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Note

Amendments to giant salvinia nitrogen content increase salvinia weevil density at field sites

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INTRODUCTION

In the United States, giant salvinia (*Salvinia molesta* D.S. Mitchell) infestations can be found in 16 states, Puerto Rico, and the Virgin Islands (U.S. Geological Survey 2018). Salvinia weevils (*Cyrtobagous salviniae* Calder and Sands) have successfully managed giant salvinia in at least 13 countries worldwide (Julien and Griffiths 1998), yet success remains low in temperate regions of the United States. Limited success can be primarily attributed to unsuccessful salvinia weevil overwintering when exposed to subfreezing temperatures (Mukherjee et al. 2014). However, there are other factors, such as plant nutritive quality, that could play a vital role in successful biological control of giant salvinia.

Previous studies have highlighted the importance of plant nitrogen content to herbivorous insect growth rate, size, fecundity, productivity, and overall population size (Brunsting and Heil 1985, McNeil and Southwood 1978, Keesing 1993). Studies have also investigated the role of giant salvinia nitrogen content and salvinia weevil productivity. Increased giant salvinia nitrogen content decreases larval developmental time (Sands et al. 1983), increases egg production (Sands et al. 1986), and increases weevil growth rate (Sands et al. 1986). At field sites where salvinia weevils did not establish under unamended conditions, nitrogen amendments increased nitrogen content from 1 to 1.75% and were successful at increasing weevil densities fivefold in 11 wk (Room and Thomas 1985).

A recent, 2-yr field study was conducted to examine a possible correlation between plant nitrogen content and salvinia weevil densities at four waterbodies across Louisiana, including Saline Lake (Nachtrieb, in press). Data collected during the first year identified a positive correlation between plant nitrogen content and both adult and larval weevil densities, but correlation analyses from the second year were not significant. Results from this study provided background knowledge of giant salvinia nitrogen content in a Louisiana lake. In particular, 69% of giant salvinia collected ranged from 1.5 to 2.5% dry weight nitrogen, 9% of samples contained greater than 3% dry weight nitrogen, and 22% of samples contained less than 1.5% dry weight nitrogen (Nachtrieb, in press).

Nachtrieb (in press) also found giant salvinia on Saline Lake had consistently less nitrogen than all other sites, ranging from 1.02 to 1.73% dry weight, and despite repeated salvinia weevil introductions, increases in salvinia weevil density were not achieved. Due to the consistently low plant nitrogen content and absence of salvinia weevil establishment success, Saline Lake was identified as a suitable location for a controlled and replicated field study to investigate potential improvements to salvinia weevil establishment and giant salvinia management as a result of nitrogen amendments. By standardizing initial salvinia weevil release densities and manipulating plant nitrogen, this study aims to specifically pinpoint the direct effect of plant nitrogen content to salvinia weevil success at field sites. This information will provide a better understanding of the environmental parameters effecting weevil establishment in the southern United States and will aid natural resource agencies in determining suitable sites for salvinia weevil establishment.

MATERIALS AND METHODS

In 2016–2017, a field trial was conducted on Saline Lake, Louisiana (31°53.964′N, 92°53.416′W) to determine the impact of fertilization on salvinia weevil establishment. Ten floating containment booms[1] were deployed in April 2016 to contain an already present infestation of tertiary (final, matt-forming stage with large folded fronds) giant salvinia into 10 replicate research plots. Each circular boom encompassed an area of 19 m^2 (diameter of 4.9 m) and contained a 1.5 m submersed curtain made of vinyl laminated fabric. Tertiary giant salvinia existed as a monoculture, covering 100% of the water's surface, both inside each boom and in the surrounding area. Five of the research plots were randomly designated as controls (no fertilizer amendments) and five as fertilizer treatments.

Salvinia weevils were stocked in each research plot on 26 April 2016 at the rate of 46 adults and 170 larvae per m^2 or approximately 884 ± 71 adults and 3,222 ± 429 weevil larvae per research plot. In total, for the 10 plots, approximately 41,000 salvinia weevils were released. Salvi-

