
Salvinia Weevil Rearing.....	14
Design of Rearing Containers.....	14
Culture Box Specifications and Costs.....	16
Plant Cultures.....	16
Cost of Supplies.....	16
Weevil Inoculation.....	16

Population Monitoring.....	18
Berlese Funnel Costs	19
Weevil Harvesting for Field Release, Cleaning Boxes, and Initiating New Cultures.....	19
Salvinia/Weevil Augmentations and Thinning of Cultures	19
Overwintering.....	20
Cold Frame Specifications and Pricing.....	20
Weevil Production Estimates	20
Cost Analysis.....	20
References.....	23
Chapter 4 Rearing the Salvinia Weevil in Outdoor Tanks at Caddo Lake, Texas	25
Introduction.....	26
Materials and Design.....	26
Greenhouses	26
Weevil-Rearing Tanks	27
Getting Started.....	29
Water Quality.....	29
Stocking Giant Salvinia and Weevils	30
Monitoring Weevil Abundance.....	31
Harvesting Weevils for Release.....	33
Cleaning and Refilling the Tanks	34
Annual, Monthly, and Weekly Maintenance	34
Weevil Production and Labor Requirements	35
List of Supplies	36
Greenhouse and Tank Structural Equipment	36
Water Quality Monitoring Equipment	36
Water Amendment Products.....	36
Weevil Harvest and Tank Cleaning Equipment.....	36
Chapter 5 Rearing the Salvinia Weevil in the Greenhouse.....	37
Introduction.....	38
Facility Design and Construction	38

Physical Layout.....	38
Rearing Tanks.....	38
Water Quality, Filters	39
Reservoir and Pump	40
Algae Filter	40
Lighting.....	40
Temperature, Fans, Misting System	41
Berlese Funnels.....	41
Procedures to Maintain Salvinia and Weevils	41
Nitrogen Fertilization	41
Alkalinity, pH, and Trace Minerals	42
Controlling Weeds, Insect Pests, and Algae.....	42
Managing Growth of Salvinia and Weevils.....	43
Estimating Weevil Density in Rearing Tanks.....	43
Harvesting Weevils from Rearing Tanks.....	43
Production Estimate	44
List of Suppliers	45
Acknowledgments.....	45
References.....	45
Chapter 6 Rearing the Salvinia Weevil in Ponds.....	47
Temperature	48
Type of Pond.....	48
Size of Pond	48
Water Source	48
Water Depth.....	49
Fertility	49
Pest Control	49
Harvest	49
Pond Rotation	51

List of Figures

Fig. 1-1. Growth stages of giant salvinia.....	3
Fig. 1-2. Egg-beater shaped hairs on leaves of giant salvinia	4
Fig. 1-3. Primary growth stage of giant salvinia.....	4
Fig. 1-4. Tertiary growth stage of giant salvinia	4
Fig. 1-5. Adult salvinia weevil.....	5
Fig. 1-7. Larva of the salvinia weevil	5
Fig. 1-6. Adult salvinia weevils.....	5
Fig. 3-1. Above-ground culture boxes used to rear salvinia weevils	15
Fig. 3-2. Above-ground culture boxes used to rear salvinia weevils.	15
Fig. 3-3. A transfer or submersion screen, shown placed over the salvinia in a culture box.	17
Fig. 3-4. A submersion screen on top of salvinia, prior to submersion.....	18
Fig. 3-5. Berlese funnel with Mason jar attached to the bottom and light fixture on top.	19
Fig. 3-6. Culture boxes are enclosed within cold frames for winter protection.....	21
Fig. 3-7. Supply costs for rearing weevils at LAERF	22
Fig. 3-8. Labor costs for rearing weevils at LAERF.....	22
Fig. 3-9. Proportion of supply and labor costs by season at the LAERF facility	23
Fig. 3-10. Facilities and equipment costs for the LAERF facility	23
Fig. 4-1. <i>C. salviniae</i> greenhouse at the Caddo Lake National Wildlife Refuge.....	27
Fig. 4-2. <i>Cyrtobagous salviniae</i> infested giant salvinia in tanks	27
Fig. 4-3. Aerial view of the two greenhouse structures.....	28
Fig. 4-4. Installing tank liners in the above-ground weevil rearing tanks	28
Fig. 4-5. The first two weeks of starting a weevil rearing tank.....	31
Fig. 4-6. The salvinia has reached secondary and tertiary stage.....	31
Fig. 4-7. Screen placement over weevil tanks.....	32
Fig. 4-8. Berlese funnels at the Caddo Lake Facility.....	32
Fig. 4-9. Heavy weevil infestation showing the characteristic darkening of the plants.....	33
Fig. 4-10. Larval stage of the Samea moth	35
Fig. 4-11. Adult stage of the Samea moth	35

Fig. 5-1. Interior of the Greenhouse.....	38
Fig. 5-2. Salvinia in growth tanks.....	39
Fig. 5-3. RO System.	39
Fig. 5-4. Algae filter.	41
Fig. 5-5. Berlese Funnels.....	42
Fig. 5-6. Screen Removal Tool (SRT).....	44
Fig. 6-1. Moving salvinia plants to conveyor belt.	50
Fig. 6-3. Loading salvinia into totes.	50
Fig. 6-2. Loading salvinia into totes.	50
Fig. 6-4. Transporting totes to release site.	50

List of Tables

Table 1-1. Mean number of days for development of salvinia weevil life stages	6
Table 1-2. Mean number of days for development of salvinia weevil and survival	6
Table 3-1. Weevil production data for 2011.....	21

List of Acronyms and Abbreviations

LAERF	USACE Lewisville Aquatic Ecosystem Research Facility
LSU	Louisiana State University
RO	Reverse Osmosis
TPWD	Texas Parks and Wildlife Department
USACE	U.S. Army Corps of Engineers
USDA	U.S. Department of Agriculture
USDA-APHIS	USDA Animal and Plant Health Inspection Service
USDA-ARS	USDA Agricultural Research Service

Preface

Biological control using the exotic weevil *Cyrtobagous salviniae* is part of the integrated pest management approach presently underway to control giant salvinia in Texas and Louisiana. This approach involves the large-scale rearing and release of *C. salviniae* to suppress the growth of giant salvinia alone and in an integrated program using herbicides. The purpose of this publication is to provide guidelines for constructing mass-rearing facilities and efficiently rearing large numbers of *C. salviniae* for release as a biological control agent of giant salvinia. The need for a guide on rearing giant salvinia weevils was identified by the Inter-agency Giant Salvinia Task Force meeting held in 2011 in Karnack, Texas. This task force is part of the Gulf and South Atlantic Regional Panel of the Aquatic Nuisance Species Task Force. Support for the development of this document was provided in part by Congressional support through the USDA's Natural Resources Conservation Service.

Allen Knutson and Julie Nachtrieb, Project Coordinators

A Note About Permits

Giant salvinia is legally classified as an exotic, harmful, or potentially harmful species by the State of Texas. No person may import, possess, sell, or place giant salvinia into water of this state except as authorized by rule or permit issued by the Texas Parks and Wildlife Department. In order to transport or hold giant salvinia in Texas, an Exotic Species Permit must be obtained each year by the Texas Parks and Wildlife Department. As of this writing, no permit is required in Louisiana to move salvinia or salvinia weevils within the state. Contact state officials to determine current requirements for transporting salvinia.

Interstate movement of live plant pests (salvinia weevil) and host plants (salvinia) for environmental release must be authorized by the U. S. Department of Agriculture (USDA) Animal and Plant Health Inspection Service (USDA-APHIS) with the issue of a Permit to Move Live Plant Pests (PPQ form 526). A second Permit to Move Live Plant Pests (PPQ form 586) from USDA-APHIS is required for each state of origin and destination. Apply for permits at www.aphis.usda.gov/permits.

Disclaimer

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Chapter I

Giant Salvinia, Salvinia Weevil, and Biological Control of Giant Salvinia

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Giant salvinia, *Salvinia molesta* Mitchell, is a free-floating aquatic fern native to southern Brazil that is a weedy invader of lakes, reservoirs, and ponds in the southeastern United States. Giant salvinia grows rapidly, forming dense mats that interfere with boating, fishing, and other water recreation. These extensive mats can displace native vegetation, reduce oxygen content of the water, degrade aquatic ecosystems, and also interfere with electrical generation and water control and supply operations. Giant salvinia was introduced into the United States through the nursery trade as an ornamental plant for water gardens. The first natural populations were reported in South Carolina in 1995, and it has been well established in Texas since 1998. Common salvinia, *S. minima* Baker, is another closely related exotic, floating fern that is widely distributed in the southeastern United States. It was first reported in the United States in Florida in 1928 and is now found in Louisiana and Texas. Common salvinia can also form large mats but is generally less of a problem in the United States than giant salvinia (Madeira et al. 2006).

Giant salvinia is easily transported from lake to lake by infested boats, boat trailers, and other equipment; removing all the salvinia from boats, trailers, and gear is important to prevent new infestations. Rapid detection and eradication of new infestations can also be effective in minimizing spread. Once a water body is infested with giant salvinia, control options include herbicides, mechanical removal, water draw-down, and biological control. Biological control, using the commonly known salvinia weevil, *Cyrtobagous salviniae* Calder & Sands, has been used to control giant salvinia in many areas of the world. This publication focuses on facilities and procedures necessary for mass rearing the salvinia weevil, *C. salviniae*, for release as the biological control agent of giant salvinia.

Rearing large numbers of healthy salvinia weevils requires an understanding of the specific requirements needed to maintain both the host plant and the insect. The artificial conditions typical of mass-rearing facilities create unique problems generally not encountered in natural settings. These problems include large fluctuations in water quality and nutrient content, contamination of rearing facilities with

other plants, and infestation by generalist insects that feed on salvinia. Dan Flores of the U.S. Department of Agriculture Animal and Plant Health Inspection Service (USDA-APHIS) first developed procedures for large-scale rearing of the salvinia weevil in the United States at a facility in Mission, Texas (Flores and Carlson 2006, Flores no date). Methods for maintaining water quality necessary for optimum plant growth and managing plant growth for optimum weevil production are detailed in the following chapters.

Salvinia weevils have been mass reared in small and large tanks as well as in small ponds. Indoor rearing facilities, especially in northern areas, can maintain warmer temperatures needed for rapid reproduction, avoiding winter mortality of weevils due to extreme cold. However, indoor facilities increase construction costs. The following chapters provide detailed instructions on constructing and maintaining weevil-rearing facilities both indoors (greenhouse) and outdoors. The optimum facility and production practices for a particular project will depend on budget limitations, existing facilities, and the number of weevils needed to achieve a project's goals.

Giant Salvinia: Plant Structure, Growth, and Reproduction

A giant salvinia plant consists of nodes (sites where leaves attach and buds grow) interconnected by branching stems called rhizomes. Two floating leaves and a third modified leaf, referred to as a root, are present at each node along the rhizome. A node, the 2 attached leaves, and the root is termed a ramet, and a plant consists of a series of connected ramets. A terminal bud is present between the newly formed leaves at the end of each rhizome. Lateral buds are present at the base of the leaves and give rise to new branches (Sands et al. 1983) (Fig. 1-1).

The upper surface of the leaves is covered with rows of tiny hairs that split and then fuse at the tip, creating an egg-beater shape, an identifying characteristic of giant salvinia (Fig. 1-2). Common salvinia also has rows of hairs on the leaves, but the hair tips do not form the egg-beater shape. The “root” is suspended in the water and consists of a mass of

fine filaments. Sporocarps, which appear as rows of beads, are found within the root filaments. Spores within the sporocarp are sterile, and reproduction is only by vegetative growth and fragmentation (Julien et al. 2009). As the rhizome breaks apart with age and damage, rhizome pieces with apical buds give rise to new plants. Lateral or axillary buds also grow and form new ramets, especially at high nitrogen levels. Plants disperse naturally through currents and floods.

Giant salvinia plants appear in 3 forms, depending on degree of crowding and nutrient availability (Fig. 1-1). Primary-form plants have small leaves (up to 15 millimeters wide) lying flat on the water surface and appear singly in open water (Fig. 1-3). Secondary plants have slightly folded leaves (15–25 millimeters wide) and appear when plants begin to clump together. The tertiary plants have large leaves that fold upward above the water surface and are densely packed along the rhizome (Fig. 1-4). The primary plants are easily carried by water and are often the invading form, while the tertiary plants grow into dense mats. The tertiary form also produces sporocarps and is typical of mature stands that form tightly packed, thick mats of plants.

The optimum temperature for salvinia growth is about 30 °C (86 °F), and growth ceases below 10 °C (50 °F) and above 40 °C (104 °F) (Room and Thomas 1986). Plants exposed to -3 °C (26.6 °F) for

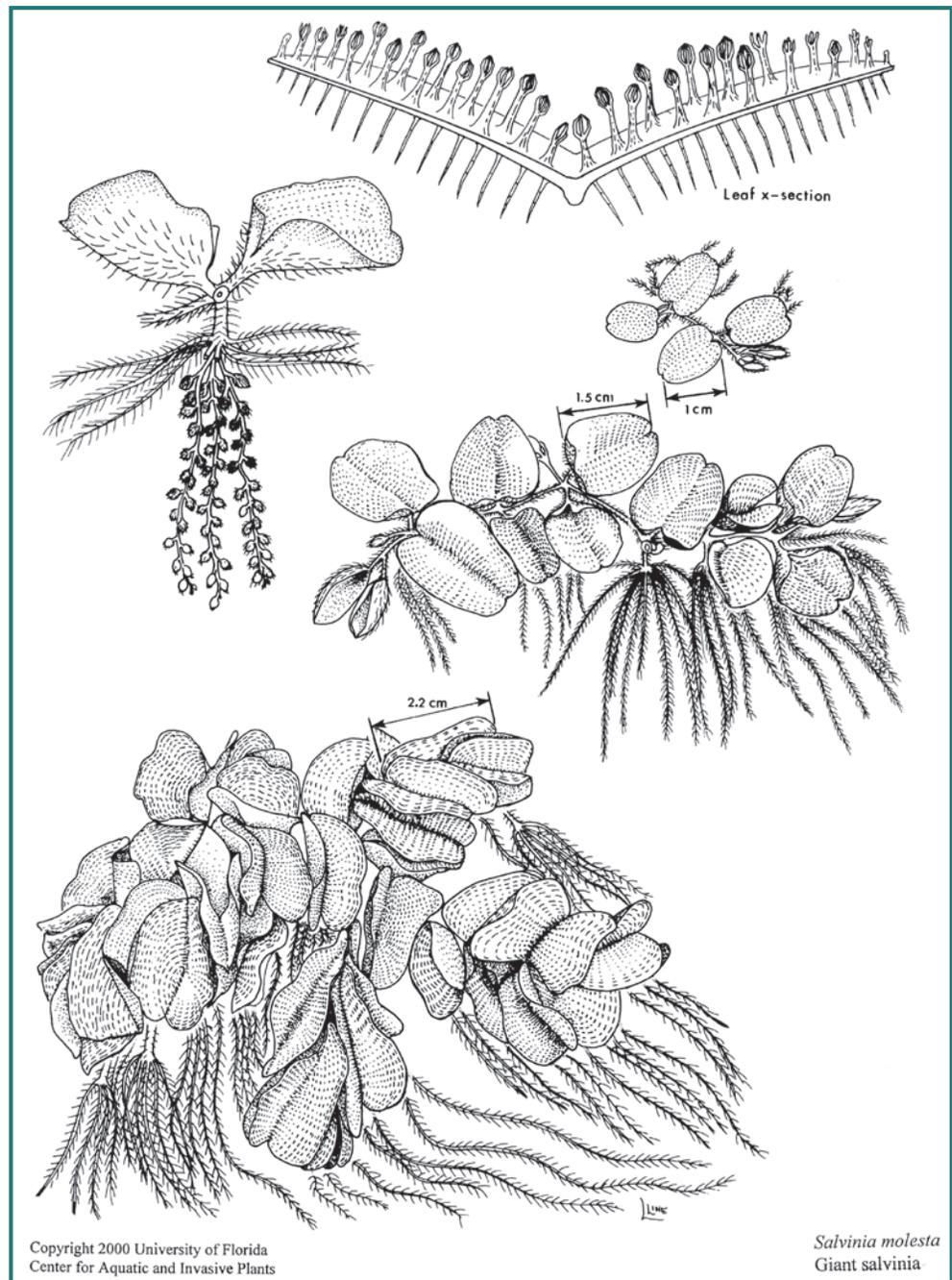


Fig. 1-1. Growth stages of giant salvinia. Copyright University of Florida.

at least several hours are killed if ice forms. However, buds are usually below the water surface and can survive frosts unless the surface water freezes (Whiteman and Room 1991; Owens et al. 2004). Buds protected within a mat of other salvinia plants can be insulated from cold and survive frosts. Salvinia growth increases with increasing light intensity up to about 4,000 kilocalories/meter/day and higher levels result in decreased growth (Rani and Bhambie 1983). In mid-summer, when temperatures and light intensity are high, salvinia growth is greater in

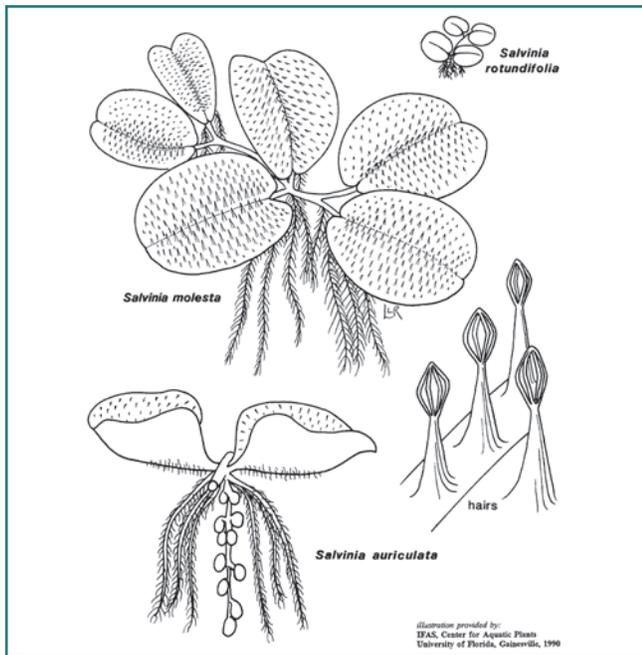


Fig. 1-2. Egg-beater shaped hairs on leaves of giant salvinia. Copyright University of Florida.

the shade than in full sun because shading moderates both temperatures and light intensity.

