Notes

Mass-rearing *Cyrtobagous salviniae* for biological control of giant salvinia: Field release implications

JULIE G. NACHTRIEB*

INTRODUCTION

Giant salvinia (*Salvinia molesta* Mitchell), a free-floating aquatic fern native to South America, is considered one of the world's worst weeds (Harley and Mitchell 1981). Infestations across the globe typically form dense mats, which clog waterways and impact recreational activities, irrigation, drainage, flood mitigation, hydroelectric production, and mosquito control, alter water quality, and displace native vegetation (Tipping 2004). In the United States, infestations have been noted in Louisiana, Texas, Alabama, Arizona, California, Florida, Georgia, Hawaii, Virginia, Mississippi, South Carolina, and North Carolina (USGS 2005).

Biological control of giant salvinia using the salvinia weevil (*Cyrtobagous salviniae* Calder and Sands) (Coleoptera: Curculionidae) has been highly successful (Julien and Griffiths 1998). Both adults and larvae impact the growth of salvinia, but the destruction of vascular tissue and subsequent impact to nutrient flow through larval tunneling in the rhizome is the most detrimental to the plant (Thomas and Room 1986, Julien et al. 1987). Adults feed on leaves and submerged "roots" (modified 3rd leaf), but prefer the nitrogen-rich buds of salvinia (Room and Thomas 1986, Julien et al. 1987). Giant salvinia has been controlled by the salvinia weevils in 13 countries (Julien and Griffiths 1998), with ongoing efforts in the United States.

Researchers at the U.S. Army Corps of Engineers' Lewisville Aquatic Ecosystem Research Facility (LAERF) in Lewisville, TX (33°04′45″N; 96°57′30″W), have been rearing salvinia weevils since 2003. Due to prior success rearing biological control agents in outdoor ponds, salvinia weevils were initially reared at the LAERF in 4 0.25-ha (0.62 acres) earthen ponds (Harms et al. 2009). Pond rearing within the northernmost range of giant salvinia proved difficult because there was no feasible means to protect plants and insects from harsh winter conditions. Both populations were commonly decimated during winter and generally had to be reestablished the following spring from field-collected material. This led to delays in rearing and subsequent release as the insect colonies typically did not build up sufficiently large populations for field releases until September or October. In fact, during the 6 yr of pond rearing, field releases occurred only 1 time, following a mild winter, due to difficulties in achieving weevil densities appropriate for collection and release. The 1 time that weevil populations were satisfactorily high, approximately 146,000 adult weevils were collected from 1 rearing pond from late July to late August 2005 (Harms et al. 2009). Due to overwintering difficulties while using ponds, it was determined that this was not the optimal method for weevil rearing in north Texas, and other rearing techniques were attempted.

Since 2008, weevils have been reared in outdoor, aboveground culture boxes in which it is easier to protect plants and weevils during winter. During 4 yr of rearing in culture boxes, many different rearing and overwintering techniques have been attempted and modified. During the first few seasons of box rearing, results were similar to pond rearing, with weevil densities peaking late in the season (J. Nachtrieb, unpub. data). The following are results from the 2011 field season, including an analysis of mass-rearing techniques, weevil population levels, and weevil production estimates.

MATERIALS AND METHODS

Salvinia weevils were reared in outdoor, aboveground culture boxes (1.5 m by 3.0 m by 0.61 m deep; 2,700-L capacity) constructed from treated lumber and lined with pond liner (5 foot by 10 foot by 2 feet deep; 713 gallons) (45-mil EPDM Firestone pond liner[1]). Boxes were supplied with city water (pH 8–9) that was amended with peat moss at a rate of 40 mL peat L^{-1} (4.5 ounces per gallon) water to facilitate a reduction in pH (Owens and Smart 2010). Water pH ranging from 5 to 7.5 provides optimum growing conditions for salvinia, allowing for the uptake of micronutrients such as iron (Owens et al. 2005). Nutrient levels (nitrogen and iron) were monitored and cultures were fertilized every 4 to 5 wk with 10 mg L^{-1} (10 ppm) nitrogen and 3 mg L^{-1} (3 ppm) iron by using Miracle-Grow® Water Soluble All Purpose Plant Food (24–8–16)[2] and Green Light Iron & Soil Acidifier®[3], respectively. During the winter of

*U.S. Army Engineer-Lewisville Aquatic Ecosystem Research Facility, 201 East Jones St., Lewisville, TX 75057. Corresponding author's E-mail: jnachtrieb@laerf.org. Received for publication October 5, 2012 and in revised form March 12, 2013.

