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# Mass-Rearing *Cyrtobagous salviniae* Calder and Sands for the Management of *Salvinia molesta* Mitchell

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**PURPOSE:** This technical note summarizes the development and application of mass-rearing techniques using ponds at the U.S. Army Engineer Research and Development Center (ERDC) Lewisville Aquatic Ecosystem Research Facility (LAERF), Lewisville, TX for the biological control agent *Cyrtobagous salviniae* Calder and Sands for use on *Salvinia molesta* Mitchell. Information is provided on rearing methods, releases, and associated costs.

**BACKGROUND:** *Salvinia molesta* (giant salvinia), family Salviniaceae, is an exotic free-floating aquatic fern native to the tropics and subtropics of South America (Harley and Mitchell 1981). During the last 50 years, giant salvinia has been both accidentally and purposely introduced in many areas of the world, becoming a major problem in Africa, India, South East Asia, Australasia (Room et al. 1990), and the U.S. (Jacono 1999). This species forms dense mats covering the water surface preventing light penetration and altering water quality. The presence of large infestations of giant salvinia negatively impacts drainage and flood mitigation (Julien et al. 1984).

*Cyrtobagous salviniae* (Coleoptera: Curculionidae) has been shown to regulate populations of giant salvinia at a variety of locations (Room 1990). For example, in Australia salvinia control was achieved relatively rapidly after introduction of the weevil; a weevil population of a few thousand grew and destroyed 30,000 tons of salvinia in one year (Room 1990). The weevil was first purposely introduced in the United States at sites in Louisiana and Texas in 2001 with subsequent impact observed at sites in Texas (Tipping 2004).[3] Although both larval and adult stages are deleterious to the plant, larvae have been shown to provide the greatest impact by tunneling within the stolons (Julien et al. 1987).

Use of mass-rearing techniques for *C. salviniae* is relatively rare in the United States. The United States Department of Agriculture Animal and Plant Health Inspection Service – Plant Protection and Quarantine Center for Plant Health Science Technology (USDA – APHIS – PPQ – CPHST) – Pest Detection, Diagnostics, and Management Laboratory (PDDML) in Mission, TX, mass produced over two million salvinia weevils for field release beginning in 2001 (Wood 2005). However, this program was discontinued in September 2006.[4] In addition, the Louisiana State University

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Agriculture Center began a *C. salviniae* mass-rearing facility in an abandoned farm aquaculture facility in May 2007.[1] This facility is located in Gheens, Louisiana and harvesting began in September 2008 for field release at sites around Louisiana.

**Mass-Rearing.** The ability to mass-produce large numbers of high quality insect biocontrol agents can be a tremendous asset when implementing a biocontrol program. Releasing high numbers of agents, in most cases, allows for more rapid population development, a larger gene pool to minimize genetic drift, and allows for variability in the population to survive changing environmental conditions.

Many challenges are encountered in a rearing program — foremost includes production of large numbers of high-quality insect agents at reasonable costs. In most cases, laboratory and/or greenhouse-based rearing is highly labor-intensive and therefore expensive, and often severe space limitations can curtail production of large numbers. In addition, there is a tendency when using such rearing methods to produce insects with genetic characteristics that may make them less suited for survival in field conditions (McKibben et al. 1988, Grodowitz et al. 1992). More field-based mass-rearing methods are needed.

*Cyrtobagous salviniae* was first introduced to the LAERF in 2003 in an attempt to develop field-based rearing procedures to maximize insect numbers, reduce costs, and maintain insect quality. While insect quality is difficult to assess, it can be determined by observing establishment success, population rate increase subsequent to establishment, and associated impact to salvinia, both at field-release sites and in rearing ponds.

The major objective of this report is to describe methods used to successfully produce and release the biocontrol agent *Cyrtobagous salviniae*. The rearing process is discussed including pond manipulations, population estimations, and insect release. In addition, a cost analysis is performed, and the estimated cost per weevil released is provided, using man-hours, equipment costs, and shipping expenses as criteria. Success is evaluated by discussing numbers of weevils produced and released, and establishment at release sites.

## MATERIALS AND METHODS

**Pond Manipulation.** Various pond manipulations were accomplished in order to maximize salvinia growth in ponds and provide a food source for *Cyrtobagous salviniae* throughout the growing season. These manipulations included pH regulation, nitrogen amendments, as well as minimal changes to water level.