The Salvinia Weevil: Life Cycle and Development

The salvinia weevil, *C. salviniae*, completes 4 development stages: egg, larva, pupa, and adult. The

adult weevil has a long snout, is about 2–3.5 millimeters long (0.08–0.14 inches), and has numerous shallow punctures on the elytra, the hard wing covers (Figs. 1-5 and 1-6). Adults are brown for the first few days after emergence but then turn shiny black. Adults are found on and between leaves, within leaf buds, and within the roots of the salvinia plant. While underwater, the weevils obtain oxygen from a thin film of air held beneath the body. Adults must periodically come to the surface to replenish this film of air. Adults feed on salvinia buds and leaves, causing ragged holes in the leaves. Feeding on buds causes the buds to turn black and die.

Male and female adults look identical, and while females are generally larger than males, sexing adults is very difficult. Adult females can live 3–5 months at temperatures above 23 °C (73 °F), and longevity is extended during cooler weather (Sands et al. 1986). Females typically deposit eggs inside shallow holes chewed in salvinia buds, leaves, and rhizomes and sometimes suspend the eggs among the roots. At a constant 25.5 °C (78 °F), females begin depositing eggs about 6–14 days after they emerge as adults (Forno et al. 1983). Under laboratory conditions, females produce an average of 208–374 eggs/female during a lifespan of 20–36 weeks (Sands et al. 1986; Jayanth 1989; Eisenberg 2011). Females produce more eggs with increasing nitrogen content of the plant, and the differences in egg production



Fig. 1-3. Primary growth stage of giant salvinia.



Fig. 1-4. Tertiary growth stage of giant salvinia.



Fig. 1-5. Adult salvinia weevil. Photo by Scott Bauer, USDA Agricultural Research Service.

in these studies is likely related to different nitrogen content. Salvinia plants with a nitrogen content of 2–3 percent dry weight are considered optimum for weevil reproduction (Sands et al. 1986).

Eggs are shaped like jelly beans and are about 0.5 millimeters (0.02 inches) long. Eggs hatch in about 10 days at 25.5 °C (78 °F) (Forno et al. 1983).

Young larvae feed externally on roots and in leaf buds during the first 1–2 weeks and then tunnel inside the rhizomes or within the base of the leaf and leaf petiole. Larvae feed on the terminal bud



Fig. 1-6. Adult salvinia weevils.

and tunnel into the new rhizome, as these sites have higher concentrations of nitrogen, needed for larval development, relative to older nodes (Sands et al. 1983). Larvae mature in about 23 days at 25.5 °C (78 °F). Full-grown larvae are white with a brown head and are about 2.6 millimeters (0.10 inches) long (Fig. 1-7). Once larvae are full-grown and feeding is complete, they exit the plant, spin a silk cocoon around themselves, and then transform into the pupal stage. The cocoon, about 2–2.6 millimeters (0.08–0.10 inches), is fastened to the rhizome or at the base of the root mass below the water line.



Fig. 1-7. Larva of the salvinia weevil. Photo by Peggy Greb, USDA Agricultural Research Service, Bugwood.org.

Table 1-1. Mean number of days for development of salvinia weevil life stages at a constant temperature of 25.5 °C (78 °F) (Forno et al. 1983).

Stage	Days in Stage	Range
Egg	10	9–11
Larva	23	17–28
Pupa	13	10–15
Egg to Adult	45	36–54
Egg to Egg	55	46–64

The cocoon is interwoven with the root hairs and acquires the color of the brown root mass. As a result, the cocoon is well camouflaged and difficult to find. The pupa inside the cocoon is inactive and does not feed. During the pupal stage, the larva transforms into the adult weevil. The pupal stage lasts about 10–15 days at 25.5 °C (78 °F), after which the adult weevil emerges from the cocoon. Adults are light brown after emerging from the pupa and then darken to black 5–6 days later.

Development from egg to adult requires about 45 days at 25.5 °C (78 °F) (Forno et al. 1983). Adding 10 days for mating and initiation of egg-laying yields a total generation time from egg to egg of about 55 days at 25.5 °C (78 °F) (see Table 1-1). As the development rate is temperature dependent, generation time is longer during cool weather and shorter in mid-summer (Table 1-2). An optimum temperature range for weevil reproduction is about 23 °C to 27 °C (73 °F to 81 °F) and a photoperiod of 14 hours of light/day. At 31 °C (88 °F), female longevity is reduced (females lay the same number of eggs/week but live fewer weeks) and egg mortality is greater than at lower temperatures (Sands et al. 1986).

In laboratory studies, females did not deposit eggs at water temperatures below 21 °C (70 °F) and eggs failed to hatch at or below 19 °C (66 °F) (Forno et

al. 1983). Larvae fail to develop at water temperatures below 17 °C (62.6 °F) (Sands et al. 1983). Therefore, reproduction ceases when temperatures fall below 21 °C (70 °F) in the fall, but adults will continue to feed until temperatures fall below 13 °C (55 °F). Weevils overwinter as adults and probably feed on warm days during the winter. Mating and egg-laying resume in the spring once temperatures are consistently above 21 °C (70 °F). At a constant 31 °C (88 °F), survival from egg to adult declined from about 42 percent to 28 percent due to reduced egg survival (Table 1-2).

Weevils have wings but apparently do not fly unless food (young salvinia buds) is limited because of high weevil populations or death of plants from drought, herbicides, etc. Flight has not been observed in the United States but has been reported in Australia.

Biological Control of Giant Salvinia

Biological control is the science of using living organisms, usually an insect or pathogen, to suppress a pest population. Many weeds, including giant salvinia, were introduced into the United States without their specialized natural enemies. Biological control seeks to reunite these natural enemies with their food plant, the weed, and to establish populations of the natural enemies that feed on the plants, thus reducing the abundance of the weeds. Biological control never eradicates the weeds.

The salvinia weevil feeds only on giant salvinia and will die of starvation if salvinia is not present. The adults preferentially feed on tender apical buds, leaves and roots, and the larvae feed on leaf buds and tunnel within the rhizomes. Although feeding by both adults and larvae is important, tunneling in the rhizomes by weevil larvae is more important,

Table 1-2. Mean number of days for development of salvinia weevil and survival at constant temperatures (Sands et al. 1986).

Temperature	Egg		Larva		Pupa		Total egg to adult	
	Days	Survival	Days	Survival	Days	Survival	Days	Survival
23 °C (73 °F)	14	75	25	77	19	75	58	42
27 °C (81 °F)	8	72	16	77	12	75	36	41
31 °C (88 °F)	6	49	14	77	9	75	29	28

as it disconnects the root-shoot link, thereby preventing nutrients from reaching leaves and buds. Because of this feeding, the leaves and buds die, the rhizomes decay and break apart, and the plants die and disintegrate (Sands et al. 1983).

The salvinia weevil, first collected from Brazil and released in 1980 in Australia, rapidly increased and reduced giant salvinia infestations in many areas of Australia (Room et al. 1981). Since then, researchers have successfully introduced the salvinia weevil for biological control of giant salvinia in at least 15 tropical and subtropical countries.

The salvinia weevil was first reported in the United States in Florida in 1960. It was apparently accidentally introduced with common salvinia, which was first reported in Florida in 1928. Adults of the Florida population are smaller than the adults from Brazil and Australia and are described as the Florida ecotype of *C. salviniae* (Madeira et al. 2006). The Brazilian weevil and the Florida weevil feed on and reproduce equally well on both common and giant salvinia, although the Brazilian weevil prefers larger (tertiary) plants without regard to host species (Tipping and Center 2005; Tewari and Johnson 2011). This preference is possibly due to the smaller diameter of the rhizome in common salvinia, relative to giant salvinia, which restricts movement of the larger Brazilian larvae.

Beginning in 1999, U.S. Department of Agriculture Agricultural Research Service (USDA-ARS) entomologists collected salvinia weevils from Florida and released them in Texas and Louisiana for biological control of giant salvinia. Results were discouraging, and the effort was then refocused on introducing Australian salvinia weevils originally from Brazil. Entomologists released salvinia weevils obtained from Australia in 2001 in Texas and Louisiana. Studies during 1999–2005 found the Brazilian salvinia weevil could overwinter and reduce salvinia by up to 99 percent at 2 research sites, one near Houston, Texas, and the other in Louisiana on Toledo Bend (Tipping et al. 2008, Tipping and Center 2003).

This success led to the need to develop mass-rearing procedures to supply large numbers of salvinia weevils for release against giant salvinia in Texas and Louisiana. A USDA-APHIS project developed

rearing procedures, and in 2001–2005, more than 2 million salvinia weevils were reared in an outdoor facility near Mission, Texas (Flores and Carlson 2006). Many of these weevils were released in the Beaumont/Port Arthur area of southeast Texas. At 4 of 5 sites, weevils reduced the salvinia by 90 percent within 9 months, and the dissolved oxygen content of the water increased. The USDA-APHIS weevil-rearing facility was closed in 2005. In 2003, a weevil-rearing facility was initiated at the Lewisville Aquatic Ecosystem Research Facility (LAERF) operated by the U.S. Army Corps of Engineers Research and Development Center in Lewisville, Texas (see Chapter 3). In 2007, the Louisiana State University AgCenter began mass rearing salvinia weevils in greenhouses for research (see Chapter 5) and in small ponds for redistribution (see Chapter 6). In 2010, the Center for Invasive Species Eradication, part of the Texas A&M University System, in cooperation with the Texas Parks and Wildlife Department (TPWD) and the Caddo Lake Institute, constructed a weevil-rearing facility near Karnack, Texas (see Chapter 4). TPWD constructed a similar mass-rearing facility at Jasper, Texas. The methods and procedures used to rear salvinia weevils at these mass-rearing facilities are summarized in this manual.

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Chapter 2

Water Quality Parameters Important to Growing Giant Salvinia

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Growth of giant salvinia is independently affected by water temperature, salinity, nutrients, and pH (Owens and Smart 2010). Each of these water quality parameters are discussed below. Information on water quality parameters and testing is taken from the U.S. Environmental Protection Agency report “Water: Monitoring and Assessment,” <http://water.epa.gov/type/rs/monitoring/vms59.cfm>

pH

The optimum pH for maximum growth of giant salvinia ranges from 5.5–7.5 (Owens et al. 2005). The water solubility of micronutrients such as iron and manganese in water declines with increasing pH. Since salvinia is a floating plant and therefore does not have roots in the soil, it must extract nutrients from the water column. The decline in the availability of micronutrients with increasing pH accounts for the reduction in salvinia growth at pH 8.0 and above. Adding peat moss and using products such as Green Light Iron and Soil Acidifier can be used to maintain iron levels at 3 milligram/liter and pH below 7.0.

The pH scale is used to indicate how acidic or basic a solution is on a scale from 1.0 to 14.0. The pH scale measures the logarithmic concentration of hydrogen (H⁺) and hydroxide (OH⁻) ions, which make up water (H⁺ + OH⁻ = H₂O). When both types of ions are in equal concentration, the pH is 7.0 or neutral. Below 7.0, the water is acidic (there are more hydrogen ions than hydroxide ions). When the pH is above 7.0, the water is basic (there are more hydroxide ions than hydrogen ions). Since the scale is logarithmic, a drop in the pH by 1.0 unit is equivalent to a 10-fold increase in acidity. So a water sample with a pH of 5.0 is 10 times as acidic as one with a pH of 6.0, and pH 4.0 is 100 times as acidic as pH 6.0. The pH affects many chemical and biological processes in the water. Most aquatic organisms prefer a pH range of 6.5–8.0.

A pH meter measures the electric potential (millivolts) across an electrode when immersed in water. This electric potential is a function of the hydrogen ion activity in the sample. Infrequently used or improperly maintained electrodes are subject to corro-

sion, which makes them highly inaccurate. The pH can be measured with electronic hand-held “pens” that are dipped in the water and provide a digital readout of the pH. They are calibrated with a single standard. Lab meters can be calibrated with 2 or more standard solutions and thus are more accurate over a wide range of pH measurements. The color change of pH paper when dipped into the water can also measure pH as the intensity of the color is proportional to the pH of the sample. The pH can be determined by matching the colors from the chart to the color of the sample. The pH should be measured within 2 hours of the sample collection; otherwise, the formation of a weak acid, caused by the dissolution of carbon dioxide from the air in the water, will lower the pH.

Nitrogen

Nitrogen is required for plant growth and the production of new buds on which salvinia weevil larvae and adults feed. For optimum salvinia growth, Flores (no date) recommended a water nitrogen content of 10 milligram/liter (parts per million) with a range of 7–16 milligram/liter. Water must be periodically tested to determine the nitrogen content, and if necessary, nitrogen fertilizer must be added to maintain optimum nitrogen concentration during the growing season. Phosphorous is also required, and many of the nitrogen fertilizers suggested in the following chapters also include phosphorous. Miracle-Gro Water Soluble All Purpose Plant Food 24-8-16 contains 24 percent nitrogen (N), 8 percent phosphorous (P), and 16 percent potassium (K) and provides macronutrients (N, P, K), iron, and other trace elements need for salvinia growth. Do not use lawn fertilizers that contain herbicides, as these weed killers may be toxic to the salvinia plants.

Nitrogen is present in water in 1 of 3 forms: ammonia (NH₃), nitrates (NO₃), and nitrites (NO₂). Most of the nitrogen in surface water is present as nitrate, and the level of ammonia or nitrate in surface water is typically low (less than 1 milligram/liter). This low level is because in the presence of oxygen, ammonia is quickly oxidized by bacteria into nitrites and then to nitrates. The decomposi-

tion of organic matter can lower the oxygen level, allowing the concentration of ammonia and nitrites to increase. These 2 forms of nitrogen are more toxic to aquatic life than nitrates.

Nitrate and nitrite content can be easily measured using paper test strips that change color when dipped into the water sample. Nitrate can also be measured using either the cadmium reduction method or a nitrate electrode. The cadmium reduction method produces a color reaction that is then measured either by comparing to a color chart or by using a spectrophotometer. The nitrate electrode can measure in the range of 0 to 100 milligram/liter nitrate. Nitrate electrodes are fragile and must be carefully maintained and calibrated before each sample run. Collect water samples to be tested for nitrate in glass or polyethylene containers. Factory-sealed, disposable Whirl-Pak bags work well. Test samples for nitrates within 48 hours of collection and keep them in the dark and on ice or refrigerated.