2010/2011, weevils were overwintered in 15 boxes within 2 greenhouses[4] (9.1 m wide by 3.6 m high by 24.4 m long) (30 feet wide by 12 feet high by 80 feet long) constructed of triple-galvanized structural steel tubing and enclosed with 6-mil greenhouse film. Although not heated, during warm, sunny days, air temperatures commonly reached 25 to 30 C (75 to 90 F). Cultures were kept in greenhouses during spring to provide artificially warm environments as a means to increase weevil feeding and reproduction early in the season. Greenhouses were opened on 19 April 2011, at which time outdoor temperatures were warm enough to support plant and insect growth.

To initiate cultures for the 2011 field season, weevils and plants were harvested during spring from overwintered boxes and new cultures were started. New boxes were supplied with giant salvinia and weevils from overwintered cultures that were harvested as plants and weevils became available. Giant salvinia was added to 25 boxes between 28 February and 28 March 2011 and to 4 boxes on 12 May 2011 for a total of 29 individual cultures. Initially, enough giant salvinia was added to each box to occupy approximately 25% of the water's surface. Plants were acclimated for 7 d and then fertilized as described previously. Once plants covered 75 to 100% of the water's surface (typically within 7 d), weevils were added to the cultures via infested plant material. Weevils were supplied to 21 boxes from 8 March to 28 March 2011 and to 2 boxes on 12 May 2011 for a total of 23 boxes with weevils. Six culture boxes were randomly selected as nonweevil controls.

Plant samples were collected 4 times during the 2011 growing season to monitor weevil population levels. Four salvinia samples were randomly collected from each culture box on 26 April, 27 May, 5 July, and 1 August 2011. To obtain weevil population estimates based on both surface area and fresh weight of giant salvinia, plant samples were collected randomly using a 1/35-m^2 (1/3.25 square feet) (16.9 cm by 16.9 cm) (6.6 inches by 6.6 inches) quadrat and fresh weights were determined. Insect extractions were accomplished using Berlese funnels constructed of galvanized sheet metal (30.5-cm diam) (12 inches) with a platform made of ¼-inch (nominal size) mesh poultry netting located 27 cm (11 inches) from the top. A light fixture with a single 40- to 60-W bulb was placed on top of the funnel and a mason jar was attached to the funnel below the platform. Mason jars were filled with approximately 200 mL (6.8 ounces) of 70% ethanol to preserve collected insects. Plants were removed from funnels when completely dry, and larvae and adults were collected from mason jars. Numbers of adults and larvae were enumerated. Adult and larval densities were determined for each box based on the 4 replicate samples, and adults and larvae m^{-2} box^{-1} were averaged for each sampling date.

Overall weevil production values were estimated based on a single harvest of all weevil-infested salvinia from the entire surface area of a box (4.6 m^2) using 2 different criteria. The maximum potential for weevil production was estimated by using the maximum value of adults and larvae m^{-2} for each box. To estimate weevil production if boxes were harvested early in the season, output from each box was recorded as soon as the box reached at least 300 adults and larvae m^{-2}, which was a weevil density representative of early-season levels. For instance, a box might peak in July at 800 weevils m^{-2}, but reach 350 weevils m^{-2} in May. In actuality, boxes were not harvested in order to monitor weevil populations for an entire season.

Weevil population levels also were used to determine the effects of a rearing method, termed thinning, on adult and larval weevil population growth. To sustain weevil populations long term, cultures must be augmented with weevil-free giant salvinia (control) and weevil-infested plant material must be "thinned" from the cultures as infested plants become highly damaged and new plant growth slows. In an attempt to eliminate any decrease in weevil levels as a result of this process, an extraction technique for removing the larvae and adults was used.

Approximately 25% of the weevil-infested material in a box was removed and the box was replenished with insect-free plants. The weevil-infested plant material was arranged in a thin layer on screens directly over the same box. Transfer screens provide the same results as Berlese funnels; as the sun and warm outdoor conditions dried the plants, weevils dropped onto the giant salvinia below. Screens were cleared off when the salvinia was completely dry. Transfer screens were constructed from ¼-inch (nominal size) mesh poultry netting with treated wood frames, and were sized to span widthwise across each box (1.7 m by 4.5 m) (5.5 feet by 15 feet).