One factor limiting growth of giant salvinia is pH (Owens et al. 2005). It has been shown that natural pond chemistry at the LAERF produces a pH of 8.5-10 (Smart et al. 1995). Studies have found optimal giant salvinia growth at pH of less than 7.5 (Owens et al. 2005, Cary and Weerts 1984). In order to provide the best possible growing conditions, pH was closely monitored during the summer growing seasons of 2004 and 2005. Ten bales of hay were added to each pond, and 30 gallons of muriatic acid (HCl) were applied to each pond to obtain a pH of less than 7. This was done once as ponds were initially filled, and before salvinia introduction. Measurements were taken weekly to

[1] Personal communication. 2008. Dearl Sanders, LSU Agriculture Center, Baton Rouge, LA.

ensure that pH remained below 7.0. Although pH was closely monitored, only nutrient additions were tested experimentally during summer 2004.

In this case, four salvinia rearing ponds were supplemented with a specific quantity of ammonium sulfate in the following amounts: 0 lb, 10 lb, 40 lb, and 100 lb every two weeks, and number of weevils were enumerated using Berlese funnel extraction procedures at the end of October 2004. Three samples (0.10 $m^2$) of salvinia were taken from both green, obviously healthy salvinia, as well as brown, less healthy salvinia in each pond. The salvinia in the pond with no nitrogen additions exhibited changes in growth and morphology. In this case, the plants had unusually long submersed fronds that were, in some cases, in contact with the sediment. Since such contact may introduce variation in plant nitrogen content, the pond with no addition of ammonium sulfate was eliminated from consideration.

Based on observations from the first year nutrient addition experiment, second-year (2005) fertilization continued with two ponds receiving 100 lb every two weeks and two ponds receiving 0 lb. This regime was changed when two ponds became highly damaged by weevil feeding and giant salvinia declined to minimal levels (Figure 1). All fertilization efforts for the remainder of the growing season were focused on the two ponds with full salvinia coverage, at 100 lb every two weeks.


Figure 1. *Cyrtobagous salviniae* rearing pond at the LAERF. Photograph (a) in July 2004, (b) one year later, in July 2005 after decimation by *C. salviniae*.

Winter months at the LAERF provide challenges to rearing operations. Successive days of freezing temperatures tend to eliminate salvinia from ponds; therefore, alternative winter strategies were employed. Two small cinderblock cells were used to rear weevils year round. One cell was used to overwinter healthy, weevil-free salvinia during the winter months; the other contained weevil-infested salvinia. This supplemental facility provided salvinia to reinoculate rearing ponds in the event low temperatures should destroy pond populations over the course of the winter. These precautions would likely be unnecessary in a more southern locale. Smaller cells provide an area which can be maintained above freezing using stock tank de-icers (Allied Precision Industries, Elburn, Illinois) during winter months. In addition, cells may also be used as a source for release in the field.

**Mass Rearing/ Release.** *Cyrtobagous salviniae* is reared at the LAERF in four 0.25-ha (Smart et al. 1995) earthen ponds. Ponds were prepared by introducing a small quantity of giant salvinia and allowing it to grow until 100-percent surface coverage was obtained. With 100-percent surface coverage in September 2003, ponds were inoculated with 150 adult weevils obtained from APHIS/USDA, Mission, TX. The following year (i.e., June 2004), weevils of all life stages were again received from APHIS/USDA (within infested plant material), and approximately 20,000 were released into each of the four ponds. Chain-link fencing was constructed around all four ponds in order to prevent salvinia spread by turtle, nutria, etc.

During the summer and fall of 2005, five random 0.01-$m^2$ samples of salvinia from each rearing pond were taken monthly. Fresh weights were recorded for each plant sample, plants were placed in Berlese funnels, and adult weevils were extracted and enumerated. Weevils per fresh weight was then calculated to estimate numbers released in infested plant material, which was packaged and transported in insulated ice-coolers for introduction. At all times, only adults were counted and these numbers were used to estimate numbers shipped for release on a per unit weight basis.

In July and August 2005, weevils were released at five sites in Texas and Mississippi (Table 1). Of these, three (Lake Sheldon, Toledo Bend Reservoir, and Lake Conroe) were introduced with weevils that were hand-collected by personnel of the Texas Parks and Wildlife Department. In the other two sites, weevils were collected in infested plant material released from insulated ice-coolers, as mentioned previously.