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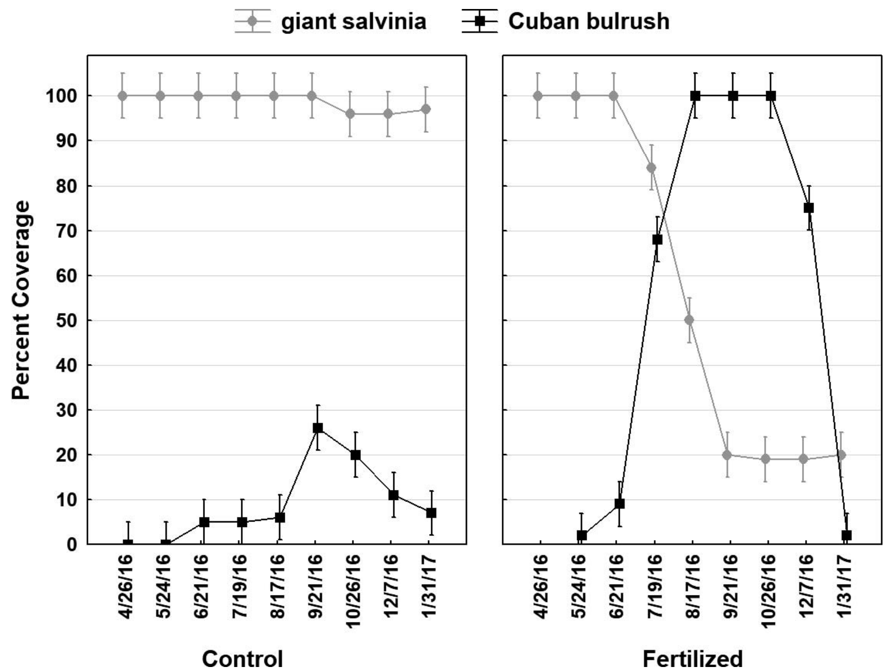

Figure 1. Mean percent coverage (± 0.95 confidence interval) of giant salvinia and Cuban bulrush in control and fertilized plots in Saline Lake, Louisiana, from April 2016 to January 2017; $n = 10$.

nia weevils were cultured at the U.S. Army Engineer Research and Development Center, Lewisville Aquatic Ecosystem Research Facility (LAERF) in Lewisville, Texas. Salvinia weevil rearing, harvesting, and field releases of weevil-laden plant material were conducted using methods described in Nachtrieb (2012). Each plot received approximately 45 L of weevil-laden plant material transported to the site inside an insulated cooler. Although salvinia weevils have the ability to fly, based on years of field trials (Nachtrieb, unpublished data), salvinia weevils are rarely detected outside the booms. Therefore, it was expected that during this study, introduced salvinia weevils would be contained within the booms. Also, immigration of nearby feral salvinia weevils was considered unlikely due to the absence of a detected salvinia weevil population elsewhere on Saline Lake.

Fertilized plots received 240 g (3.04 g N per m^2 giant salvinia) of Miracle-Gro® All Purpose Plant Food[2] (24-8-16) dissolved into 13 L of water and evenly applied to the foliage using a backpack sprayer.[3] Initial fertilizer treatments were applied after the salvinia weevil release on 26 April 2016. Additional fertilization treatments were scheduled every 4 to 5 wk from April through October 2016.

During site visits, plant species percent surface coverage, water temperature, salvinia weevil densities, and giant salvinia nitrogen content were monitored. Percent coverage for all present plant species was recorded by visual observations of each plot during each site visit. Water temperature loggers[4] were placed 5 cm beneath the salvinia mat and programmed to continuously record hourly temperature in three control and three fertilized treatment plots. Giant salvinia samples were collected from each plot for salvinia weevil extraction and plant nitrogen analysis. A 14-tine bow rake was used to indiscriminately collect giant salvinia from within each plot to fill three 3.8 L plastic bags. Samples were returned to the lab and individually placed into a Berlese funnel (Nachtrieb 2012) for 48 h to extract adult and larval weevils into 70% ethanol for insect preservation. Adult and larval salvinia weevils per kg dry weight giant salvinia were identified and quantified by using a dissecting microscope at 10× magnification. Following weevil extraction, giant salvinia samples were placed into paper bags and dried in a forced air oven at 55 C for 48 h to obtain dry weights. Dried floating fronds were separated from submersed fronds (i.e., roots), and all floating fronds were mailed to the Pennsylvania State University Agricultural Analytical Services Laboratory. Floating fronds were ground to less than 20 mesh size before analysis by a combustion method using an Elementar Vario Max C/N Analyzer[5] to determine percent dry weight total nitrogen content. Data collections began on 26 April 2016 (the same day as salvinia weevil release and first fertilizer application) and were scheduled for every 4 to 5 wk from April through October 2016 and every 6 to 8 wk from December 2016 through late January 2017.

Fertilizer treatments were conducted as planned during the first three site visits. Before the fourth sampling trip in July, May plant nitrogen results (4 wk after initial fertilizer application) were received from the laboratory, and plant nitrogen content remained at a reduced quantity, approximately 1.3% dry weight nitrogen. Due to the less than desirable plant nitrogen content, it was decided to increase fertilizer rates by 1.5-fold during the July sampling period to ensure adequate nitrogen levels. When sites were visited in August, the invasive emergent Cuban bulrush (*Oxycaryum cubense* (Peopp. & Kunth) Lye) covered 100% of the surface