Boxes were chosen for thinning based on population data coupled with visual observations of the health of salvinia. Unhealthy or brown salvinia typically coincided with weevil population levels of $> 1{,}000$ adults and larvae m^{-2}. Fourteen boxes were thinned and 8 were not in June 2011, and 16 boxes were thinned and 6 were not in July 2011. Although it was known that larval and adult weevils would vacate drying plants, as evidenced in Berlese funnels, it was unknown if the insects, particularly larvae, would survive for long periods of time after this process. Adult and larval densities were compared at sampling dates before and after thinning and between thinned and nonthinned cultures to determine if weevil populations had increased, decreased, or stabilized. Percent population change between thinned and nonthinned boxes was compared by a 1-way ANOVA. Experimental data were analyzed at a significance level of $P < 0.05$ using STATISTICA version 9.0[5].

RESULTS AND DISCUSSION

Successful overwintering and heightened spring temperatures within cold frames facilitated a rapid increase in weevil populations (Figure 1). When first sampled on 26 April, adult and larval densities were similar and averaged 133 and 175 m^{-2}, respectively. When sampled 4 wk later on 27 May, larval densities had increased 5-fold to 90 m^{-2}, the highest level for the 2011 growing season. At this same sampling date, adults had only increased 2-fold to 285 m^{-2}. As larvae matured to adults, adult weevil densities peaked at 614 m^{-2} on 5 July. Larval densities began to decrease in July, and by August were reduced by 59% from densities recorded in May. Similarly, adult densities also decreased from July to August by 24%.