**Table 1. *Cyrtobagous salviniae* release sites and number released.**

| Date Released | Release Site | Approx. # Adult *C. salviniae* Released |
|---|---|---|
| 7/28/2005 | Lake Sheldon, TX | 31,300 |
| 7/28/2005 | Toledo Bend Reservoir, TX | 37,400 |
| 7/28/2005 | Lake Conroe, TX | 4,550 |
| 8/3/2005 | Toledo Bend Reservoir, TX | 32,450 |
| 8/3/2005 | Lake Conroe, TX | 20,000 |
| 8/3/2005 | Lake Sheldon, TX | 13,050 |
| 8/16/2005 | Biloxi, MS | 3,650 |
| 8/22/2005 | Lake Texana, MS | 3,926 |

**Costs.** Total rearing costs were determined using the criteria previously used with *Hydrellia* spp. (Harms et al. 2009) and include both rearing and field release costs. These include pond maintenance, insect enumeration, fertilization, and insect collection for shipment. Costs associated with population monitoring included time spent collecting samples from rearing ponds, weighing, and enumerating adults collected via Berlese funnel extraction for release estimates. Costs for pond maintenance include mowing the vegetation along the banks of the ponds and maintaining consistent water levels. Shipping supplies were also included in the total cost (ice coolers, packing tape, and plastic trash-can liners). Shipping costs were estimated using approximate weight of full shipping containers, the “Priority Overnight” FedEx™ shipping option, and actual origin and destination ZIP codes.

## RESULTS/DISCUSSION

**Pond Manipulation.** Fertilization was successful in producing vigorously growing salvinia throughout the growing season, and high levels of fertilization produced significantly more weevils than low levels of fertilization (Figures 2 and 3). Overall numbers of weevils produced at the 100-lb fertilization level was 1.75-fold greater than at the 40-lb level, and 2.09-fold higher than at the 10-lb level. Whole-pond weevil populations were estimated to be 870,000 for October with the 100-lb fertilization, including both green and brown salvinia.

When concentrating on only brown, apparently damaged salvinia, the 100-lb fertilization produced 1.98-fold greater weevil numbers than 40 lb, and 2.65-fold greater numbers than 40-lb fertilization (Figure 2). This equates to approximately 1,608,630 weevils total in the 100-lb treatment pond. This is evidenced in Figure 3, in which salvinia that received more fertilization produced more weevils, which led to more damaged plants. As many as 620 weevils/m$^2$ were found on brown, damaged plants in the pond treated with 100-lb fertilizer.