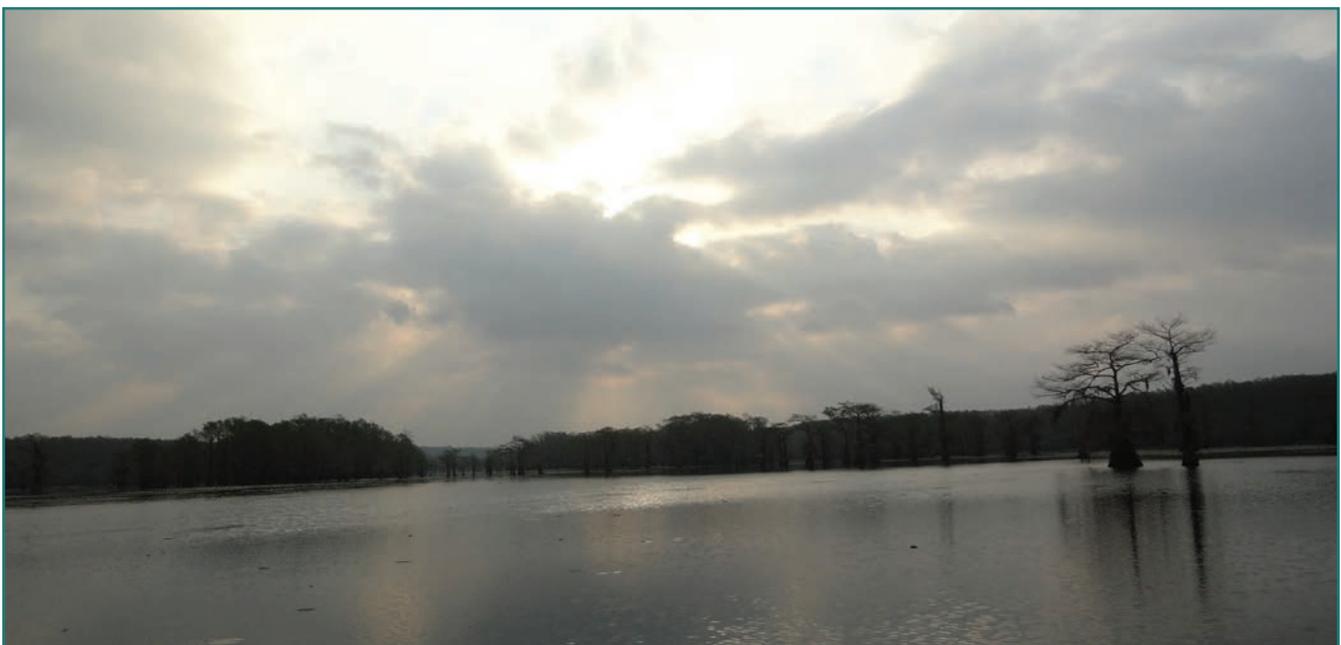
Conductivity and Salinity

Giant salvinia can grow in waters with a wide range of conductivity. Salvinia has infested sewage lagoons with a conductivity of 1375 microsiemens/centimeter but conductivity levels of 2000–4800 microsiemens/centimeter have significantly reduced salvinia growth (Owens and Smart 2010; Divarkaran et al. 1980; Room and Gill 1985). Conduc-

tivity can increase due to accumulation of salts from fertilization. Conductivity levels that exceed 1500 microsiemens/centimeter may be a concern, especially if salvinia growth is reduced, and is best corrected by replacing the water in the rearing tank.

Conductivity is a measure of the ability of water to conduct an electrical current. It increases with a greater concentration of inorganic dissolved solids such as chloride, nitrate, sulfate, and phosphate anions (ions that carry a negative charge) and sodium, magnesium, calcium, iron, and aluminum cations (ions that carry a positive charge). In water, salts dissolve into positively charged ions and negatively charged ions, which conduct electricity. Thus, conductivity is related to the concentration of salts dissolved in water and is therefore a measure of salinity. Organic compounds do not conduct electrical current very well and therefore have a low conductivity in water. Conductivity is also affected by temperature: the warmer the water, the higher the conductivity. For this reason, conductivity is reported as conductivity at 25 °C (77 °F).

Conductivity is measured as microsiemens per centimeter and micromhos per centimeter, and both units are interchangeable as 1 micromhos/centimeter = 1 microsiemens/centimeter. The conductivity of distilled water is 0.5 to 3 microsiemens/centimeter. The conductivity of potable waters and rivers in the United States generally ranges from 50 to 1500 microsiemens/centimeter. Conductivity is measured



with a probe and a meter. Some conductivity meters can also be used to test for total dissolved solids and salinity.

Samples are collected for later measurement in a glass or polyethylene bottle that has been washed in phosphate-free detergent and rinsed thoroughly with both tap and distilled water. Factory-prepared Whirl-Pak bags may also be used.

Alkalinity

Alkalinity is a measure of the capacity of water to neutralize acids (see pH description). Alkalinity is important when it is necessary to add acid or peat moss to water to lower the pH to 7.0 as required for optimum growth of salvinia. Adding acidifiers to highly alkaline water may provide only a temporary reduction in pH. In such cases, it may be necessary to use a different water source.

Alkaline compounds in the water such as bicarbonates (baking soda is one type), carbonates, and hydroxides remove H⁺ ions and increase pH. They usually do this by combining with the H⁺ ions to make new compounds. Without this acid-neutralizing capacity, any acid added to water would cause an immediate change in the pH. Measuring alkalinity is important in determining the water's ability to neutralize acidic fertilizers and other acidic compounds. Alkalinity is influenced by rocks and soils, salts, certain plant activities, and certain industrial wastewater discharges. Total alkalinity is measured by measuring the amount of acid (e.g., sulfuric acid) needed to bring the sample to a pH of 4.2. At this pH, all the alkaline compounds in the sample are "used up." The result is reported as milligrams per liter of calcium carbonate (milligram/liter CaCO₃).

Measuring alkalinity requires training and a digital titrator or buret. If these are not available, send the water sample to a water-testing laboratory to determine alkalinity. Alkalinity samples must be analyzed within 24 hours of their collection. Keep the samples on ice and take them to the laboratory as soon as possible.

Iron and Other Micronutrients

The addition of iron (Green Light Iron and Soil Acidifier) may also be necessary to maintain an opti-

imum iron concentration of about 3 milligram/liter in the rearing tanks. The addition of iron and other micronutrients is especially important if reverse osmosis (RO) water is used (see Chapter 5). Paper test strips can be used to measure iron concentration.

Water Testing Laboratories

- Louisiana State University:
http://www.lsuagcenter.com/en/our_offices/departments/SPESS/ServiceLabs/soil_testing_lab/
- Texas A&M University:
<http://soiltesting.tamu.edu/>

List of Suppliers

- HANNA H19813-6, pH, conductivity, ppm, temperature unit: www.hannainst.com/usa/
- HANNA Nitrate photometer, measures nitrate levels: www.hannainst.com/usa/
- AquaCheck Nitrate and Iron Test Strips: www.aquachek.com/

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Chapter 3

Rearing the *Salvinia* Weevil for Biological Control of Giant *Salvinia* at the U.S. Army Corps of Engineers Lewisville Aquatic Ecosystem Research Facility

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Introduction

Researchers at the U.S. Army Corps of Engineers Lewisville Aquatic Ecosystem Research Facility (LAERF) in Lewisville, Texas, have reared salvinia weevils (*Cyrtobagous salviniae*) in culture boxes since 2008. The salvinia weevils reared at LAERF were obtained from the Animal and Plant Health Inspection Service (USDA-APHIS) rearing facility at Mission, Texas, and originated from weevils introduced into the United States from Australia by the U.S. Department of Agriculture Agricultural Research Service (USDA-ARS) (Tipping and Center 2003). LAERF is located within the northernmost range of giant salvinia, making it difficult to maintain plant and insect cultures during winter. Rearing in small, above-ground boxes—as opposed to large ponds—provided the most practical means of protecting plants and weevils during winter.

Successful overwintering can lead to early season weevil population peaks and, in turn, early field releases. Early season field releases provide the weevils with more time to acclimate to their new environment, increasing their numbers through subsequent generations before winter. Additionally, in spring and early summer, there is typically less giant salvinia present at field sites, making it more likely that the weevils can reduce the growth and spread of the plants.

During 4 years of rearing in culture boxes, Corps researchers have attempted and modified many different rearing and overwintering techniques with the ultimate goal of producing early season weevil population peaks. During the first few seasons of box rearing, results were similar to pond rearing, with weevil densities peaking late in the season. Approximately 10,000 and 16,000 adult weevils were produced, primarily during the months of September through November, in 2009 and 2010 respectively. During the fourth year of box rearing, 2011, overwintering was successful and early-season population peaks were attained. Approximately 28,000 adults and 55,000 larvae were produced from late April to early July 2011. All of the above figures are estimated from 1 harvest/box (23 boxes total/season).

Rearing in culture boxes has been the most reliable and feasible method for rearing at LAERF within the northernmost range of giant salvinia. The following is an overview describing current rearing facilities and procedures developed and used by the LAERF.

Salvinia Weevil Rearing

Design of Rearing Containers

At the LAERF, salvinia weevils are reared in 30 above-ground culture boxes (measuring 5 feet × 10 feet × 2 feet) (Figs. 3-1 and 3-2). Box depth was set at 2 feet to accommodate high rates of evapotranspiration, which can quickly deplete the water. Multiple small boxes were built instead of a few large boxes because culture maintenance, sampling, and harvesting can be accomplished with less time and manpower when working on a smaller scale. For instance, with small boxes facility personnel can reach all areas from outside the boxes and do not have to enter the water. Weevil-inhabited salvinia is harvested for field release with nets and samples are typically collected by hand. Population estimates may also be more accurate when multiple samples are collected from a smaller area.

Having multiple small boxes also facilitates a quicker recovery time following unpredictable problems. When working in outdoor environments with plants and insects, many unexpected problems can arise and cultures can rapidly become stressed beyond recovery. Some of the commonly encountered problems include pest insect invasions, incorrect water chemistry or fertilization, accidental draining of water due to liner damage, and an overabundance of salvinia weevils leading to salvinia depletion and a lack of food for the weevil colony. Using multiple culture boxes provides a safety net. If an unpredicted and irreversible problem occurs, the entire insect and/or plant colony is not lost because other insect and plant cultures exist. Furthermore, small boxes can be easily drained and restarted with a relatively small amount of plants and insects.



Fig. 3-1. Above-ground culture boxes used to rear salvinia weevils.



Fig. 3-2. Above-ground culture boxes used to rear salvinia weevils.

Culture Box Specifications and Costs

- Boxes, 5 feet × 10 feet × 2 feet, constructed from treated lumber, lined with 45-mil EPDM Firestone pond liner, and stabilized by attaching cables between opposite t-posts along the 10-foot long sides (Figs. 3-1 and 3-2)
- Treated lumber, 60 feet of 2 inch × 12 inch × 12 feet = \$80: lowes.com
- Pond liner, 10 feet × 15 feet = \$78: www.azponds.com
- T-posts: Six 6-foot posts = \$24: lowes.com
- Miscellaneous supplies, such as wire and turn bolts = \$20
- Estimated cost of each culture box = \$202

Plant Cultures

Giant salvinia grows best in water with pH ranging from 5.0 to 7.0 (Owens et al. 2005) in which micronutrients, such as iron, are available for uptake. Culture boxes (2,700 liters or 713-gallon capacity) are supplied with city water (pH 8.0–9.0) that is adjusted to a pH of less than 7.5 and amended with iron and nitrogen. Peat moss is added to each box while dry at an approximate rate of 42 milliliter/liter water (5.5 ounces/gallon) (Owens and Smart 2010). Each box is filled with city water and through peat decomposition and release of humic acid, the water's pH is reduced. Peat will initially float but will sink upon complete saturation. The pH is monitored at least once per week and giant salvinia is added once pH is less than 7.5 and the peat has sunk.

Enough giant salvinia is added to each box to occupy approximately 25 percent of the surface area. It is preferable to initially fill the culture box with a small amount of giant salvinia, as opposed to filling 100 percent of the box with mature salvinia, so that the box is filled with new growth of the initial plants. When salvinia is actively growing, buds (the new growth and the part of the plant preferred by adult weevils) are plentiful and high in nitrogen (Forno and Bourne 1988), which helps increase weevil production (Forno and Semple 1987).

Plants are given 1 week to acclimate and then each box is fertilized with Miracle-Gro Water Soluble All Purpose Plant Food (24-8-16) and Green Light Iron

and Soil Acidifier. The nitrogen and iron content of the water are measured, and then each product is added until the water analysis shows a concentration of 10 milligram/liter nitrogen and 3 milligram/liter iron. To maintain these levels of nitrogen and iron, boxes are fertilized approximately every 3–5 weeks depending on nutrient levels and amount of active giant salvinia growth in each box. Plants typically require more nutrients during periods of active growth, such as when boxes are originally started. Nutrient uptake will decrease once boxes are established. Nutrients can be monitored with test kits or through visual observations; for example, plant yellowing may indicate an iron deficiency. When first starting a rearing effort, it can be helpful to monitor nutrients every 3–4 weeks until a sense of how often nutrients need replenishing is gained.

Cost of Supplies

- Peat moss, 3 cubic feet (treats approximately 4 boxes) = \$11.84: lowes.com
- Estimate for initial peat moss additions for 30 boxes = \$88.80
- Miracle-Gro Water Soluble All Purpose Plant Food, 6.25 pounds (treats 22.5 boxes for 1 fertilizing event) = \$12.27: lowes.com
- Green Light Iron and Soil Acidifier, 1 gallon (treats 7.5 boxes for 1 fertilizing event) = \$10.97: lowes.com
- Estimate per fertilizing event for all 30 boxes = \$60

Weevil Inoculation

Weevils should be released into each box once giant salvinia covers 75–100 percent of the water surface. Approximately 20 percent of the culture boxes do not receive weevils and are used as giant salvinia cultures to provide fresh, healthy plants when starting new boxes or providing a fresh food source to sustain boxes with large weevil populations.

Culture boxes are typically inoculated with weevils through the addition of weevil-inhabited salvinia collected from the field and from other rearing organizations, or supplied from preexisting cultures at the LAERF. The facility uses three different inoculation methods. In one method, a portion of

the weevil-free salvinia in each box is removed and weevil-inhabited salvinia mixed in. Integrating the weevil-free and weevil-inhabited salvinia is important so that weevils can quickly and easily find the new food source.

The second method, plant submersion, is used to transfer weevils without plants into prepared cultures. With this method, plants in a weevil-inhabited box are submersed, forcing adult weevils to vacate the submersed plants and come to the surface for air. A screen is placed inside the box to cover the entire surface of the water. Screens are constructed from 1/4-inch-mesh poultry netting with a 2-inch \times 2-inch treated wood frame (Fig. 3-3). Two to three tension rods (shower curtain rods) are placed on top of the screen, parallel to the screen, and expanded to fit snugly inside the box (Fig. 3-4). The tension rods are used to submerge the screen and the salvinia approximately 6 inches below the water surface. Weevil-free salvinia is scattered across the water's surface to act as a floating island on which adult weevils congregate once they vacate the submersed plants. The floating salvinia can then be collected and placed into a different culture box. With this method, adults are extracted from plants

while larvae and pupae remain in the box. Salvinia is typically submersed for 3–5 days to recover the maximum number of adults. However, some adults will appear on the surface after 1 day. The exact time plants need to be submersed to force weevils to the surface is unknown as weevils are able to breathe from a film of air on their ventral surface (Forno et al. 1983). Weevils may also find air bubbles in the mass of submersed plants and continue to survive submersed for several days. Larvae may have an increased risk of dying from the lack of oxygen if plants (and larvae) are submersed for long periods.

The third method, the use of transfer screens to dry out plants, also transfers weevils without plants into prepared cultures. Transfer screens are constructed the same way as submersion screens, except the screens are sized to span widthwise across each box. A thin layer of weevil-inhabited salvinia is placed on each screen directly over the weevil-free salvinia in each box. As the weevil-inhabited salvinia dries, adult and some larval weevils drop into the salvinia within the new culture box. It is important to have boxes filled with water to maximum depth so that the giant salvinia is as close as possible to the transfer screens. Screens are removed when the sal-



Fig. 3-3. A transfer or submersion screen, shown placed over the salvinia in a culture box.

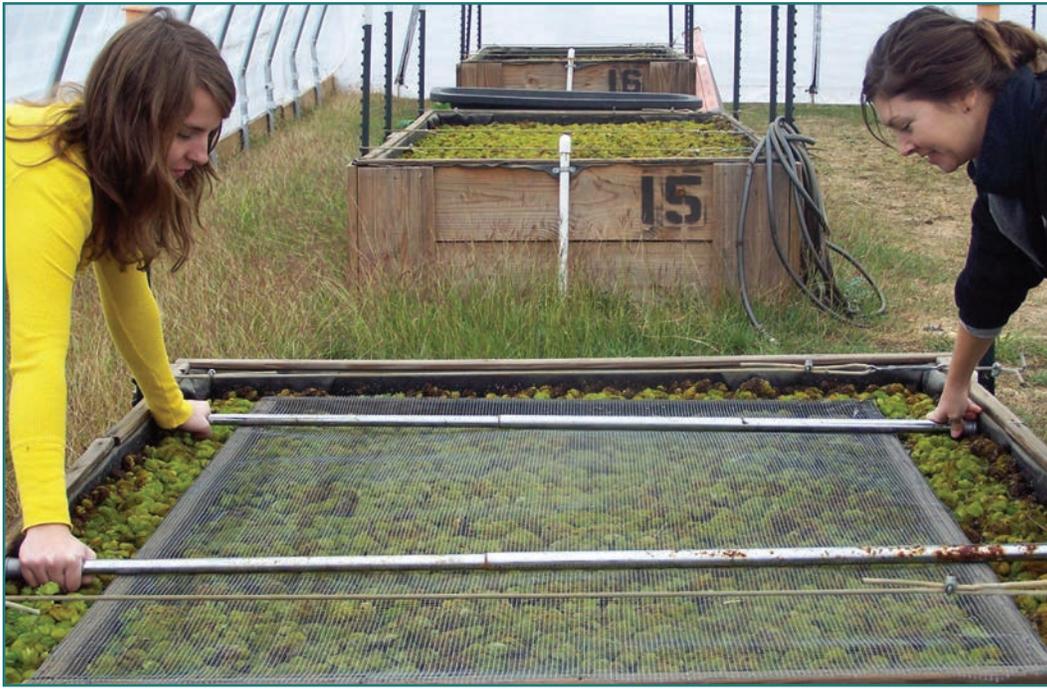


Fig. 3-4. A submersion screen on top of salvinia, prior to submersion. Tension rods (shower curtain rods) are placed on top of the screen, parallel to the screen, and are expanded to fit snugly inside the box. The tension rods are used to submerge and hold the screen and the salvinia approximately 6 inches below the water surface.

vinia has completely dried out. This method quickly and easily transfers weevils into the culture boxes and onto fresh salvinia without introducing infested salvinia plants.