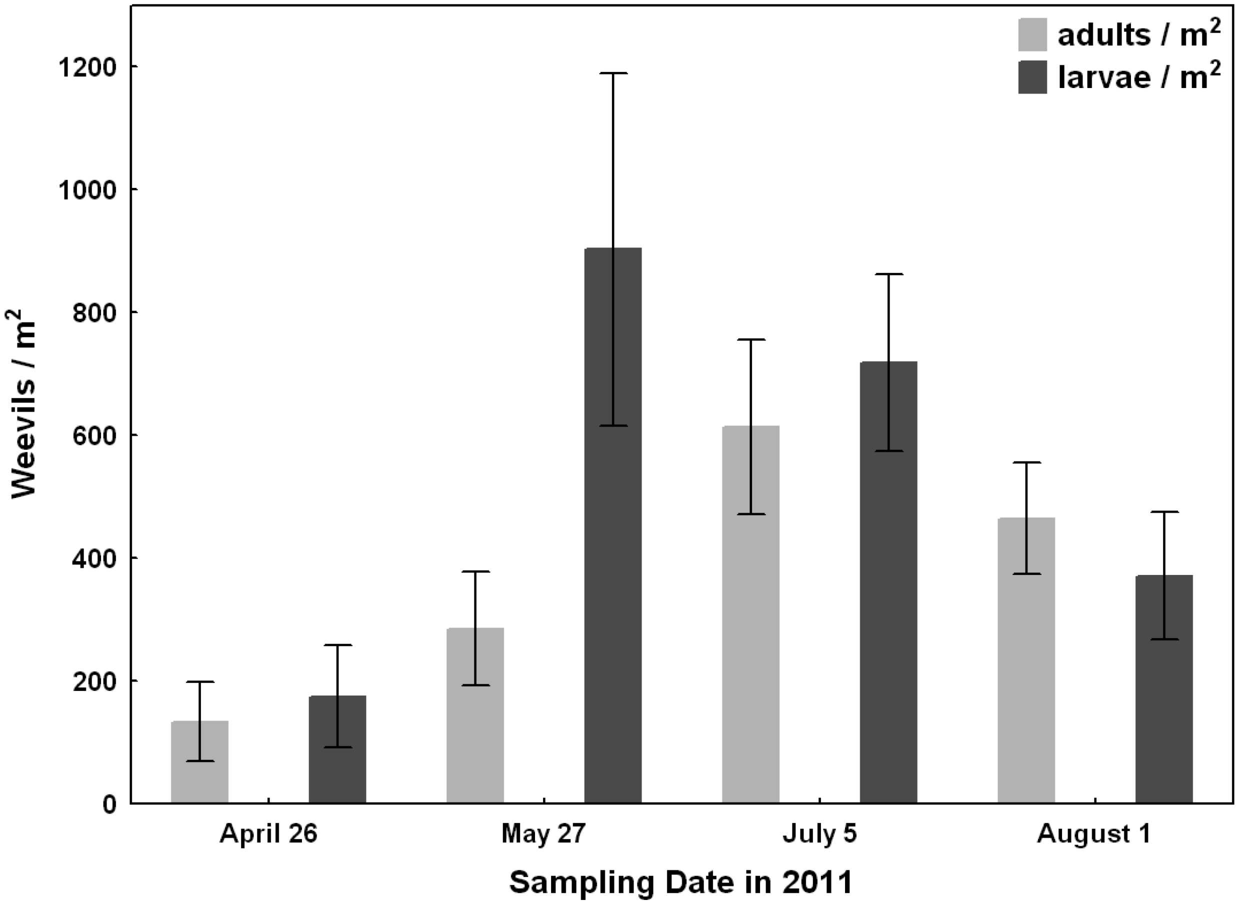

Figure 1. Mean (± 0.95 confidence interval) adult and larval weevils m^{-2} extracted from giant salvinia at each sampling date.

These data display a trend of a single population peak in larvae, approximately 1–2 mo after weather warmed, followed by a decrease in larval numbers. Previous research also indicates that weevil reproduction begins early in the field season. For instance, Forno et al. (1983) found that oviposition was greatest at 23 and 27 C, and Sullivan and Postle (2010) detected eggs at field sites at temperatures as low as 16 C. Therefore, it appears that *C. salviniae* is most fecund at the beginning of the warm season followed by reduced reproduction or population growth.

Population reductions during July and August could be due to decreased egg laying due to increased summer temperatures and high levels of plant damage. Previous research found that when *C. salviniae* was reared at a constant temperature of 31 C, as opposed to 23 to 27 C, there was a 45% reduction in oviposition (Sands et al. 1986). When samples were collected on 5 July and 1 August, there had been 40 and 67 consecutive days, respectively, with maximum air temperatures at or above 31 C. Furthermore, by 1 August there had been 31 consecutive days with maximum air temperatures > 38 C (Weather Underground 2011).

Reduced oviposition rates also have been documented when weevils are reared on damaged plants. For instance, Forno and Bourne (1988) monitored egg production when females were reared on weevil-damaged plants with and without new, undamaged growth and when reared on plants that were manually disbudded. There was a 93% reduction in eggs laid when reared on damaged plants with no new growth (1 bud $female^{-1}$), a 57% reduction when reared on damaged plants with new growth (6 buds $female^{-1}$), and a 31 to 55% reduction when bud levels were manually decreased to 1 and 0 buds $female^{-1}$, respectively. From these data, Forno and Bourne (1988) concluded that insect damage had a greater effect on oviposition than bud supply, possibly due to a biochemical defense mechanism induced by insect damage that would deter oviposition. During the 2011 field season, weevils reached densities of > 1,000 adults and larvae m^{-2} when confined to culture boxes, and although plant damage was not quantified, plants were highly damaged based on visual observations. Future research should attempt to determine if population decreases were temperate or plant damage dependent, by restarting cultures on fresh plants during high temperatures.

Thinning proved to be detrimental to larval populations and have no effect on adult populations. From 27 May to 5 July, larval densities in thinned boxes decreased by 34.86%, whereas nonthinned boxes increased by 533.45% (Figure 2A). From 5 July to 1 August, regardless of thinning or nonthinning, larval levels decreased (Figure 2C), but thinned boxes decreased at a significantly higher rate (–54.07%) than nonthinned boxes (–19.02%). Significant