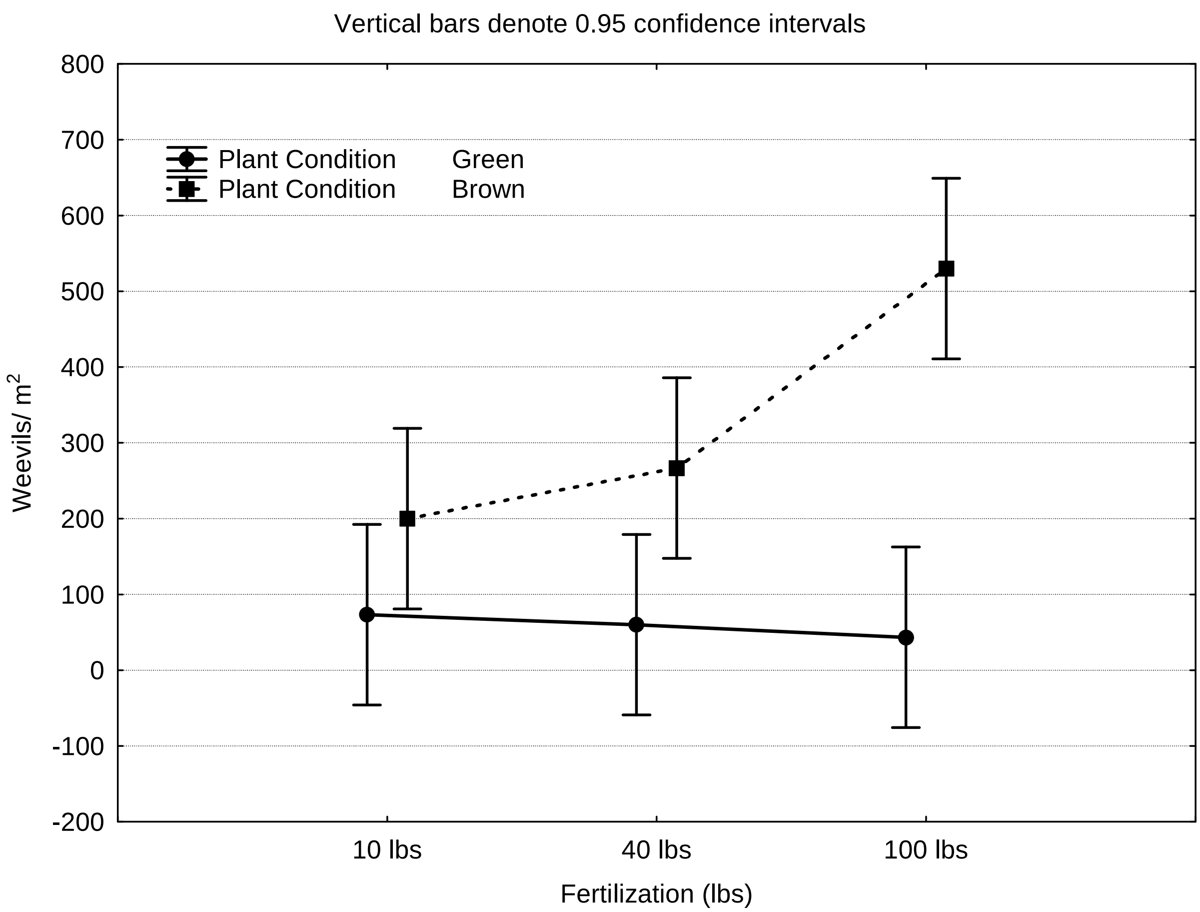


Figure 2. The amount of *Cyrtobagous salviniae* weevils/m$^2$ was significantly different between fertilization levels and different plant health conditions (Two-way ANOVA, F= 5.97, p=0.015, DF=2). Vertical bars denote 0.95 confidence intervals.