Not all weevils will make it through the transfer screens and into the culture box. Recent research (Nachtrieb, in review) suggests that transfer screens may have a negative effect on weevil survival, especially larvae. Weevils either do not vacate the drying plants or do not survive for long periods after vacating plants. It is not advisable to use transfer screens as a frequent means of manipulating culture boxes, but at least adults can be transferred with a high retention rate.

Population Monitoring

Population monitoring is the most important procedure while rearing weevils in containers, as population counts determine all future culture activities such as weevil/salvinia augmentations and weevil harvests for field releases. Weevil populations can rapidly expand, damage all available salvinia, and consequently die from lack of food or substrate.

At the LAERF, facility personnel monitor culture boxes approximately every 4 weeks with the use of Berlese funnels (Fig. 3-5). Berlese funnels use light to provide heat to plant samples, effectively drying them out and causing insects to flee. Berlese funnels are the most cost effective and efficient means to extract adult salvinia weevils for population counts (Boland and Room 1983; Room and Thomas 1985; and Tipping and Center 2003). Boland and Room (1983) found that 95 percent of salvinia weevil adults were extracted by Berlese funnels within 24 hours. However, the recovery rate of larvae using Berlese funnels has not been documented.

Berlese funnels used at the LAERF consist of a galvanized sheet metal cylinder (30.5 centimeter diameter) with an inside platform made of 1/4-inch-mesh poultry netting located 27 centimeters from the top. A light fixture with a single 40–60 Watt bulb is placed on top of the funnel and a Mason jar (pint or quart capacity) is attached to the funnel below the platform (Fig. 3-5). Plant samples (approximately 300–500 grams) are collected from the culture boxes, drained on mesh screens, weighed, and placed on the wire screen platform within the



Fig. 3-5. Berlese funnel with Mason jar attached to the bottom and light fixture on top.

funnel. Then the light is turned on. Mason jars are filled with approximately 5 centimeters (enough to cover insects and allow for some evaporation) of 70 percent ethanol to kill and preserve collected insects. The samples are dried for 24–48 hours, or until plants are completely dry and brittle, and then the number of weevils (larvae and adults) are counted in each jar.

Four samples of salvinia are collected monthly from each culture box to monitor weevil populations. To obtain weevil populations estimates based on both surface area and fresh weight of salvinia, plant samples are collected from a 1/35 square meter quadrat (16.9 centimeters × 16.9 centimeters) and the fresh weight is recorded prior to placement in funnels.

Berlese Funnel Costs

- Berlese funnels = \$40 each: Lake Cities Fabrica-tion, 972-420-7900
- Lights = \$32.56 each: mustangelectric.com
- Wide-mouth Mason jars, 12 pack = \$10.44: walmart.com

Weevil Harvesting for Field Release, Cleaning Boxes, and Initiating New Cultures

Cultures are designated for field releases when weevil populations reach approximately 60 adult and larval weevils/kilogram salvinia fresh weight, a density representative of early-season levels. Releases are made by collection of weevil-inhabited giant salvinia from rearing cultures. Weevils are released with plants because releasing weevil-inhabited giant salvinia 1) provides a larger number of weevils over-all since infested plants contain all weevil life stages (eggs, larvae, pupae, adults) and 2) increases weevil survival during transport by providing their normal habitat and a food source. Infested plant material is drained of most excess water, weighed (for weevil release estimates), placed in plastic bags, and packed in ice chests. The salvinia is then transported to release sites by overnight shipping or hand carry-ing. At the release site, the infested plant material is placed directly into the water body allowing free mixing with existing salvinia plants.

A portion of the infested plant material (approx-imately 25 percent of each box) is not used for field releases and is used to restart cultures. Fresh salvinia is added to the box so that 50–75 percent of the sur-face area is covered, allowing room for plant growth. Boxes should be drained, completely cleaned of debris, and refilled with water and peat moss at least once per year. This can be done in early spring or immediately following harvests for field releases. Typically, boxes are cleaned out in the spring when the weather is more favorable. If spring cleaning of culture boxes is not conducted, salt accumulations from excess fertilizer can create conditions less favor-able for salvinia growth, such as high conductivity levels.

Salvinia/Weevil Augmentations and Thinning of Cultures

If weevil cultures are not designated for immedi-ate harvest and released once populations reach 60 adult and larval weevils/kilogram salvinia fresh weight, the weevils may need fresh salvinia as plants become highly damaged and new plant growth slows. Depending on weevil population densities

and damage to salvinia plants, 25–50 percent of the infested plant material should be removed (a process called thinning) and replenished with weevil-free salvinia. The removed weevil-inhabited salvinia can be disposed of, released at a field site, placed on a transfer screen over the same box to transfer weevils, or used to augment boxes with few weevils.

It is common for some weevil boxes to have more or less weevils than the others. Salvinia weevils can be transferred from boxes with high populations to boxes with low populations by using transfer screens, submersion, or movement of weevil-inhabited plant material using the same weevil-inoculation methods as previously discussed.

Overwintering

Salvinia weevils live naturally in tropical South American environments; therefore, they do not have a known winter diapause (hibernation) or overwintering behavior that allows them to withstand freezing temperatures. Past research has shown that larvae do not survive below 17 °C (62.6 °F) (Sands et al. 1983), and adult feeding slows substantially at 15 °C (59 °F) (Forno and Bourne 1985). During winter at the LAERF, weevils have been observed to feed minimally and not reproduce. When rearing in areas where freezing temperatures are possible, it is necessary to protect the weevils during winter to ensure survival. Overwintering procedures, as discussed below, are advantageous to the rearing program because they help eliminate delays in weevil population peaks and subsequent field releases the following spring. A general rule of thumb is that if the plants are able to survive winter intact, the weevils should also be able to survive.

During winter, 16 of the culture boxes are enclosed in 2 separate cold frames (Fig. 3-6). While not heated, during warm, sunny days temperatures can easily reach 25 °C to 30 °C (75 °F to 85 °F) inside the cold frames. It is important that temperatures are not constantly maintained at optimum summer conditions so that the weevil populations do not expand at a time when high populations are not needed for field releases. Fertilization is unnecessary as salvinia growth slows with decreases in temperature and photoperiod. Additionally, only minimal water additions are needed during this

time due to reduced evapotranspiration. Culture boxes overwintered in cold frames will require fertilization earlier than boxes that are not enclosed in cold frames, due to temperatures heating up sooner than in an open-air situation. Fertilization typically begins in March for cold frame enclosed boxes. It is important to first observe salvinia growth prior to fertilizing as this indicates water temperatures are high enough for active plant growth and the uptake of nutrients.

Cold Frame Specifications and Pricing

- Each cold frame: 30 feet × 12 feet × 80 feet, constructed of triple galvanized structural steel tubing, enclosed with 6-milliliter greenhouse film
- Premium Round Style High Tunnel Kit for 30 feet × 12 feet × 80 feet = \$6,419; www.farmtek.com

Weevil Production Estimates

During the field season of 2011, 23 culture boxes were used for weevil production and 82,889 weevils were produced. With successful overwintering, all boxes reached releasable population levels (60 adult and larval weevils/kilogram salvinia fresh weight) by July, with initial boxes ready in April. On average, 3,604 total weevils (larvae and adults) were produced per box and each box was harvested once during the growing season. Results are detailed in Table 3-1.

Cost Analysis

A cost analysis was performed to determine the total cost of supplies, labor, facilities, and equipment to maintain 30 culture boxes for an entire year. A weevil production year is considered to begin with overwintering of the weevil cultures and to conclude with the final harvest of the last box for field releases. Costs of supplies and labor were analyzed both overall and divided into the 3 seasons of winter, spring, and summer, as culture maintenance differed based on time of year. Winter procedures included preparing outdoor boxes for winter and



Fig. 3-6. Culture boxes are enclosed within cold frames for winter protection.

monitoring outdoor weevil populations during November, December, and January. Spring activities, performed during February and March, primarily involved cleaning out culture boxes and restarting plant and weevil cultures. Summer included weevil population monitoring, thinning, fertilizing, watering, and mowing from April until early August, when all weevils were harvested. Supplies for all seasons included items such as fertilizer and ethanol. Cost of facilities and equipment used in weevil rearing included capital costs such as culture box, cold frame construction, and Berlese funnels. All values for supplies, facilities, and equipment were calculated in cost/weevil produced using total adults and larvae from Table 3-1. Labor was calculated in units of man-hours/weevil produced to ease comparison to other agencies with various hourly rates, overhead fees, etc.

An estimated total of \$1,209.40 was spent on supplies during the 2010–2011 weevil-production season (Fig. 3-7). Ethanol, used during Berlese funnel extractions, accounted for the largest amount of supply costs, accounting for 57 percent of total supply costs. Twenty percent of the supply budget was spent on the purchase of a pH probe, which was included in yearly supplies, as opposed to capital costs, because probes are generally replaced each year. Fertilizer, peat moss, and miscellaneous supplies, such as light bulbs and plastic bags for sample collections, represented 23 percent of costs. On a per-weevil basis, using total weevils produced from 1 harvest from each box, supply costs were \$0.04/adult weevil, \$0.02/larvae and \$0.01/total weevils produced (larvae and adults).

The majority of costs associated with weevil rearing were accrued through man-hours, as rear-

Table 3-1. Weevil production data for 2011.

Month	Number of Boxes	Adult Weevils	Larval Weevils	Total Weevils	Average Weevil Total/Box
April	9	10,342	13,206	23,548	2,616
May	12	11,206	35,491	46,697	3,891
July	2	6,723	5,921	12,644	6,322
Totals	23	28,271	54,618	82,889	3,604

ing is a labor-intensive process. An estimated 464 hours were spent rearing salvinia weevils during the 2010–2011 weevil production season. Weevil population sampling, which included sample collection, Berlese funnel procedures, and counting weevils represented the largest portion of labor, 36 percent. Twenty-one percent of labor was devoted to general culture management, defined as conducting observations of the cultures to determine future maintenance procedures, as well as any meetings with staff or outside entities concerning the weevil cultures and possible projects or field releases. Cleaning and restarting plant and weevil cultures post-winter also consumed a significant portion of labor, 20 percent. The remaining 23 percent of labor was devoted to culture maintenance such as fertilizing and watering (7 percent), mowing grass around the culture boxes (7 percent), harvesting for field releases (5 percent), overwintering preparations (2 percent), and thinning (2 percent). On a per-weevil basis, using total weevils produced from 1 harvest from each box, labor costs were \$0.02 hour/adult, \$0.008 hour/larvae, and \$0.006 hour/total weevils produced (larvae and adults).

Labor costs (Fig. 3-8) can be reduced significantly by increasing weevil production mainly through multiple harvests throughout the growing season. In future years, an attempt will be made to harvest weevils multiple times from individual boxes during the growing season.

On a per-season basis, summer rearing procedures (Fig. 3-9) accounted for the highest percentage of

supply and labor costs at 44 percent and 46 percent, respectively. Costs for the summer is expected to be the highest since it is the peak weevil-production season, all 30 boxes are in use, and all activities increase such as fertilization and sample collection. Labor is also increased during summer as 3 activities are typically only conducted during summer: harvesting for field releases, mowing grass around the boxes, and thinning salvinia cultures. Supply costs for summer were greater than other seasons primarily due to the amount of fertilizer and ethanol used to maintain and sample 30 boxes.

Supply costs for winter and spring were similar, but winter labor costs were half those of spring. During winter, cultures are not typically watered or fertilized and only 16 culture boxes are maintained, reducing the demand for labor. In contrast, spring is a labor-intensive time, as each culture box must be drained, cleaned out, and repopulated with plants and insects.

The LAERF spent approximately \$60,000 on capital costs for facilities and equipment (Fig. 3-10). The majority of costs, 65 percent, were for the purchase and construction of the 2 cold frames. Although expensive, cold frames were necessary to sustain weevil and plant populations through the winter at this location. Twenty percent of the funds were spent on construction of the 30 rearing boxes and 15 percent were spent on Berlese funnel supplies and storage. This cost included having 34 funnels built, purchasing lights and Mason jars, and purchasing a storage building to house the 34

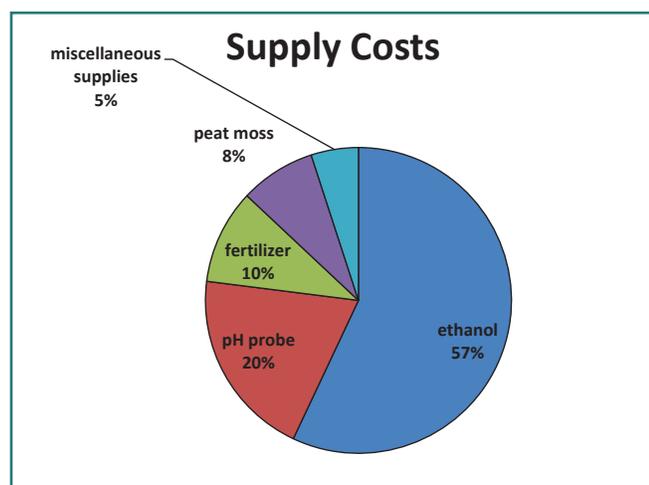


Fig. 3-7. Supply costs for rearing weevils at LAERF.

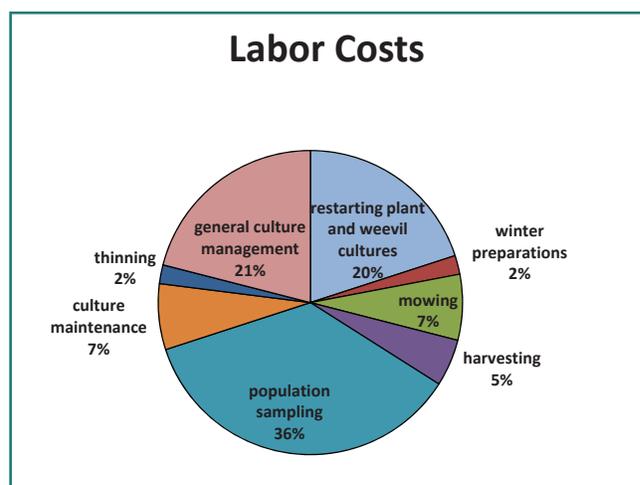


Fig. 3-8. Labor costs for rearing weevils at LAERF.

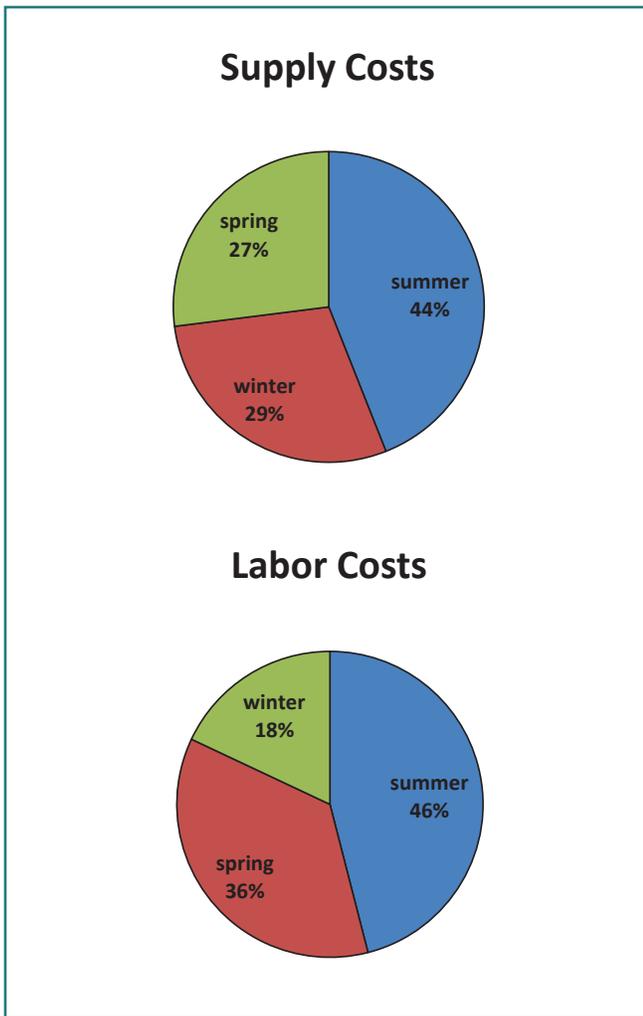


Fig. 3-9. Proportion of supply and labor costs by season at the LAERF facility.

Berlese funnels. If all the above-mentioned items (except the greenhouse film) remain in usable condition for 15 years, then \$4,000 would be contributed per year to weevil production costs. If greenhouse film, based on the length of its warranty, needs to be replaced every 4 years, \$1,500 (materials and installation labor) would be spent every 4 years, adding \$375/year to costs for a total of \$4,375/year. During 2010–2011 the costs of facilities and equipment on a per-weevil basis using total weevils produced from 1 harvest from each box would be \$0.15/adult, \$0.08/larvae, and \$0.05/total weevils produced (larvae and adults).