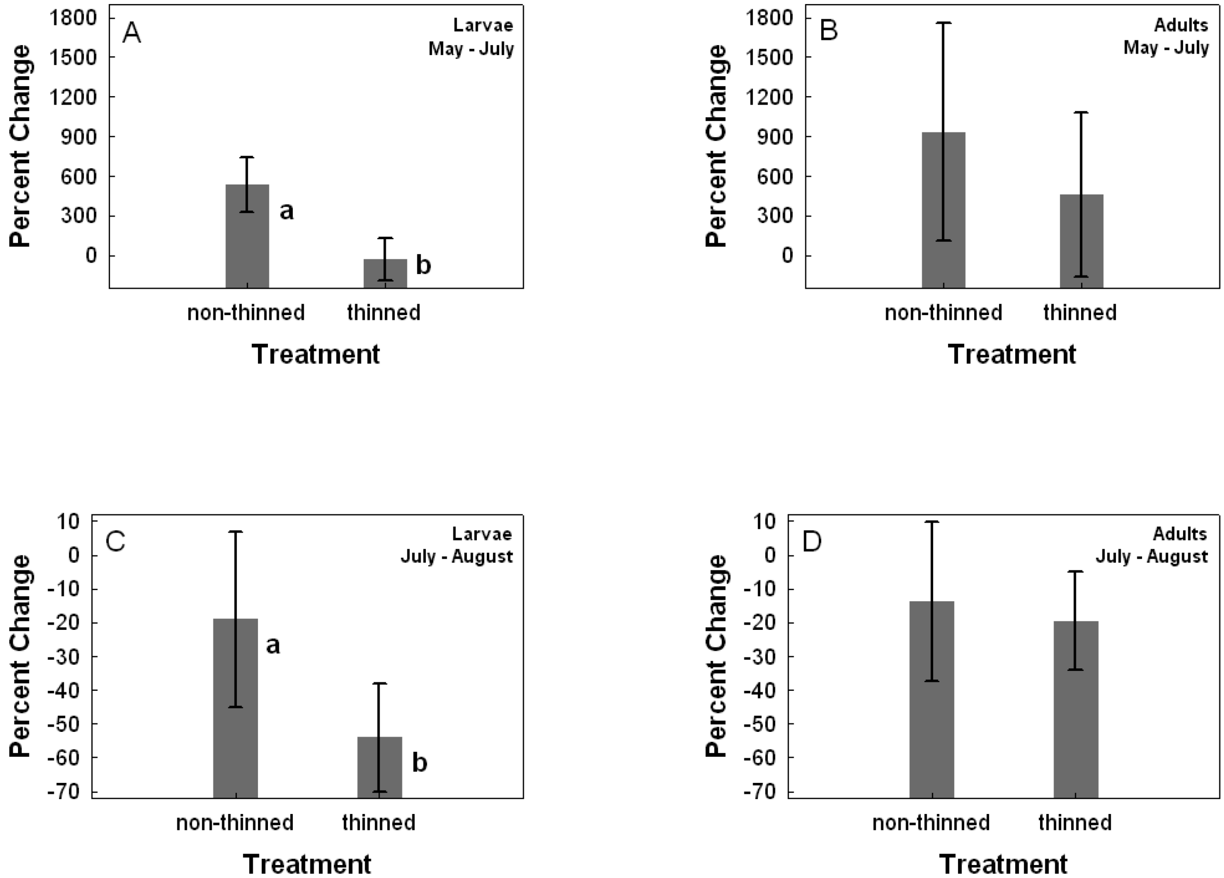

Figure 2. Mean (± 0.95 confidence interval) percent change of adult and larval weevils between thinned and nonthinned cultures from May to July and July to August. Means with the same letter are not statistically different. One-way ANOVA, (A) larvae May–July: $P < 0.001$, $F = 20.213$, degrees of freedom (df) = 1, 20; (B) adults May–July: $P = 0.347$, $F = 0.927$, df = 1, 20; (C) larvae July–August: $P = 0.026$, $F = 5.746$, df = 1, 20; (D) adults July–August: $P = 0.663$, $F = 0.196$, df = 1, 20.

differences between thinned and nonthinned boxes were not detected for adult population growth at either sampling date (Figures 2B and 2D). Adult populations in both thinned and nonthinned boxes increased from 27 May to 5 July by 457.37 and 933.02%, respectively, and decreased from 5 July to 1 August by −19.52 and −13.66%, respectively, regardless of treatment (Figures 2B and 2D).

It appears that although larvae are able to vacate drying plants and move onto substrates below, as evidenced in Berlese funnels, many of the larvae do not survive. Weevil population levels would have increased at a much higher rate from May to July and would have decreased at a reduced rate from July to August if not manipulated through thinning. It is unknown if the possibly traumatic thinning process could have negative physiological impacts to adults, such as decreased egg laying, which would increase larval reductions. Yet, it seems that any negative effects would be minimal since adults typically vacate drying plants and are back in a suitable environment within 24 hr (Boland and Room 1983; J. Nachtrieb, unpub. data). It is also possible that reductions in population growth in thinned boxes were magnified by reduced egg laying due to high levels of plant damage and insect crowding. While approximately 25% of infested plants were removed during thinning, 75% of the highly damaged plants remained in the box. If oviposition rate is reduced when reared on damaged plants, oviposition rates could have been negatively affected even after thinning. Future research should evaluate the effects of thinning in cultures with both low and high weevil populations to distinguish thinning effects from other parameters.