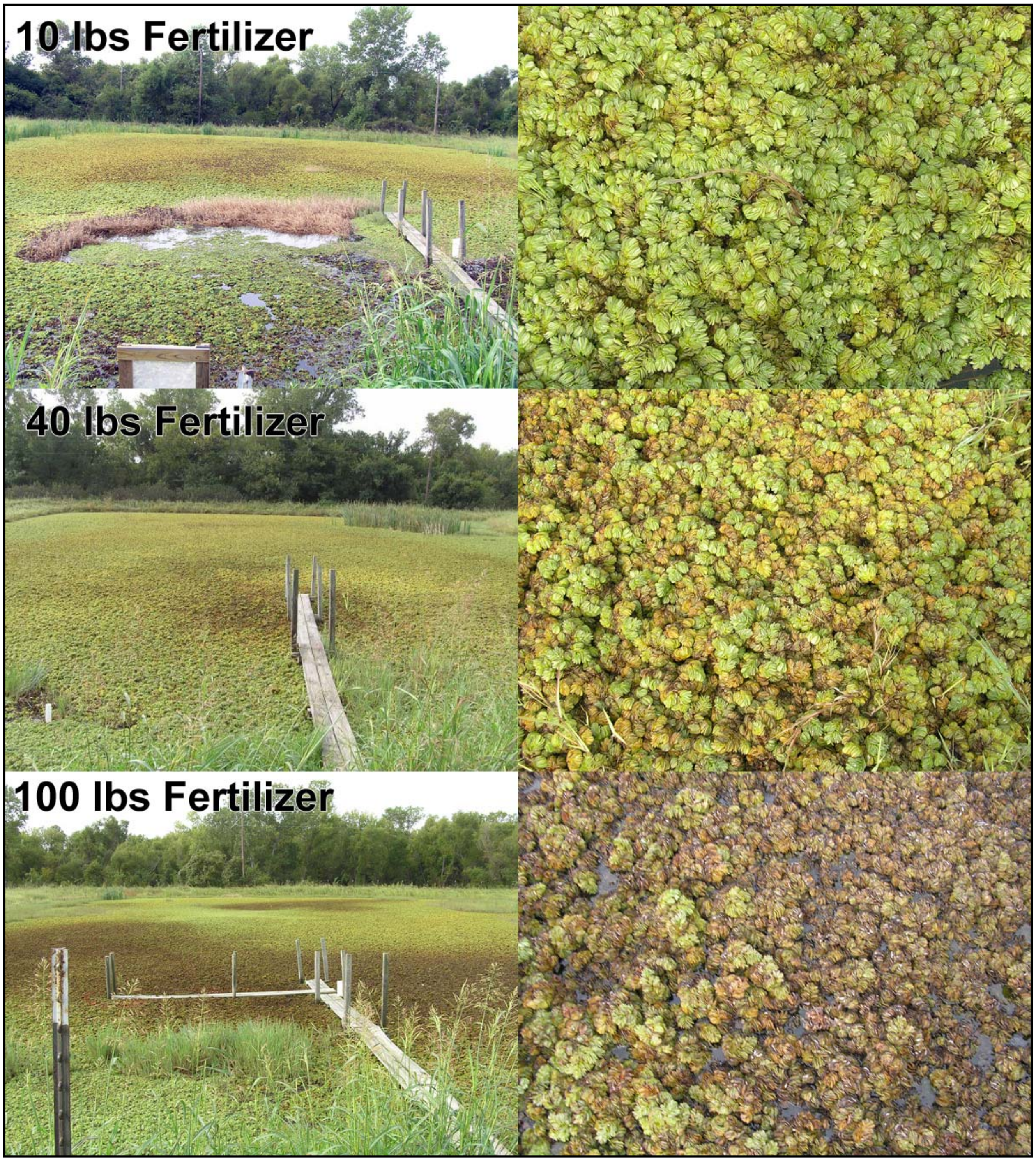


Figure 3. Various fertilization levels and corresponding damage to salvinia plants. Notice higher fertilization corresponds to higher weevil numbers (Figure 2), which in turn produce more damaged plants.