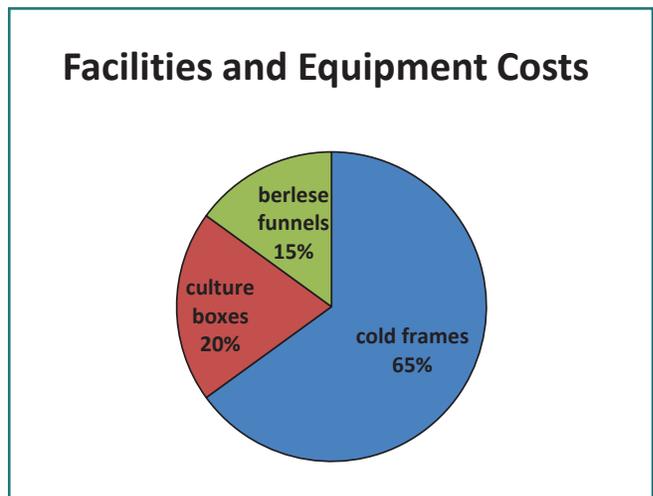
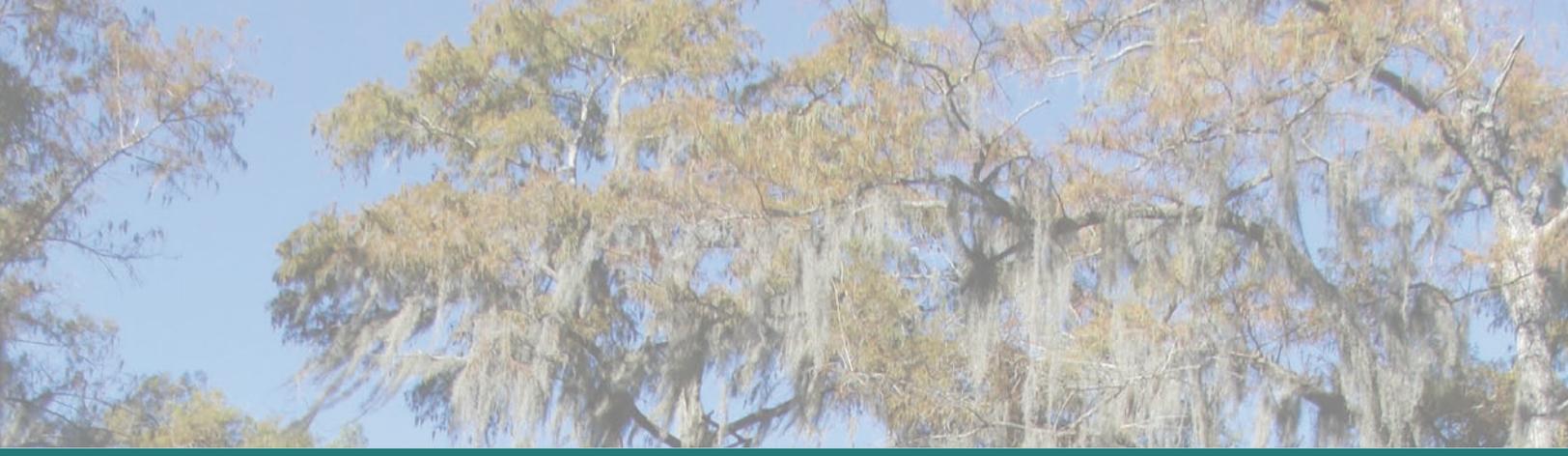


Fig. 3-10. Facilities and equipment costs for the LAERF facility.

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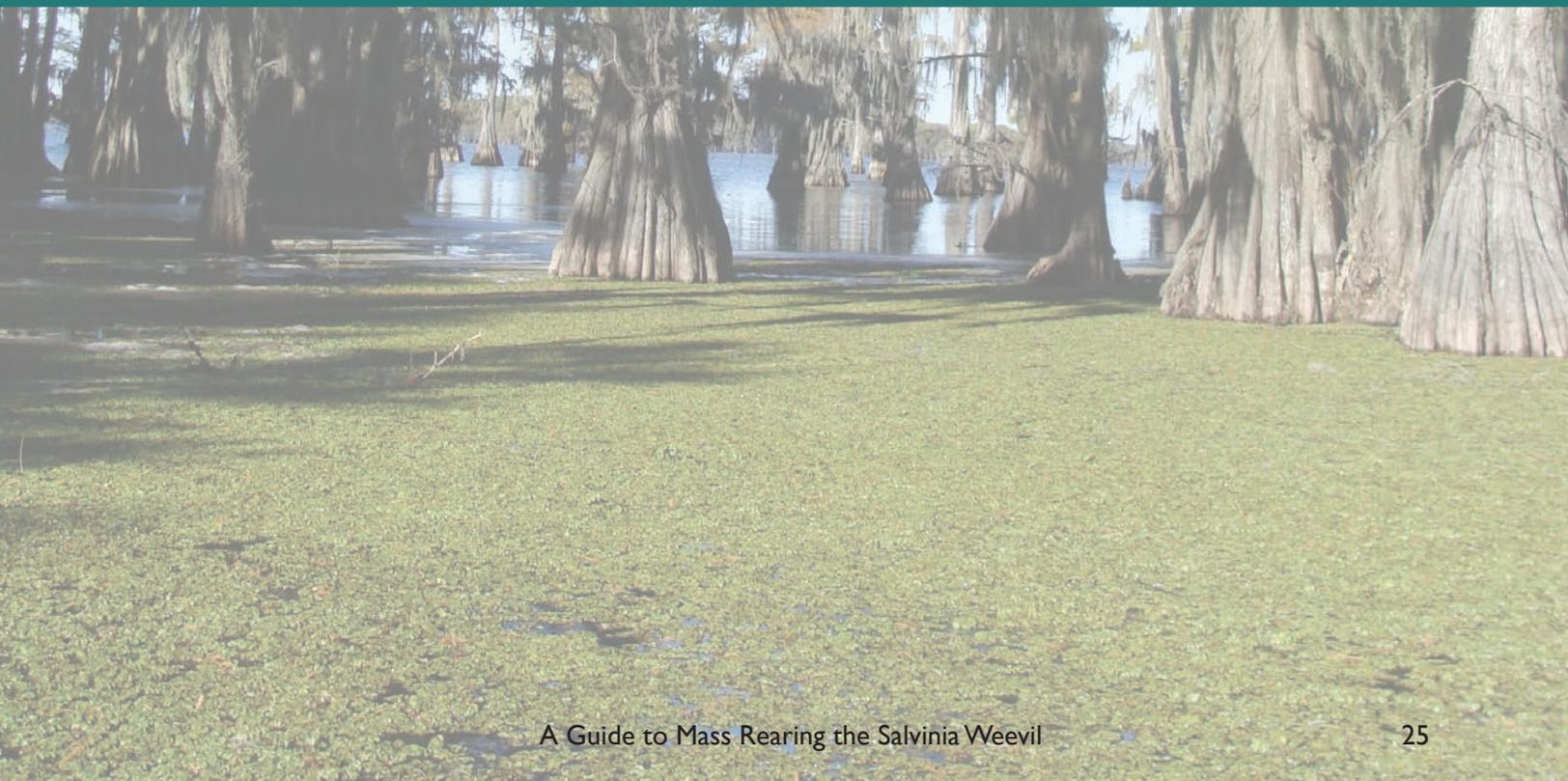
Chapter 4

Rearing the *Salvinia* Weevil in Outdoor Tanks at Caddo Lake, Texas

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Introduction

The following guidelines are based upon the mass-rearing techniques for salvinia weevils developed at the Salvinia Weevil-Rearing Facility at the Caddo Lake National Wildlife Refuge in Harrison County, Texas (32°39'59.06"N; 94°9'53.52"W). The Center for Invasive Species Eradication (CISE), of the Texas A&M AgriLife Extension Service, Texas A&M AgriLife Research, and the Texas Water Resources Institute, operates the facility, which is located in a large asphalt parking lot near the refuge headquarters. The refuge is situated on the decommissioned Longhorn Army Ammunition Plant near Caddo Lake. The giant salvinia project of CISE is funded by Congressional support through the USDA's Natural Resources Conservation Service

The goal of this salvinia weevil-rearing operation is the production and release of salvinia weevils that result in self-sustaining populations that increase to sufficient numbers to biologically control giant salvinia. This facility produced large numbers of salvinia weevils in the early spring of 2011 following multiple periods of below-freezing winter weather. The success is attributed to the greenhouses' ability to buffer against temperature dips and prevent wind chill in the weevil tanks during cold periods, therefore ensuring weevil survival through typical and abnormally cold winters. In addition, the greenhouse micro-climate allows the giant salvinia to grow and generate new buds weeks before the plants begin to grow outside of the greenhouses. This situation provides an early start for salvinia weevil egg-deposition, which results in an early spring population increase.

Materials and Design

Greenhouses

The Salvinia Weevil-Rearing Facility at Caddo Lake contains 2 identical greenhouses (Figs. 4-1 and 4-2). The greenhouses, manufactured by ClearSpan are "Premium Round Style High Tunnels" purchased from FarmTek (www.farmtek.com). Each greenhouse is 30 feet × 120 feet, a custom size to allow more space for research activities. The cost of each greenhouse, the recommended ribbon

board and shipping, was about \$11,000 in 2010. However, these greenhouses can be purchased in 4 standard sizes depending on the size of the weevil tanks being housed. The structures are 12 feet high with a rafter spacing of 4 feet and are covered with 6-mil clear greenhouse film, which is guaranteed for 4 years. This particular model has zippered end panels made from 22-mil woven plastic sheeting that can be rolled up by hand.

A team of at least 3 people is needed to construct the greenhouses following the instructions provided; however, 4 or more people are preferred as some portions of the frame are quite bulky. A 50-foot boom lift/cherry picker (rental costs \$400/day for 2 days) was used and is highly recommended to expedite the assembly process and improve the safety of the crew; however, a stepladder may suffice.

Other tools used in assembling the greenhouse frames were:

- cordless and corded drills
- 2 socket sets
- drill bit set
- tape measures
- permanent markers
- 22-ounce claw hammers
- 8-pound sledge hammers
- reciprocal saw with a metal cutting blade
- several stepladders

Additionally, 8 screw-type anchors were concreted into the ground and attached to the greenhouse frames with 3/16 inch stainless steel cable and galvanized turnbuckles rated at 1,000 pounds working load. Other supplies, including replacement screws, bolts, cable clamps, duct tape, and lumber for baseboards, can be purchased at local hardware stores. About \$1,500 was spent on these miscellaneous supplies.

Shade cloth was also installed on each greenhouse to reduce summertime heating and extend the life of the plastic greenhouse cover. A 30 percent shade cloth was preferred; however, only 40 percent shade cloth (Fig. 4-1) and higher could be fashioned into large panels. For these purposes, 2 custom fabricated shade cloth panels (32 feet × 110 feet) were purchased from FarmTek at a cost of approximately \$1,150 each including shipping. Nylon rope pur-



Fig. 4-1. *C. salviniae* greenhouse at the Caddo Lake National Wildlife Refuge with sides lowered during early spring of 2011.



Fig. 4-2. *Cyrtobagous salviniae* infested giant salvinia in tanks under the greenhouse structure.

chased separately was used to secure these panels to the greenhouses.

Weevil-Rearing Tanks

Each greenhouse covers 2 identical above-ground water tanks (each 15 feet × 48 feet with walls 20

inches high) used to grow and rear giant salvinia and salvinia weevils (Fig. 4-3). Tanks were constructed of 2-inch × 10-inch × 10-foot treated lumber. Boards were stacked on top of each other edge-to-edge and arranged in a checkerboard fashion so that the joints on the top occurred at the mid-point of the board on the bottom. The boards were fastened together



Fig. 4-3. Aerial view of the two greenhouse structures before the plastic was placed over the frames.



Fig. 4-4. Installing tank liners in the above-ground weevil rearing tanks.

by butting the ends together and affixing a 12-inch \times 18-inch piece of 5/8-inch treated plywood to the outside of the box with 1 1/2-inch ceramic deck screws. In the corners of each tank, the boards were fastened with a 5-inch \times 5-inch galvanized steel bracket secured with 1 1/4-inch ceramic deck

screws. At each of these splices, half of a 6-foot steel t-post was driven into the ground such that the top of the post was approximately 1 inch below the top of the board frame (Fig. 4-4). The wooden tank frames were then leveled by shimming them up with wooden blocks.

At this point the frames were lined with a 110-foot × 25-foot section of 30-millimeter antiskid pond liner (www.coloradolining.com/). Two liners, \$880.00 each, were cut in half to yield four 55-foot × 25-foot liners that fit each tank. A bed of sandy loam was placed at the bottom of each tank prior to liner installation. Soil depth varied due to variations in the base under the tanks but typically ranged from 2–4 inches deep. A 2-inch minimum depth was maintained above the lower extent of the tank frame to prevent the liner from extending under the frame. The primary purpose of this soil base was to provide a relatively level surface on which to place the liner that was free of items that may puncture the liner. Soil was also placed around the outside of the tanks to further stabilize the tank bases.

Once liners were installed, water was slowly added and the edges of the liner were wrapped over the top edges of the tank. Ideally, about 12 inches of liner were left over the top of the tank, which were folded over such that several layers of the liner extended down the outside wall of the tank. This portion of the liner was tucked between the tank frames and steel t-posts and secured to the outside of the tank with 2-inch × 12-inch strips of 5/8-inch treated plywood or 1 1/2-inch × 3/4-inch treated batten strips about 12 inches long attached with 1 1/2-inch ceramic deck screws. To prevent direct contact between the t-posts and the pond liner, as well as to prevent injuries to workers and visitors, an 8-inch length of 1 1/2-inch PVC pipe fitted with a pipe cap was placed over each t-post. Galvanized pipe straps were then secured over the PVC pipe to the tank frames with 1 1/2-inch ceramic deck screws.

Tanks were filled with water to a depth of about 18 inches for a total capacity of about 7,500 gallons. Construction of the tanks and installation of the sand and liners requires about 400–500 man-hours of labor. Total cost for liners, wood, and additional materials was approximately \$5,000.

One 2,500-gallon water storage tank was placed at one end inside each greenhouse and was connected to each salvinia tank via PVC piping. Gravity is used to supply water to the tanks; however, an inline pump could easily be incorporated as well. These tanks are filled with lake water and used to replace water in the salvinia tanks lost to evaporation and to help in refilling the rearing tanks after periodic

cleanouts. These tanks are made from black high-density polyethylene plastic, which prevents algae growth and retains heat absorbed from the sun. This heat sink may help maintain warmer air temperatures inside the greenhouse during the winter. Tanks were purchased from Tractor Supply for approximately \$1,200 each.

The material costs for the rearing tanks, 2 greenhouses, shade cloth panels, 2 water storage tanks, and incidental materials and supplies were about \$30,000 when purchased in 2010. Labor costs were not accounted for in this estimate.

Getting Started

Following completion of the greenhouse and tank construction, the initial procedures for large-scale production of *C. salviniae* are summarized in 4 steps:

1. Provide suitable water quality for optimal giant salvinia growth.
2. Inoculate tanks with weevil-free salvinia plants and allow the plants to rapidly grow.
3. Stock weevils in sufficient density to allow a rapid population expansion.
4. Maintain water quality and monitor weevil population growth until weevil numbers are sufficient for harvest and release.

Water Quality

Salvinia requires specific conditions of water quality and fertility to grow rapidly and provide a nutritious food source necessary for production of large numbers of salvinia weevils. Thus, the importance of maintaining appropriate water conditions for giant salvinia growth cannot be overstated. The most important water quality parameters are pH, nitrogen (typically measured as nitrate), iron (measured as total iron), conductivity, and total dissolved solids. Water quality should be frequently monitored to determine what amendments are necessary to optimize growth of salvinia (Chapter 2).

pH

At this facility, the well water has a pH of 8.5–9.0 and must be adjusted to a pH range of 5.5–7.0

needed for optimum growth of giant salvinia. Muriatic acid (HCl) is added in 1-gallon increments to each of the tanks to lower the pH below 7.0. For each 7,500-gallon tank, about 2 gallons of muriatic acid are needed to lower the pH to around 6.5. Once the acid is added, it is mixed in the tank by circulating water using a boat paddle. A pH as low as 5.0 has not resulted in observed ill effects to the giant salvinia but a pH above 7.0 negatively affects the plant's growth.

To determine how much muriatic acid to add to a large-volume tank, small increments (tablespoons/teaspoons) of muriatic acid can be added to a 5-gallon bucket filled with source water until the desired pH is attained. These results can then be scaled-up to determine how much acid is needed for the large-volume tank.

The pH must be monitored at least weekly through the growing season if the source water has a high pH. At the Caddo Lake facility, the pH will gradually rise and additional muriatic acid (typically a half gallon every 2 weeks/tank) is necessary to maintain a pH below 7.0. Also, peat moss is added to acidify the water and maintain pH below 7.0. Two large mesh laundry bags are filled with peat moss and placed on each end of each tank where the bags slowly absorb water and sink to the bottom of the tanks. Peat moss should not be poured into the tank directly because it will float on the surface and take several weeks to absorb water and sink. In addition, a layer of peat moss at the bottom of the tank will create additional labor when cleaning the tanks after weevil harvest.