During the field season of 2011, 23 culture boxes were used for weevil production and a maximum of 193,028 weevils (larvae and adults) were produced. Peak populations in each box ranged from 814 to > 2,700 adults and larvae m^{-2}. Due to successful overwintering and heightened spring temperatures while enclosed in cold frames, all boxes reached peak population levels by 5 July. Eleven boxes reached peak levels by 27 May and produced 98,457 weevils. An additional 9,571 weevils were produced by the remaining 12 boxes by 5 July. On average, 8,392 weevils were produced per box. During previous pond-rearing efforts at the LAERF, without a means of overwintering, cultures did not peak until September or October (J. Nachtrieb, unpub. data).

Although high production levels are important to a mass-rearing operation, conclusions from this research (weevil population increases early in the growing season, the negative effects of thinning, and population decreases later in the season) suggest that it may be more prudent to harvest and release weevils early in the field season even if population levels might not have peaked. Although low quantities of weevils were available for release in April, those available had a high potential for rapid population development at field sites as shown by the 5-fold increase in larval densities by 27 May in the culture boxes. At field sites, where larval development and egg production would not be limited by space and food, the potential for population increase should be even greater. As larvae provide the most damage to giant salvinia (Julien et al. 1987), it is most advantageous to target field releases to coincide with high egg production so that larvae can emerge at field sites and begin to impact infestations. Sullivan and Postle (2010) also suggest that releases in early spring are important in temperate regions as it allows more time for weevil population development before winter. Furthermore, soon after populations were sampled on 27 May, 14 boxes had to be thinned due to degrading salvinia conditions caused by weevil densities $> 1{,}000$ adults and larvae m^{-2}. As thinning has been found to decrease adult and larval densities, it would be prudent to harvest for field releases instead of trying to maintain cultures through repeated thinning.

To eliminate the need for thinning and to harvest for field releases prior to larval population peaks, a low weevil population level was chosen as an indicator of when to harvest. On average, cultures that were at 1,000 adults and larvae m^{-2} (in need of thinning) on 27 May, were at 450 adults and larvae m^{-2} 4 wk prior. To be conservative, 300 adults and larvae m^{-2} was set as the target population density for field releases. Releasing early in the season, when populations were at low densities, would help to ensure that oviposition and larval peaks occur at field sites.

If each box was harvested during the 2011 season when boxes reached 300 adults and larvae m^{-2}, approximately 83,000 weevils would have been collected between 26 April and 5 July. Nine boxes reached releasable levels by 26 April, 12 boxes by 27 May, and 2 boxes by 5 July. Although this represents a 57% reduction in weevil output when compared to maximum release estimates, it is important to note that although initial field release quantities will be low, populations should quickly increase at field sites. As an example, the cultures that reached 300 adults and larvae m^{-2} on 26 April increased on average 3.8-fold by 27 May. The 23,548 weevils that would have been released in April grew to $> 89{,}000$ within 4 wk while in culture. The potential for weevil population expansion in field infestations of salvinia should far exceed those seen in cultures as space and food would not be limited.

When managing a mass-rearing program, it is important to not only maximize yield, but to pay attention to the life cycle of the reared insect and host plant. In the case of *C. salviniae*, it appears that field releases in spring would maximize the insect's ability to establish and impact field infestations of giant salvinia through rapid population growth. Furthermore, spring may be the most opportune time for field releases in regards to seasonal growth patterns of giant salvinia. Harsh winters can reduce infestations of $> 1{,}000$ (400 hectares) acres to < 100 acres (40 hectares) in early spring. If weevils are released onto relatively small amounts of giant salvinia at the most opportune time, when oviposition is highest, more substantial impacts could be achieved at field sites.

SOURCES OF MATERIALS

[1]AZ Ponds and Supplies, Inc., 4274 Perkiomen Ave., Reading, PA 19606.

[2]The Scott's Company, 14111 Scottslawn Road, Marysville, OH 43041.

[3]The Green Light Company, 10511 Westmore Road, San Antonio, TX 78216.

[4]ClearSpan, 1395 John Fitch Blvd, South Windsor, CT 06074.

[5]StatSoft, Inc., 2300 East 14th Street, Tulsa, OK 74104.

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