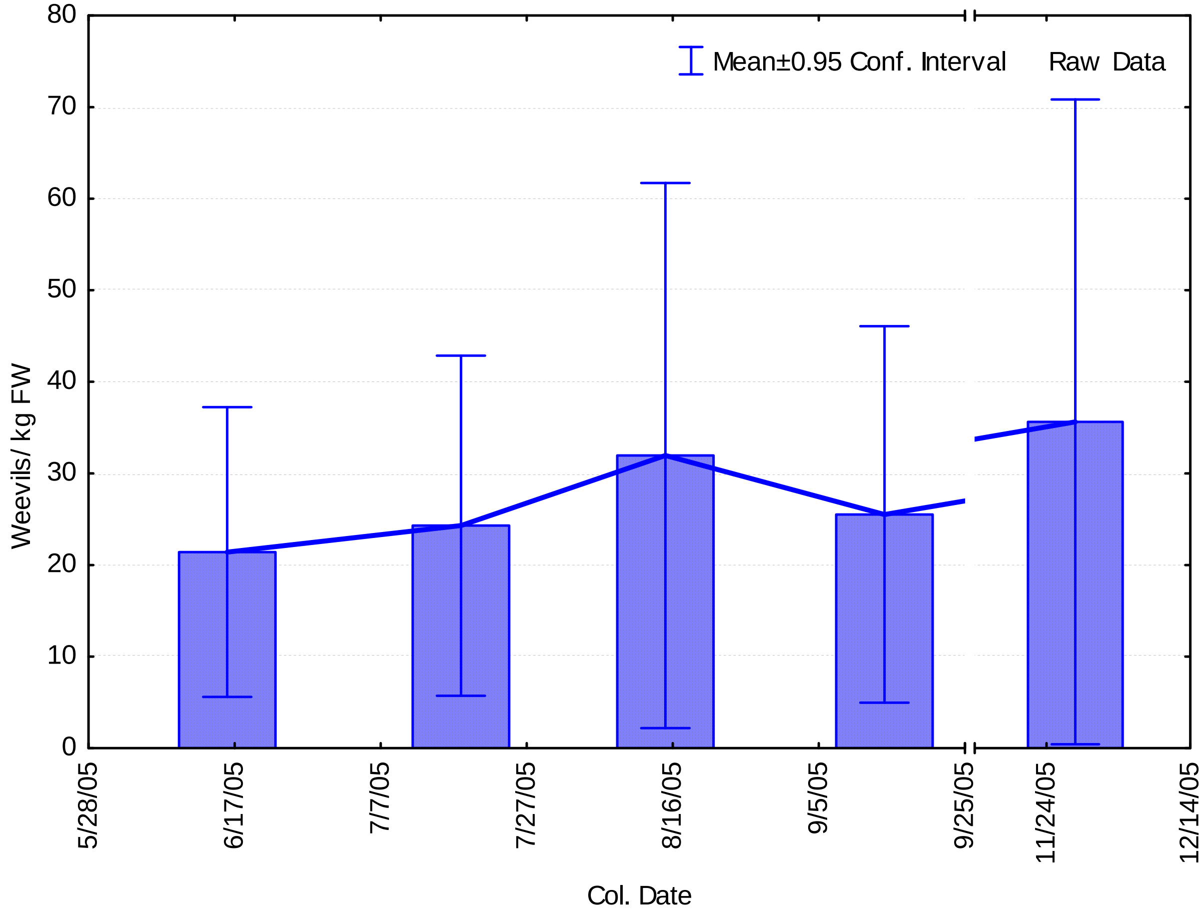


Figure 4. Mean adult *C. salviniae* weevils/kg fresh weight salvinia during 2005.

Based on this information, it is apparently better to release weevils collected from brown, damaged material to maximize weevil/plant weight numbers. Because drying conditions can also produce brown plants, populations should be estimated prior to harvest to be sure of population hot spots. Also, weevil numbers tend to increase as long as temperatures allow for plant growth, so releases in southern locales could take place late in the summer and still provide establishment and growth.

A colony of *C. salviniae* was successfully maintained throughout the winter with the use of stock tank de-icers. These prevented formation of ice in the salvinia mat and allowed the weevils to survive all winter long. It is not known how many, if any, generations of weevils were produced during the winter months, yet in February 2008 one cell produced an estimated 100 adult weevils/kg salvinia wet weight. This allowed transfer of infested salvinia into rearing ponds to supplement the spring weevil populations.

**Weevil Production/Establishment.** Weevil numbers were closely monitored during the summer of 2005 (Figure 4). Population estimates were calculated to reach as high as approximately 70 adult weevils/kg salvinia wet weight in one pond. In August 2005, whole-pond weevil numbers were estimated as high as 1,456,000 adults in one rearing pond.

At the time of inoculation with weevils, the giant salvinia cultures were green and healthy, and completely covered the surfaces of the four ponds. Midway through summer 2005, two years after initial inoculation, two of the four ponds were damaged by insect feeding to the point there was insufficient plant material available for sampling (Figure 1), so only the remaining two ponds were monitored from that point on. New methods to replenish the *S. molesta* supply are being pursued (see Future Plans).

During the summer of 2005, approximately 147,000 adult *C. salviniae* weevils were released in infested plant material at field sites in Texas and Mississippi. On July 27, 2005 Texas Parks and Wildlife Department (TPWD) biologists collected approximately 73,000 adult weevils for release at several sites in Texas. They returned the following week and collected roughly 45,500 more adult weevils.

Establishment has been confirmed in at least two of the Texas release sites, and declines in salvinia were observed in at least one site. This site, Lake Conroe, TX, was monitored by field personnel, and with an integrated approach, which included herbicide applications in weevil-free areas, management has been very successful. More demand for the salvinia weevil is anticipated as availability and success become more widely known.

**Costs.** Cost analyses were performed for *C. salviniae* rearing during 2005. Other years were not considered because insufficient data exist regarding man-hours, etc., and 2005 was the first year of releases. The cost of rearing *C. salviniae* and maintaining a salvinia culture was reasonable. Pond maintenance contributed the largest amount to the cost estimate; i.e., $2500.00 out of $4189.00, or approximately 60 percent (Figure 5). Shipping contributed about 25 percent. Alternative shipping containers are currently under examination in order to reduce shipping weights (Styrofoam™ shipping containers vs. traditional heavy-plastic ice-coolers), though the "Priority Overnight" option via FedEx™ is very costly. Estimated cost per adult weevil released from the LAERF during the first year of rearing efforts is $0.028. Taking into account that there are probably three larvae per adult (Julien et al. 1987) in infested plant material, cost per weevil (all life stages) is substantially less at approximately $0.0082, or $8.20 per 1000 weevils.

There are various ways to decrease costs associated with mass rearing *Cyrtobagous salviniae*. Data indicate that with various pond manipulations weevil numbers will increase so that per weevil associated costs will decrease. By collecting brown, damaged salvinia, weevil numbers are maximized, especially in conjunction with a fertilization regime. Costs can also be alleviated by allowing reservoir managers the opportunity to hand collect infested plant material at the rearing facility. This cuts down substantially on shipping and packaging costs, as well as man-hours associated with shipping.