Conductivity

Conductivity is a measure of the water's salt concentration, which can increase in rearing tanks because of accumulation of salts from fertilizers. Conductivity should be measured first when the tanks are filled with water and then periodically. A high conductivity level is best addressed by draining the tank and filling the tank with water having a conductivity level within the appropriate range.

Fertilization: Nitrogen and Iron

Nitrogen fertilizer must be added to stimulate salvinia growth and production of buds on which

the weevils feed. Nitrogen level must be measured weekly so that deficiencies can be addressed quickly. Miracle-Gro Water Soluble All Purpose Plant Food 24-8-16 containing 24 percent nitrogen, 8 percent phosphorous, and 16 percent potassium, iron, and other trace elements has shown the best results at the Caddo Lake facility. The nitrogen is provided as ammonium sulfate and urea phosphate. The target for nitrate nitrogen in the tanks is 10 (range 7–16) parts per million (milligrams/liter). Rapidly growing plants can quickly deplete the nitrate, and fertilizer is added any time the nitrate level dips below 7 parts per million. When the tank is covered with giant salvinia and plant growth is slow, the nitrate level is easier to maintain in the 7–10 parts per million range.

When a new tank is started with fresh water, 5 packs (for a total of 6.25 pounds) of Miracle-Gro All Purpose Fertilizer are added to each 7,500-gallon tank. The fertilizer is mixed in a 5-gallon bucket of water and poured along the perimeter of the tank. In addition, 6 cups of Green Light Iron/Acidifier are added to the water. A boat paddle is used to mix these materials into the water column. Green algae will quickly cover the surface of the tank following the addition of fertilizer. The algae growth is only a temporary issue that is quickly reduced once giant salvinia begins to cover the tank. Also, small floating plants such as duckweed and watermeal will be reduced once the salvinia begins to grow, and no treatment for these plants has been necessary.

Iron is also an important nutrient for salvinia, with optimum concentrations about 3 milligrams/liter. The iron concentration can be measured with test strips, and adding iron (Green Light Soil Iron Acidifier) can correct a deficiency.

Stocking Giant Salvinia and Weevils

Once the water quality is appropriate as previously described, salvinia can be added to the tank. Before giant salvinia can be added, appropriate permits are required to handle and transport the plant. In Texas, an Exotic Species Permit is needed to possess and transport salvinia.

Salvinia should be moved in secure tubs (with sealed lids) to prevent material from spreading to new sites. Thirty-gallon plastic tubs with zip-tied

lids work well. *Salvinia* used to inoculate new tanks should be free of weevils to allow the *salvinia* to grow rapidly. At the Caddo Lake facility, weevil tanks can be started with fresh *salvinia* from early March to the end of September. Although tanks can be stocked in fall and winter, the growth rate will be very slow during this time of the year. Because of its rapid growth rate, very little plant matter is needed to inoculate a new tank. During the summer, one 30-gallon tub of *salvinia* placed in a tank should expand to cover the tank in less than a month, assuming that the water has adequate fertilization. As the plant expands across the tank, it will transition from the uncrowded primary structure (flat leaves floating on the water) to a more crowded tertiary structure (leaves and buds begin to grow upward and stack on each other) (Fig. 4-5). Once tertiary-structure plants appear across the tank, *salvinia* weevils can be added (Fig. 4-6). Typically, infested *salvinia* plants containing about 1,000 adult weevils, determined by Berlese samples, are added to each tank and distributed evenly around the perimeter.

As the *salvinia* continues to grow and crowd the tank, some of the *salvinia* plants must be removed to maintain adequate bud growth needed for weevil feeding and reproduction. Approximately 25 percent of the giant *salvinia* from a tank can be removed and placed on the screens and allowed to dry for several days (Fig. 4-7). The adult weevils, along with some larvae, will fall back into the tank

as the plants dry on the screens. The screens are constructed using 2-inch × 4-inch lumber and 1/4-inch hardware cloth available at hardware stores.

Monitoring Weevil Abundance

The density of adult weevils needs to be estimated every 1–2 weeks to monitor population increases and determine when to harvest the weevils. Weevils must be harvested before they consume all of the *salvinia* plants as the weevil population will then dramatically decline. Weevils densities in each rearing tank are determined by drying plants in a Berlese funnel, which extracts the adults and some larvae from the plants. (See Chapter 3, “Population Monitoring” for details on constructing Berlese funnels.) Results are reported as the average number of adult weevils per kilogram of fresh *salvinia*. The average number of larvae can also be reported but it is less reliable than that for adults. To determine the density of weevils in a tank, a sample of *salvinia* plants weighing about 800–900 grams is collected from each of 8 locations/tank, and each sample is held in a sieve to allow water to thoroughly drain from the plants. A subsample of 500 grams is then taken from each of the larger samples and weighed using an electronic scale. This 500-gram sample is placed in a Berlese funnel. A Mason jar containing about 2–3 inches of water is fastened to the bottom of the Berlese funnel. Also, 5 to 6 *salvinia* leaves



Fig. 4-5. The first two weeks of starting a weevil rearing tank in which one thirty gallon tub of clean *salvinia* was added and after one week, the *salvinia* is beginning to cover the tank, along with green algae.



Fig. 4-6. The *salvinia* has reached secondary and tertiary stage by week three and has crowded out all the algae. By week four, the tank has topped out with budding tertiary plants, and weevil infested material was added (the dark material seen in the picture).

are added to each jar where the leaves float on the surface. The plants are left to dry in the Berlese funnel for at least 24 hours or longer if necessary to ensure all of the plants are completely dry. During

this time, the adult weevils and some larvae escape from the plants and heat and fall into the jar below. Once the salvinia plants are dry, the adult weevils, which accumulate on the floating leaves and are



Fig. 4-7. Screen placement over weevil tanks. Excess giant salvinia growth is placed on screens and adult salvinia weevils will fall back into the tank as the giant salvinia dries.



Fig. 4-8. Berlese funnels at the Caddo Lake Facility. Weevil infested salvinia plants are placed inside the funnel and allowed to dry for 24 hours under a 40-Watt light bulb. Adult weevils will fall into the jar for counting.

easily seen, are counted along with the larvae, which will remain in the water (Fig. 4-8). The average number of weevil adults per 500 grams is then determined for the 8 samples per rearing tank. This average is multiplied by 2 to yield the average number of adult weevils per kilogram (1000 grams) of fresh salvinia. The density of larvae per kilogram can also be estimated using the same calculation.

Weevils densities in the rearing tanks are determined at 2-week intervals from spring to fall (March–September). During December to early February, sampling with the Berlese funnels once a month is adequate because of the slowdown in weevil population expansion.

Harvesting Weevils for Release

Weevils are collected for field release by removing the infested salvinia from the tanks, weighing the salvinia, and then transporting it to the weevil release site. By moving infested salvinia, the entire population of eggs, larvae, pupae, and adult weevils is released undisturbed at field release sites.

Tanks are harvested when the weevil density reaches approximately 30–40 adult weevils/kilogram, although densities at harvest have been as high as 160 adults/kilogram. If weevil population surpasses 100 weevils/kilogram, salvinia in rearing

tanks may begin to turn brown and fragment from the intensive weevil feeding. In such a case, harvest the tank quickly before the weevil population begins to decline from lack of food. As weevil density and plant damage increase, plants will become brown and bud damage will become common (Fig. 4-9). These visual evidence along with Berlese sample counts should aid in deciding when to harvest a tank. Rapid increases in weevil density can be expected early in spring when the overwintered adults begin laying eggs as the tank water warms. Rapid increases in weevil density can also occur within 2 months of restarting a tank later in the summer so long as the weather is warm and the giant salvinia is actively growing. Weevil density seems to stabilize and decrease slightly during late fall and winter months.

All or some of the salvinia may be removed from a tank depending on the demand for weevils to release in the field and the need to clean the tank of accumulated sediment. To harvest the salvinia and weevils, 1 or 2 individuals enter the tank and manually remove the salvinia using mesh fish-landing nets and place the plants into 30-gallon tote-tubs with 1/4-inch holes drilled along the bottom to allow excess water to drain. The lids are fastened using cable-ties and the tubs loaded onto a truck for delivery to the release site. At the release site, the tubs are weighed



Fig. 4-9. Heavy weevil infestation showing the characteristic darkening of the plants from weevil damage.

to determine the weight of salvinia. Weighing the tubs at this time allows water to drain from the tubs during the drive to the release site, providing a more accurate estimate of weevil density per kilogram of salvinia plants. Weevil density estimates from recent Berlese samples taken from the same tank are then used to calculate an estimated number of adult weevils per tub. A small boat is usually used to shuttle tubs of salvinia from the truck out to release sites. Take care to transport the tubs to the location as quickly as possible to minimize the amount of time spent under direct sunlight and heat, which can be fatal to the weevils.

Two individuals can remove all the material from one 15-foot × 48-foot tank in about 30 minutes. Typically, all of the salvinia from 1 tank will fill 10–14 thirty-gallon tote-tubs and weigh 400–650 pounds. If all of the salvinia is removed, the tank is then drained, cleaned, and refilled to allow the production of another crop of salvinia weevils. It is important, however, to maintain a reserve population of salvinia weevils in another tank or water-filled tubs to re-inoculate the tank once the tank is refilled and restocked with giant salvinia.

About 70–80 percent of the infested salvinia is usually removed and the balance is left to serve as a reservoir of weevils to repopulate the tank. Weevil-free salvinia is then added to the tank and will regrow, filling in the space and providing an abundance of new buds on which the weevils will rapidly increase. This approach can lead to a faster increase in weevils, since a large resident population remains, relative to starting a new tank with a release of about 1,000 adults as described earlier.

When this approach is used, a balance between the increase in weevil numbers and salvinia growth is sometimes difficult to achieve. As weevil feeding begins to damage the salvinia, its growth is slowed, minimizing the plant's ability to rapidly cover the tank's surface. In this case, additional weevil-free salvinia should be added to the tank to feed the growing weevil population. If this cannot be done, a last resort is to harvest the tank before it has reached its full weevil production. Adding salvinia plants is tedious, and if field-collected plants are used, other plants and insect pests can be introduced. For this reason, it is easier to remove all the infested material from a tank and start the tank over from scratch.

Partial removal will also delay the draining and cleaning of the tank causing additional accumulation of decaying organic matter.

Cleaning and Refilling the Tanks

The process of rearing salvinia weevils in tanks creates a large accumulation of decaying organic matter at the bottom of the tanks. This buildup can reach 3–4 inches deep during a growing season and can foul the water and altering the cycling of nutrients. The accumulation of this material also reduces the effectiveness of water quality amendments. In addition, dissolved salts accumulate in the water because of consistent fertilization of the tank. Salts accumulation can increase the water conductivity, which is detrimental to the growth of giant salvinia.

Consequently, the tanks should be drained, the sediment removed, and new water added once in the fall and again following the first harvest of salvinia in the spring. A 1/3-horsepower submersible sump pump and garden hose (available at most hardware stores) is a simple way to drain a tank in less than a day. Once drained, the layer of decaying organic matter at the bottom of the tank can be scooped up, using a small mesh landing net, and then disposed of on a compost pile. Once clean, which should take about half a day for a single tank, the tank can be refilled and the entire weevil-rearing process can start over. If planning to clean the tanks in advance, water levels can be allowed to drop to minimize the amount of water that must be removed.

Annual, Monthly, and Weekly Maintenance

Every 2 weeks during the growing season, giant salvinia in the tanks should be checked for damage by small caterpillars of the *Samea* moth (*Samea multiplicalis*) (Fig. 4-10). The adult stage of this insect is a small moth (Fig. 4-11), which can fly into rearing tanks at night and lay eggs on the salvinia plants. Small larvae hatch from the eggs and eat the salvinia leaves and buds. Plants with winding feeding scars and dark masses of caterpillar excreta on the leaves indicate that *Samea* larvae are present. These caterpillars can be easily seen on and between leaves. Left

uncontrolled, *Samea* moth larvae can quickly inflict massive damage to the giant salvinia plants. Applications of insecticides that contain *Bacillus thuringiensis* will control these caterpillars and are not toxic to salvinia weevil adults or larvae. These insecticides are applied as a spray to the salvinia plants and frequent applications (every 2–3 days) are often necessary. An example of an insecticide containing *B. thuringiensis* is Thuricide (Southern Agricultural Insecticides, Inc., Hendersonville, North Carolina).

During hot, dry weather, fire ant colonies can appear around tanks, and in addition to being a hazard to workers, fire ants can invade the tanks and attack salvinia weevils. Colonies should be treated immediately using ant baits commonly available at feed, hardware, or landscaping stores. Be extremely careful not to get ant bait or other insecticides in the rearing tanks, as they are toxic to the salvinia weevils.

Test the water quality in each tank weekly to determine pH, nitrate, iron, and conductivity, and to determine if amendments or other action is needed to keep conditions favorable for salvinia growth and weevil reproduction. In addition, observe giant salvinia in the tanks for feeding by *Samea* caterpillars and discoloration. Yellowing of the leaves may indicate an iron deficiency, while brown leaves and dying plants may indicate high weevil damage and the need to harvest weevils from the tank. Determine weevil densities at a minimum of once a

month using samples processed through the Berlese funnels.

In early spring, when nighttime low temperatures no longer dip near freezing, it is essential to fully open both end doors and roll up the sides of the greenhouse to allow good air circulation and reduce interior heating, which can be detrimental to salvinia growth. At the Caddo Lake facility, in late fall, around the time of the first freeze, the sides are lowered back to the ground and the doors at each end closed with zippers. Once closed and sealed, the greenhouse maintains a warmer air and water temperature than the outside. During the coldest winter months, water temperatures in the tanks ranged from 11 °C to 18.8 °C (52 °F to 66 °F) and stabilized around 12 °C (54 °F). The air temperature under the greenhouses during the winter months ranged from a high of 26.6 °C (80 °F) during sunny afternoons to a low of -3 °C (26 °F) during an evening in a winter storm. A digital “High-Low” thermometer, available at any home improvement store, can be used to monitor daytime high and low air temperatures under the greenhouses and in the tanks.

Weevil Production and Labor Requirements

The capital investment of the rearing facility is relatively high, but the operation costs are low and



Fig. 4-10. Larval stage of the *Samea* moth. Photo by Peter Room, The Queensland University, Bugwood.org.



Fig. 4-11. Adult stage of the *Samea* moth. Bugwood image #1929045. Photo by Richard Chan, Commonwealth Scientific and Industrial Research Organization, Bugwood.org.

consist primarily of labor and fertilizer. Once constructed and operational, a single, part-time worker can maintain the Caddo Lake facility. Additional labor is necessary to harvest, transport, and release the weevil-infested salvinia.

In 2011, the Salvinia Weevil-Rearing Facility at Caddo Lake produced and released an estimated 130,000 adult weevils within 2,700 pounds of infested salvinia. Weevil larvae estimates were not included in this total; however, it is believed that the ratio of larvae to adults is approximately 3 to 1. Thus, it is possible that the facility harvested and released an estimated 390,000 salvinia weevil larvae, bringing the total weevils released in 2011 to 520,000 larvae and adults. Each tank was harvested twice in 2011 and generated about 32,500 adult weevils (130,000/4 tanks) or about 16,000 adults/harvest/tank.