**FUTURE PLANS:** Because a major problem with mass rearing *Cyrtobagous salviniae* is lack of salvinia available to feed weevils during the growing season, a weevil-free culture is being developed. This will be used to supplement ponds in which plants become badly damaged. The insect-free culture method will also be useful in future rearing applications. A potential problem with this weevil-free culture is size limitation. It is possible that transferring weevil-free salvinia to a salvinia-sparse pond will still result in early destruction of plant populations; therefore, field collection of salvinia is an option. However, field collection must be undertaken carefully, so as not to introduce other invasives into the research ponds.

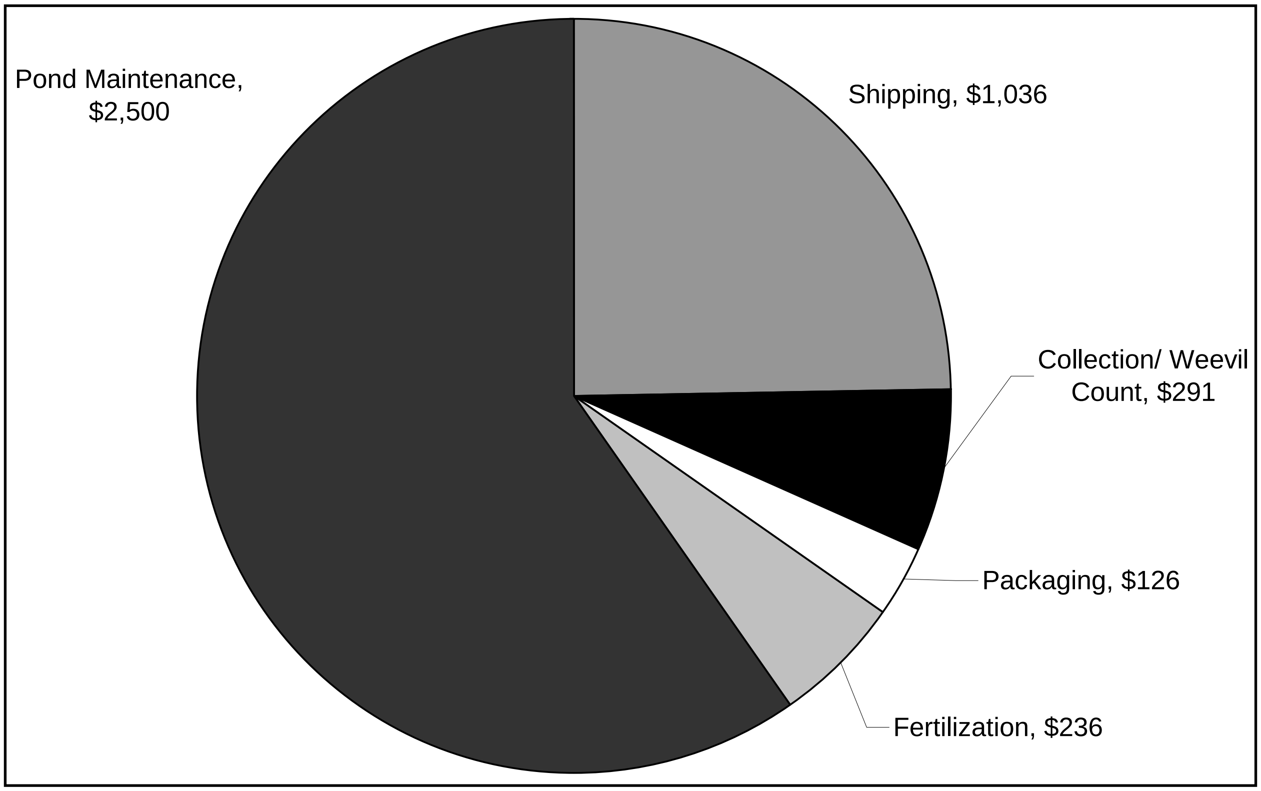


Figure 5. Cost breakdown of *C. salviniae* rearing at the LAERF. Approximately 118,500 adult weevils were released from the LAERF in 2005.

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> Harms, N., M. J. Grodowitz, and J. Nachtrieb. 2009. Development of a multi-attribute utility analysis model for selecting aquatic plant restoration sites in reservoirs. APCRP Technical Notes Collection. ERDC/TN APCRP-BC-16. Vicksburg, MS: U.S. Army Engineer Research and Development Center.

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