List of Supplies

Greenhouse and Tank Structural Equipment

- Greenhouse structures: www.farmtek.com/
- Tank liners: www.coloradolining.com/
- Miscellaneous lumber, t-posts, bolts and screws: Lowe's and Tractor Supply

Water Quality Monitoring Equipment

- HANNA H19813-6, pH, conductivity, ppm, temperature: www.hannainst.com/usa/
- HANNA Nitrate photometer, measures nitrate levels: www.hannainst.com/usa/
- AquaCheck Nitrate and Iron Test Strips: www.aquachek.com/
- AcuRite High Low Outdoor Thermometer: Lowe's

Water Amendment Products

- Miracle-Gro All Purpose Plant Food (24-8-16): hardware and garden stores

- Green Light Soil Iron Acidifier: available at Lowe's and Home Depot
- Muriatic acid, 1-gallon containers: available at most hardware stores
- Peat moss bales: available at any garden or landscaping store

Weevil Harvest and Tank Cleaning Equipment

- Small mesh landing net: available at Academy Sporting Goods and Wal-Mart
- 30-gallon tote tubs and zip-ties: available at Wal-Mart, Lowe's, and Home Depot
- 1/3 horsepower sump pump and hose: available at Lowe's, Tractor Supply, and Home Depot

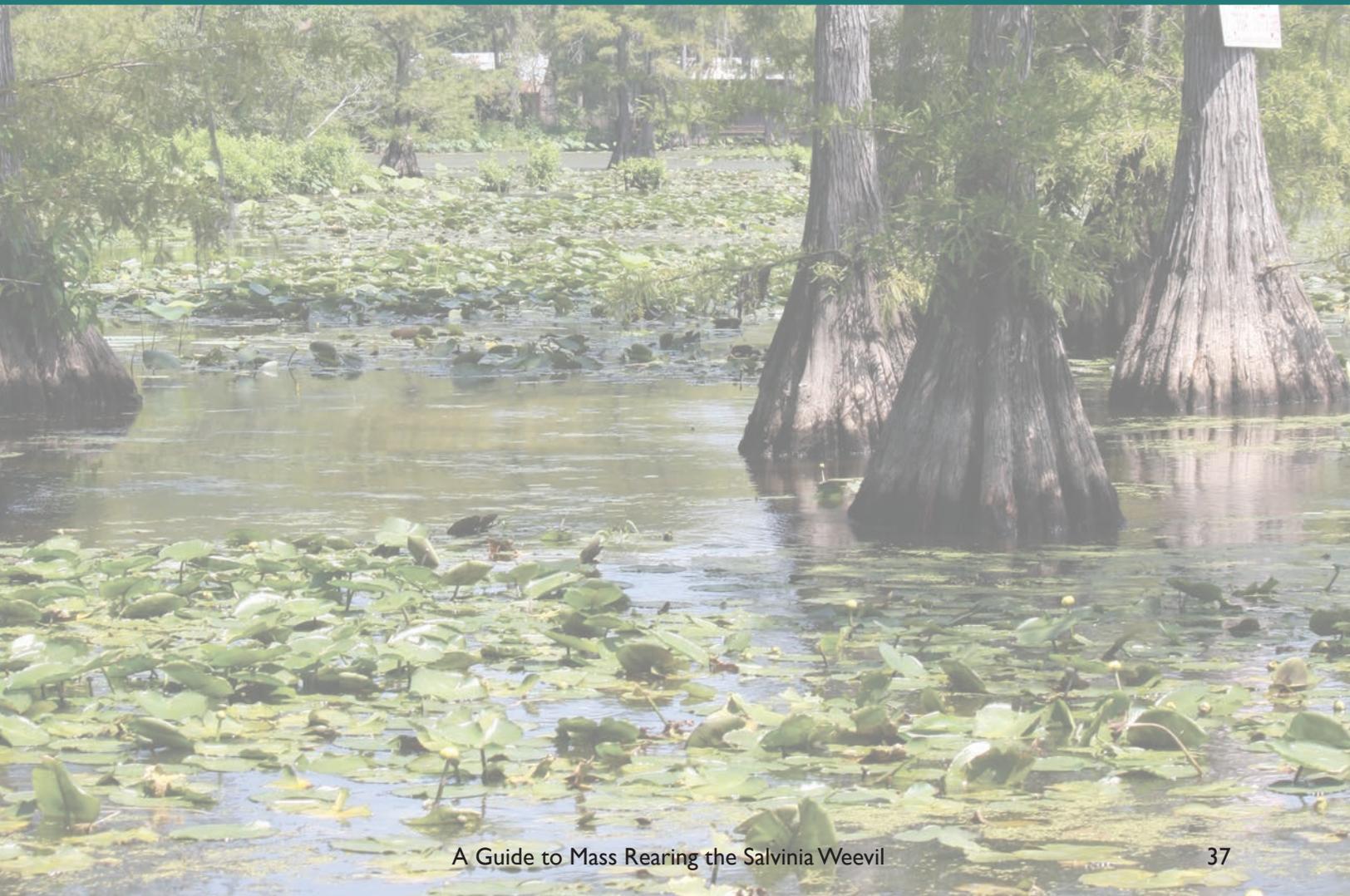


Chapter 5

Rearing the *Salvinia* Weevil in the Greenhouse

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Introduction

Louisiana State University (LSU) researchers have reared salvinia weevils for research since 2007 in a greenhouse on the LSU campus in Baton Rouge, Louisiana. The colony did not survive during the destruction and disruption of work schedules during Hurricane Gustav but was restarted with salvinia weevils collected from a release site in Ghens, Louisiana, near Golden Ranch Plantation, between October 2008 and March 2009. Additional weevils were collected from a release site in Larose, Louisiana. All of these field populations originated from Brazilian weevils originally released by Tipping and Center (2003) at Toledo Bend Reservoir.

Salvinia weevils reared at the LSU facility are used for research on weevil biology and for field studies. As a research colony, production costs are greater as the facility does not benefit from the economy of scale achieved by larger facilities. However, there is more control over a research colony, so inquiry into specific areas, such as nutrition, reproduction, and cold hardiness, can be conducted.

Facility Design and Construction

Physical Layout

Salvinia weevils are mass-reared in 2 greenhouse sections at the Campus Range Greenhouses, located on the LSU campus in Baton Rouge, Louisiana (30°24'40.870"N, 91°10'20.658"W). Both sections are 8.53 meters in both length and width (Fig. 5-1), have clear plastic sheathing that is shrouded with 60–70 percent shade cloth, and are equipped with standard heaters, fans, and evaporative coolers. One section is used to cultivate high quality, weevil-free salvinia, which is distributed to the mass-rearing tanks in the adjacent section. The shade cloth is installed at the vernal equinox (March 20) and removed at the autumnal equinox (September 20).

Rearing Tanks

Salvinia is grown and weevils are reared in 35 Rubbermaid stock tanks (150-gallon capacity) (Fig. 5-2). The tanks are oblong, 5 feet × 2.5 feet, and



Fig. 5-1. Interior of the Greenhouse.



Fig. 5-2. Salvinia in growth tanks.

are made of structural foam (high density polyethylene), which makes them light, portable, and durable. The drain is plugged with a Nerf ball. The sides and rims of the tanks are insulated with bubble wrap-foil insulation to prevent solar gain from driving water temperatures too high. Insulation, 2 feet wide, fits the sides of the tanks neatly and is secured with foil duct tape. Without insulation, rims sometimes reach temperatures of 45 °C (113 °F) or more, which can raise water temperature over 36 °C (96.8 °F) and reduce weevil fertility. Float valves and replacement drains are available for these tanks.

Water Quality, Filters

The concentration of dissolved metals, especially sodium, in the water source should be determined because these metals can accumulate to harmful levels in the host plant salvinia. State Extension Services and private laboratories can conduct these and other water quality tests (see Chapter 2).

At the LSU facility, tap water was initially used to fill the tanks, but high levels of dissolved miner-

als, especially sodium, proved to be detrimental to the salvinia. Tanks are now filled with water with a pH of 7.0 supplied by a reverse osmosis (RO) filter (Fig. 5-3). These systems can be purchased or leased. Ion-exchange filters will not remove sodium, so they are of no value for cultivating salvinia where high sodium is a problem. The system produces 200 gallons of water/day, which is sufficient to maintain this facility. The RO system has a particulate filter



Fig. 5-3. RO System.

(pre-filter) and a carbon filter that supplies water to the RO filter. Both of these filters are available from the Culligan dealer and should be changed every 6 months. If they become clogged, the system will produce less than 200 gallons of water/day, and the useful life of the RO filter may be shortened.

Replacing the pre-filter is simple; remove the housing by turning it counterclockwise and put a new filter in place of the spent one. Replacing the carbon filter is a little more difficult. Remove the RO filter before replacing the carbon filter. Secure the housing of the carbon and RO filters with 4–6 screws. After removing the carbon and RO filters, replace the carbon filter and then run the water, which will drain on the floor, to rinse out the carbon fines (dust left in the filter from the manufacturing process). Initially, the water will run black. After the water runs clear, let the water run a little longer to be sure the fines are gone. Only then, should the RO filter be reconnected. If not removed, carbon fines will shorten the useful life of the RO filter, the most expensive part of the system. The RO filter is simply removed and replaced when water production falls off. RO filters typically last about 2–2.5 years, depending on the water quality.

Reservoir and Pump

At the LSU facility, the water produced by the RO filter accumulates in a 400-gallon polyethylene reservoir. Water is supplied to the rearing and growth tanks by a 1/15-hp Little Giant Pump. The pump is located within the reservoir and connected to a short, 6-foot hose with a brass adapter (3/4-inch standard thread to hose thread). The short hose is connected to a 50-foot hose by a ball valve, which prevents water loss if the hose is left on the floor. All connections are sealed with Teflon tape. To operate the pump, the ball valve is opened, and then the pump is plugged in, starting the flow of water. To stop operation, the pump is first unplugged, and then the ball valve is turned off. Take care that the pump is not run dry or run against a closed valve, which will cause it to overheat and shorten its useful life. These pumps can be used submerged or in-line, so they can be adapted to a number of other components. Ordinarily, a float valve, installed by the

dealer, shuts off the water when the reservoir is full, so that the RO filter does not run continuously.

The LSU reservoir was acquired long after dealer installation and does not have a float valve at this time. The water supply is shut off manually when the reservoir is full. The reservoir is covered with 2 sheets of Plexiglas and a tarpaulin to keep out debris and prevent algal growth. The Plexiglas was used simply because it was available. Any material could be used to cover the reservoir, as long as it does not contaminate the water.

Algae Filter

In the greenhouse in southern Louisiana, the tanks must be monitored continuously for algal growth. Reduced UV light and phosphate-enriched water favor algae, and algae productivity far exceeds that of salvinia. If algae covers the roots of the salvinia or covers more than 1/6 of the surface of a tank, it may be necessary to remove algae by filtering. A portable algae filter was constructed from a can filter and a UV filter mounted on a portable workbench and piped together with 5/8-inch flexible tubing (Fig. 5-4). To operate, a 1/15-hp Little Giant pump is placed in an algae-infested tank and water is pumped through the tubing to the intake of the can filter and from there to the UV filter. The UV filter's outlet dumps water back into the tank. If there is a heavy sediment load, it helps to remove excess sediment from the tank with a dip net prior to filtering. The pump can also be placed in a 5-gallon bucket with numerous 1/4-inch holes drilled in it to keep debris out of the pump. This can be surrounded with hardware cloth to remove more debris. The can filter and UV filter are no longer available, but Tetra markets a combined filter, which is cheaper and easier to operate. For a system with large tanks, a sand filter would be a more practical means of removing algae and debris. Sand filters are frequently used for swimming pools.

Lighting

Sixteen Commercial gro-lights are used to extend day length to 14:10 (L:D) and keep the weevil population reproductively active during the winter



Fig. 5-4. Algae filter.

months. The lights are turned off at the vernal equinox and turned on at the autumnal equinox. (These can be seen in Fig. 5-1).

Temperature, Fans, Misting System

Extreme heat will reduce the population growth rate of the salvinia weevil. In addition to the evaporative cooler and fans installed in the greenhouse, 30-inch pedestal fans are used to increase airflow through the greenhouse and reduce the temperature early in the summer. A misting system was installed that reduced the temperature by 5 °C to 6 °C and increased weevil population growth during the hottest part of the year.

Berlese Funnels

Weevils are collected from infested salvinia using Berlese funnels fabricated by LSU Facility Services. The funnels are 2 feet in diameter, with a hardware cloth tray. Reptile lights with 125 Watt brooder lamps clamped on the top edge of the funnel provide heat to desiccate the plant matter (Fig. 5-5).

Insects are collected in a 4.5-inch × 9-inch Whirl-Pak bag secured to the stem of the funnel. The Berlese funnels are insulated with a 2-foot-wide bubble wrap/foil jacket, which is secured with cold weather foil duct tape to maintain a consistent temperature within the funnel. See Chapter 3, “Population Monitoring” for details on constructing and using Berlese funnels.

Procedures to Maintain Salvinia and Weevils

The quality of the salvinia plants is a very important factor for establishing a successful salvinia weevil colony. Temperature and nutrition influence the growth rate of salvinia, whose buds provide a crucial nitrogen source for egg-laying females (Room and Thomas 1985).

Nitrogen Fertilization

Nitrogen is a limiting nutritional factor, and salvinia with tissue nitrogen content of 1.2 parts per million or higher (dry weight) is necessary to



Fig. 5-5. Berlese Funnels.

maintain a growing population of weevils (Room et al. 1984). Higher nitrogen levels usually result in higher fecundity. At the LSU facility, tanks are fertilized twice a week (Tuesday and Friday) with Miracle-Gro Water Soluble Lawn Food, which has a NPK content of 36-0-6. To achieve a concentration of 2 parts per million (milligrams/liter), 1.2 grams of fertilizer are added to 135 gallons of water in each tank. The nitrogen content of the water is measured with Quick Dip 5-n-1 Test Strips prior to fertilization, and treatment is delayed if the nitrate concentration is over 3 parts per million. These strips are easy to use, but not precise, and are mostly used to monitor the level of nitrate and nitrite in the water in a tank. Readings are based on color changes. The API (Mars) Fishcare 34 Freshwater Master Test Kit gives more accurate readings. If a tank has a very high nitrogen concentration, it can be partially drained and diluted with fresh water or emptied and refilled. Salvinia requires some phosphorus, so 1.5 grams of rock phosphate (0-3-0) is added per tank every other week.

This fertilization regime produces enough weevils for research, but does not result in exponential growth of the population, which would quickly outgrow the limited space and cause the population to die off. Adjusting the nitrogen regime can regulate population growth. During the early summer, when demand for weevils is high, nitrogen is increased to increase weevil production. Nitrogen levels are re-

duced during the winter when weevil reproduction and feeding declines due to cooler temperatures.

Alkalinity, pH, and Trace Minerals

Salvinia grows best between a pH of 5.5 and 7.0. The nitrogen in Miracle-Gro Water Soluble Lawn Food is supplied mostly as urea, which breaks down into CO_2 and NH_4 , both of which are mild acids and help to maintain the pH below 7.0. A few ounces of peat moss is added directly to the tanks if the pH needs to be brought down. If pH is 5.5 or less, the water in a tank is pumped or siphoned out and replaced with RO water. Using RO water avoids any concerns about alkalinity. However, RO water also lacks trace elements that may be needed to support the growth of healthy salvinia. To provide trace elements, 4 ounces of Osmocote Plus Multi-Purpose Plant Food is added to each tank every 2 months.

Controlling Weeds, Insect Pests, and Algae

Sedge grasses, pigweed, alligatorweed, patches of azolla fern, and other unwanted plants are removed from the tanks by hand. To reduce the growth of algae and duckweed, weevil-free salvinia is added twice a week until there is no open water in the rearing tank. The algae filter described earlier and low phosphate fertilizer also help suppress algal growth. To remove algae from salvinia brought in from the field, salvinia plants are placed in tanks half full of water, and 15 milliliters (1 teaspoon) of Algaefix is added. After 24 hours, the growth tanks are filled to their customary level (4 inches below the rim), and allowed to sit for a week before the salvinia is used to resupply tanks containing weevils.

Caterpillars of the Samea moth and aphids are pest insects that feed on salvinia leaves. To control caterpillars of the salvinia moth, *Samea multiplicalis*, the tanks are sprayed twice weekly with Thuricide. Thuricide contains *Bacillus thuringiensis* Kurstaki (*B.t.* Kurstaki), a strain that is only toxic to caterpillars. The sprayer is rinsed out immediately after use to prevent clogging.

Aphids are small, soft-bodied insects that suck plant sap from salvinia plants. When abundant, aphids can turn salvinia leaves yellow and become

desiccated. Aphids are attacked by naturally occurring parasitic insects (tiny wasps) that can reduce aphid numbers below damaging levels. If these parasites do not appear, biocontrol agents such as *Aphidoletes aphidimyza* can be purchased from a commercial supplier and released in the greenhouse to initiate a population.

Aphids can be temporarily controlled by submerging the aphid-infested salvinia in a tank and capturing the aphids with a fine-mesh aquarium net when they come to the surface. Fire ants occasionally invade the greenhouse, especially in the spring, and are controlled with Terro Liquid Ant Baits. This bait contains boric acid, a slow acting poison that takes 2–3 weeks to work. Information on other products for fire ant control can be found at fireant.tamu.edu/

Managing Growth of Salvinia and Weevils

If not needed in rearing tanks, overcrowded salvinia from the growth tank is removed to stimulate bud development and maintain host plant quality. Salvinia from rearing tanks with the highest weevil populations is distributed to tanks with lower populations. Weevil populations are highest in the spring and fall. Weevils often emerge en masse in the early spring before there is an adequate host plant population at field sites for a release. Summer heat in the greenhouse (over 31 °C or 88 °F) reduces adult survival and egg hatch (Sands et al. 1986), but the misting system appears to be a mitigating factor. Winter populations grow slowly as low water temperatures reduce the developmental rate of the immature weevils. Fans are turned off in cold weather to avoid an influx of frigid air. A small reserve (laboratory) colony is maintained that could be used to resupply the greenhouse with weevils if the greenhouse colony is lost from a power failure or other disaster.

Estimating Weevil Density in Rearing Tanks

The greenhouse population is sampled by randomly removing 5–6 double handfuls of infested salvinia from a tank and placing the salvinia in Zip-

loc bags with an average weight \approx 0.66 kilograms. Six tanks are sampled at a time so that each sample can be immediately processed in a Berlese funnel (Fig. 5-5). The bags are drained in a wire basket lined with window screen and then weighed on an Adventurer scale. The sample of salvinia is then placed in the Berlese funnel and allowed to dry for approximately 20 hours. The weevils fall down the funnel and collect on a salvinia ramet in a Whirl-Pak bag secured to the stem of the funnel. In the lab, the ramet is searched under a Leica MZ75 dissecting microscope, and the weevils are counted and classified as either black (mature) or brown (newly emerged). The number of weevils/kilogram of giant salvinia is calculated.

Harvesting Weevils from Rearing Tanks

Tanks are harvested when weevil populations approach 40 adults/kilograms of salvinia because the weevils may be significantly stressed by declining host plant quality due to feeding damage. Adult weevils can be separated from the salvinia by fully submerging the plant, which forces the adult weevils to eventually float to the top for oxygen. A small clump of salvinia plants is placed on the water and acts as a floating island where adult weevils will collect. After 24 hours, the small clump of plants is removed and the number of weevils is determined with a microscope. Alternately, the weevils can be extracted from the small clump of plants using the Berlese funnel.

Salvinia is submerged in tanks by setting an expanded metal screen on top of the salvinia and pushing all of the of salvinia plants 6–10 inches underwater. The screen must be cut to fit the tank snugly, or the mass will not stay submerged. The edges of the screen must bind on the sides of the tank. It may be necessary to remove insulation from the rim of the tank, and workers should be careful that they do not pinch their fingers. A screen-removal tool was fabricated, which enables one person to easily remove a screen. It is essentially a lever with chains that can be hooked onto the screen and a handle that rests on the edge of the tank. When pressed down, the tool pops the screen loose (Fig. 5-6).



Fig. 5-6. Screen Removal Tool (SRT).

It is also possible to remove duckweed from a tank using screening. After the mat is submerged, the water is agitated by hand, and most of the duckweed will escape the screen and float to the surface. It can then be removed with a fine mesh aquarium net.

Weevil-infested salvinia can easily be harvested from the tanks with a dip net and placed in coolers or totes for transport. If totes are used, high temperatures and exposure to the sun may cause significant weevil mortality. Place wet paper towels on top of the plants to keep them moist and leave a 1-inch gap around the inside of the cooler or tote for ventilation. If a continuous greenhouse colony is to be maintained, 50–75 percent of the salvinia in each tank can be harvested and replaced with fresh weevil-free plants. Releasing weevil-infested salvinia ensures the presence of all life stages, which augments the chances of the population becoming established.

Alternately, salvinia plants can be submerged as described above, the adult weevils retrieved for field release, and then the screen removed. If the salvinia is submerged for only 8 hours, fewer larvae and eggs will die than if plants are submerged for 1 or more days. This method removes adults but allows imma-

ture weevils to continue to develop in the tank. If all the salvinia and weevils are removed, the tank can be drained and cleaned with a wet/dry Shop-Vac to remove organic matter and tannins before the tank is refilled.

Production Estimate

Because the LSU facility is a research colony, the weevil population is harvested before it has achieved its reproductive potential. However, the following is an estimate of the minimum production possible from this facility. During June and July 2010, the weevil density averaged 45.5 adults/kilograms in the 15 rearing tanks, each of which contained approximately 2.5 kilograms of salvinia (a total of 37.5 kilograms). Thus, these 15 tanks would yield 1,700 adult weevils if harvested in late July. Assuming 4 generations could be completed per growing season, the annual production from these 15 tanks would be 6,800 adult weevils.

List of Suppliers

- Reverse osmosis filter (model LCRO, 200 gpd), Culligan International Company, Rosemont, Illinois: find local dealer on website
- Greenhouse equipment: heaters, pedestal fans (Air King 9135, Utilitech, Inc., Reading, Pennsylvania), shade cloth (Aluminet, Polysack U.S.A., Inc., San Diego, California): Ludy's Greenhouse Manufacturing Corporation, New Madison, Ohio
- 150-gallon Rubbermaid stock tanks, Rubbermaid Commercial Products, Rubbermaid Corporation, Winchester, Virginia: available online, may be cheaper from local hardware store or farm supply if bought in bulk
- Durapride stock tank: Hawkeye Steel Products, Inc., Houghton, Iowa
- Shop-Vac (model # 63-12-27, Shop-Vac Corporation, Williamsport, Pennsylvania), foil duct tape (Cold Weather 324A, Nashua Tape Products, Toronto, Canada), bubble wrap-foil insulation (Reflectix, Inc., Markleville, Indiana): Lowe's or Home Depot
- Gro-light fixtures (fixture-LF1000 SM-DR-HPS 120 Volt): Bell Lighting Technologies, Saanich-ton, British Columbia, Canada
- Gro-light bulbs (mercury vapor bulbs-model # 1000 Watt m47/9, Phillips Lighting USA, Somerset, New Jersey): Grainger, Inc.
- Little Giant 1/15-hp pump (model # 3E-12N): Grainger, Inc. or Little Giant Pump Company, Oklahoma City, Oklahoma
- Misting system (model MC570): Mist and Cool LLC., Simi Valley, California
- Fertilizer, Miracle-Gro Liquid Lawn Food, 36-0-6 (Scotts Miracle-Gro, Marysville, Ohio): Lowe's
- Rock Phosphate fertilizer 0-3-0 (New England Hydroponics, Southampton, Massachusetts): Planet Natural sells 50 pounds for \$22.50, PlanetNatural.com
- Dip nets: Academy Sports (may only be available in the spring)
- Hoses, fittings, fire ant baits (Terro Liquid Ant Baits, Senoret Chemical Co., St. Louis, Missouri): Lowe's or Home Depot
- Whirl-Pak bags: Nasco International, Ft. Atkinson, Wisconsin
- Thuricide (*B.t.* Kurstaki pesticide, Southern Agricultural Insecticides, Inc., Hendersonville, North Carolina): Ace Hardware
- Adventurer scale: Ohaus Corporation, Pine Brook, New Jersey
- *Aphidoletes aphidimyza* biocontrol agent: Rincon-Vitova Insectaries, Ventura, California
- Pump sprayer (Gilmore Model # 2000 gpw): Robert Bosch Toll Corp., Peoria, Illinois
- API (Mars) Fishcare 34 Freshwater Master Test Kit: Mars Fishcare, Chalfont, Pennsylvania
- Quick Dip 5-n-1 Test Strips: Spectrum Jungle Lab Corporation, Cibolo, Texas
- Portable workbench: Stanley Black and Decker, New Britain, Connecticut
- Reptile lights: Fluker Farms, Port Allen, Louisiana

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Chapter 6

Rearing the *Salvinia* Weevil in Ponds

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Salvinia weevils can and have been reared in open-air ponds for more than 20 years. Rearing salvinia weevils in ponds may be the most cost-efficient method of mass-producing weevils for release, providing the climate is suitable to open-air weevil reproduction and propagation. Before starting a pond-type weevil nursery, there are a few key things to consider.

Temperature

Probably the greatest limiting factor in the successful rearing of salvinia weevils is minimum winter temperatures. The minimum temperature tolerated by the giant salvinia weevil is debatable and is currently being investigated. Since 2007, researchers at the Louisiana State University (LSU) AgCenter have successfully overwintered weevils in open-air ponds in a number of parishes (counties) in coastal Louisiana. Temperature monitors have recorded overnight temperatures as low as -6.1°C (21°F) with fair to good weevil survival. In north Louisiana, recorded temperatures below -6.6°C (20°F) for multiple hours have caused almost complete weevil mortality. Until, or if, a cold-tolerant strain of salvinia weevil is identified, open-air rearing ponds are recommended to be located in areas not usually subject to temperatures below -3.8°C (25°F).

Type of Pond

Weevils can be reared in either an excavated pond or a leveed pond. Most existing ponds suitable in size for weevil nurseries (see “Size of Pond”) are dug or excavated ponds. Using an existing excavated pond eliminates the cost of constructing a new pond. However, there are some drawbacks to excavated ponds. For example, most excavated ponds are much deeper than required for rearing weevils and require some type of boat for maintenance and harvest. Overflow following heavy rains can result in loss of giant salvinia and may contaminate surrounding environs. Another drawback is that surface water runoff can enter an excavated pond, bringing unwanted pesticides, weeds, debris, etc. Leveed ponds with an adequate stand pipe type overflow system have the advantage of more efficient depth control while eliminating problems with inflow

and outflow. Ponds with levees can be expensive to construct and maintain. The need for an external water supply, usually requiring some type of pump or well, can add to maintenance costs.

Size of Pond

Before starting a weevil nursery, careful consideration must be given to the desired size of the pond. Repeated measurements have shown that a 1 square foot sample of tertiary giant salvinia (with water expressed) from the nurseries weighs approximately 2 pounds. Since the final harvested product will be tertiary giant salvinia containing weevils, each acre harvested represents more than 43 tons of material. Because nursery ponds must be managed as a single unit (fertility, water level control, pest management, etc.), production levels in excess of what can realistically be harvested is wasteful. One-fourth- to 1/2-acre ponds work well. In some instances, floating booms have been used to divide an existing pond into a more manageable size. While this method has its application, it adds to the level of management required. The booms must be sufficiently sturdy to withstand the pressure of the giant salvinia, wind, wave action, and fluctuations in water level. And as stated above, even when dividing a pond for weevil production, the entire pond must be treated as a unit in regards to water levels, fertility, etc.

Water Source

Prior to pond selection or construction, careful consideration must be given to the availability and quality of water used to fill or supplement the pond during low rainfall periods. Surface or well water can be used, but the water source will need to be tested prior to initiating the nursery. The AgCenter has encountered problems with excess salinity in south Louisiana in both surface and well water. Salinity levels lower than 1 part per thousand are desirable, and levels greater than 2 parts per thousand are not acceptable. Alkalinity of less than 150 milligrams/liter is desirable and a neutral to slightly acidic pH is preferable. Surface water is usually so heavily buffered naturally that large changes in pH are difficult to obtain in large ponds. The range of pH of most surface water in coastal Louisiana (6.8

to 7.5) has been shown to be acceptable. The available quantity of water must be considered prior to nursery initiation. One acre-inch of water equals 27,000 gallons. Both surface re-lift pumps and well flow rates are calculated in gallons/minute. For example, a 50 gallons/minute pump will need to run 9 hours to replace 1 inch of water in a 1-acre pond. The highest evaporation and transpiration rates in the nursery ponds occur in mid-to-late summer when rainfall is often limited and water replacement demands are highest. There have been a number of nurseries lost to drought and insufficient water supply.

Water Depth

Giant salvinia can be reared in as little as 6 inches of water. To account for water loss from evaporation and transpiration, a minimum depth requirement of 18 inches is normally set. The maximum depth preferred, especially in a leveed pond, is 36 inches, which is a comfortable wading depth. The deeper the water level, the higher the input costs for fertilization and pumping.

Fertility

Salvinia weevils prefer to feed and reproduce on nitrogen-rich salvinia plants. To accomplish this, additional nitrogen fertilizer must be added to the nursery ponds on a regular basis. Regardless of the source, some nitrogen will be lost to the atmosphere through denitrification, some will be bound to the soil, and some will be continuously taken up by the salvinia plants (and any other vegetation in the pond). It is recommended that the nitrogen (N) level be maintained at 1–4 milligrams N/liter whenever weevils are present in the pond. Favorable results have been obtained using ammonium sulfate on an as-needed basis in most ponds. Conduct a water analysis monthly until confident of the interval and rate for the addition of supplemental nitrogen. Under normal weather conditions in south Louisiana, the addition of ammonium sulfate is stopped when temperatures decline in the fall. Weevil reproductive rates decline at this time, and excess nitrogen is wasted. Adding ammonium sulfate is resumed in the late winter or early spring when water temperatures reach approximately 21 °C (70 °F). If mineral-

free well water is used as the primary water source, the addition of a complete fertilizer with micro-nutrients is recommended. The type and amount needed can be determined by laboratory analysis of the water.

Pest Control

Open-air nursery ponds are subject to invasion from a number of unwanted plants and insects. Broadleaf aquatic plants can compete with giant salvinia for space, water, and nutrients. Most can be readily controlled with applications of the herbicide 2,4-D applied at the rate of 1 gallon/surface acre. Exceptions include water lettuce, duckweed, and some submerged aquatic weeds. Control of grasses and sedges is more problematic since there are no herbicides that specifically control grass and sedges and are labeled for use in water. Grasses and sedges can be removed by hand. AgCenter personnel have repeatedly encountered two problem insects: the moth *Samea multiplicalis* and the fire ant *Solenopsis invicta*. The larvae or caterpillar of the moth *Samea multiplicalis* feeds on giant salvinia to the point it can interfere with weevil production. Applications of insecticides that contain *Bacillus thuringiensis* will control the *Samea* larvae without affecting the weevils. These insecticides are applied as a spray to the salvinia plants and frequent applications are often necessary to control *Samea* caterpillars.

The red imported fire ant, *Solenopsis invicta*, feeds voraciously on adult and larval stages of the weevil. The ant can easily cross onto the mat of salvinia and has been observed feeding on weevils as far as 25 feet from the edge of the pond. The red imported fire ant can be controlled with topical insecticides or insecticidal baits placed outside the perimeter of the pond. Multiple applications during the growing season will be required. Be careful not to allow insecticides that are harmful to the weevils to enter the pond either through drift or surface runoff.

Harvest

The harvest and distribution of pond-reared salvinia weevils is accomplished by removing the entire salvinia plant containing adult, larval, pupal, and egg stages of the salvinia weevil and transplanting



Fig. 6-1. Moving salvinia plants to conveyor belt.

the plant to the desired location. It is best to harvest when the maximum number of adult weevils is present. This minimizes the effort per unit of weevils transplanted. The number of adult weevils present at a given time is most efficiently determined using a Berlese funnel (Chapter 3). Adult weevil numbers will vary across the surface of any nursery pond requiring a number of samples to determine an average. In the center's early work, an adult weevil density approaching 30 adults/pound (66 adults/kilogram), termed the "tipping" point, was determined to rapidly consume more giant salvinia than the giant salvinia population could replace. This tipping point can result in a rapid loss of giant salvinia from the pond because of weevil feeding. The rapid loss of giant salvinia also results in a rapid net loss of weevils as they deplete their food source. If the pond is small enough or the harvest can be rapid



Fig. 6-2. Loading salvinia into totes.

enough, then the harvest time should be scheduled when the weevils are approaching 30 adults/pound (tipping point). In the center's experience, harvests have never gone as rapidly as desired. The harvest and distribution of tons of giant salvinia is a labor-intensive operation with current equipment and technology. (Note that harvested giant salvinia with weevils should be transplanted within 24–36 hours of harvest to ensure maximum survival rate). To prevent loss of weevils from declining salvinia populations, a harvesting period of approximately 6 weeks is planned. Harvest should begin when the adult weevil populations reach approximately 15 adults/pound (33 adults/kilogram). Weevil-infested salvinia is transported in a container holding approximately 40 pounds of salvinia with holes drilled in the bottom of the container to allow excess water to drain. Since most of the weevil-infested salvinia



Fig. 6-3. Loading salvinia into totes.



Fig. 6-4. Transporting totes to release site.

is transported by boat, a 40-pound container was determined to be near optimum for transport and handling in a boat. At the 15-adults/pound harvest threshold, each container carries approximately 500–600 adult weevils, which is sufficient to start a weevil population at a release site. To increase harvest efficiency and reduce harvest labor inputs, a modified forage elevator with the intake end in the pond and the discharge end over whatever transport container is used. The elevator is a 30-foot electrically driven forage elevator outfitted with a belt containing 3/4-inch cleats every 12 inches, and a 1-inch hole between cleats to assist in draining excess water from the salvinia as it moves from the pond to the container. In shallow water the intake end of the elevator is loaded by hand, and in deeper water a push boat is used to crowd the salvinia onto the elevator intake (Figs. 6-1 and 6-2). Using this system makes it possible to harvest giant salvinia in excess of 3,000 pounds/hour (Figs. 6-3 and 6-4). If the Berlese sampling indicated an average of 20 adult weevils/pound of giant salvinia, then the salvinia harvested from a 1/4-acre pond would weigh about 10 tons (20,000 pounds) and contain about 400,000 adult weevils.

Pond Rotation

After first stocking a new nursery pond with weevil-free giant salvinia, it takes 2–4 months to achieve 100 percent coverage with at least 25 percent tertiary-form giant salvinia in the pond. Salvinia weevils should not be stocked into a new nursery until at least 25 percent of the salvinia is in the tertiary form. Weevils do not readily reproduce on primary or secondary form plants. Ponds are inoculated with weevils by distributing weevil-infested salvinia uniformly toward the middle of the pond (avoiding predation from fire ants on the perimeter). Optimum initial stocking rates of weevils into a new nursery have not been determined. Currently, ponds are initially stocked at a rate of about 10,000 adults/acre of pond, as determined by Berlese sampling the weevil-infested salvinia. Experience indicates that it usually takes 2 growing seasons to reach populations levels ready for harvest. Under very good conditions this has occurred in 1.5 growing seasons.

A typical timeline is:

1. stock weevil-free giant salvinia into new nursery (spring year 1),
2. stock salvinia weevils into new nursery (summer/early fall year 1),
3. manage nursery (year 2); occasionally some harvests can be made in the fall of year 2, but depending on the transplant site, this may not be advisable if overwintering at the transplant site is questionable,
4. sample nursery for adult weevil numbers beginning year 3 (March/April), and
5. begin harvest year 3 when weevil numbers approach 15 adults/pound.

To insure a constant source of weevils, it is best to initiate a new nursery pond every year. Once harvested, a nursery pond can be treated with a non-residual herbicide to rid the pond of remaining giant salvinia and weevils and then reused.

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