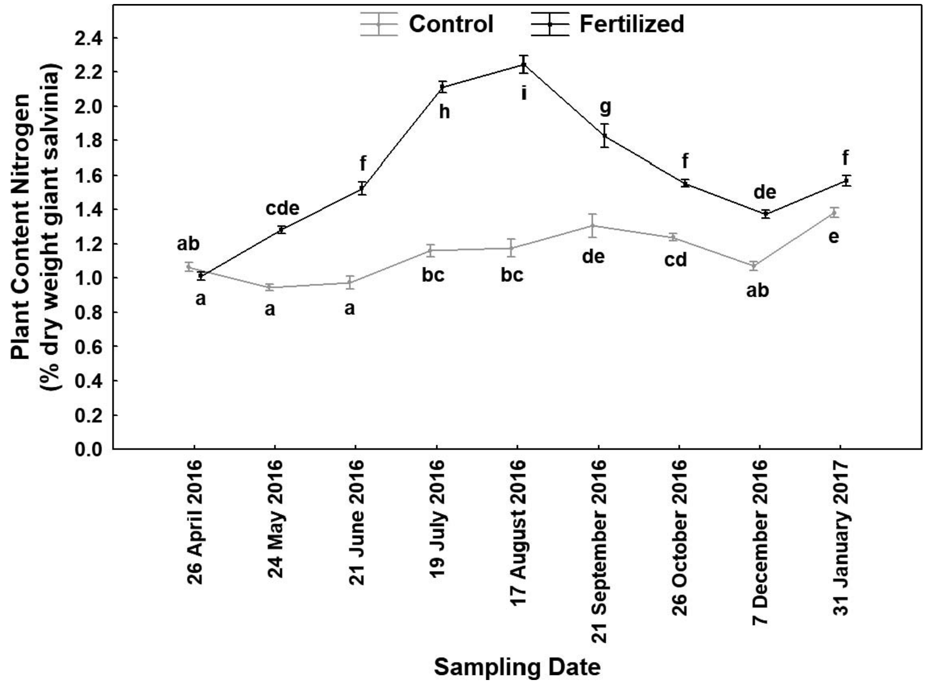

Figure 2. Mean plant nitrogen content (± standard error) in control and fertilized plots in Saline Lake, Louisiana; $n = 10$. Means with the same letter are not significantly different according to the Student-Newman-Keuls test, $\alpha = 0.05$. Repeated measures ANOVA, treatment: $P < 0.001$, $F = 292.32$; sampling date: $P < 0.001$, $F = 93.78$; interaction: $P < 0.001$, $F = 53.52$.

area of the fertilized plots (Figure 1). Although Cuban bulrush is commonly found growing on the surface and throughout stands of giant salvinia, the rapid expansion of this mat-forming species was likely attributed to the addition of fertilizer during previous site visits. In contrast, Cuban bulrush covered approximately 6% of the surface area within the control plots (i.e., nonfertilized plots) (Figure 1). As a result of the colonization of Cuban bulrush, fertilizer treatments were discontinued after July.

Statistical analyses

Data were analyzed using STATISTICA™ (version 12) software[6] and included analysis of variance (ANOVA) and Student-Newman-Keuls multiple comparison/range tests. A separate repeated measures ANOVA was performed for each of the following dependent variables: 1) adult weevil densities, 2) larval weevil densities, and 3) plant nitrogen content, in relation to the categorical predictor treatment (fertilized or control) and within plots/subjects factor of sampling date at an alpha level of 0.05.

RESULTS AND DISCUSSION

Despite the reduced frequency of fertilizer applications, plant nitrogen content was significantly greater ($P < 0.001$) in fertilized plots for all sampling dates after initial fertilizer application (May through January) (Figure 2). From May through January, fertilized plots ranged from 1.28 to 2.24 and control plots from 0.94 to 1.38% dry weight nitrogen (Figure 2). Differences between control and fertilized plots peaked in August (4 wk after final fertilizer application), with fertilized plots (2.24% dry weight nitrogen) having 1.07% more dry weight nitrogen than control plots (1.17% dry weight nitrogen). This is a substantial difference in plant nitrogen content because Sands et al. (1986) recorded that an increase of 0.1% dry weight nitrogen resulted in a 3.6% increase in weekly oviposition (egg laying) and that when nitrogen content was increased by 0.5% dry weight, weevil growth rate increased by 15% (Sands et al. 1986).

Salvinia weevil larval densities also varied between treatments ($P < 0.001$). Larval weevils were not detected in plots before introduction in late April 2016. Within 4 wk of salvinia weevil introduction and salvinia weevil adults feeding on plants of differing nitrogen levels (control or fertilized plots), adults experienced increased fecundity in fertilized plots as determined by 2.8-fold higher larval density in fertilized plots in May (Figure 3). Increased fecundity in fertilized plots in May was further illustrated by adult to larva ratios of approximately 1 : 7 for control plots and 1 : 10 for fertilized plots. From May to July, larval densities in both treatments gradually decreased. Although this decline is not fully understood, the decline may have been attributed to increased water temperatures during this time. Peak water temperatures were detected in plots from June through August, with maximum water temperatures in both control and fertilized plots at approximately 33 C (Figure 4). Optimum salvinia weevil oviposition occurs from 23 to 27 C (Forno et al. 1983) and reduced oviposition is experienced at 31 C (Sands et al. 1986). It is possible that high summer water temperatures caused a reduction in oviposition and subsequently in larval densities. During the

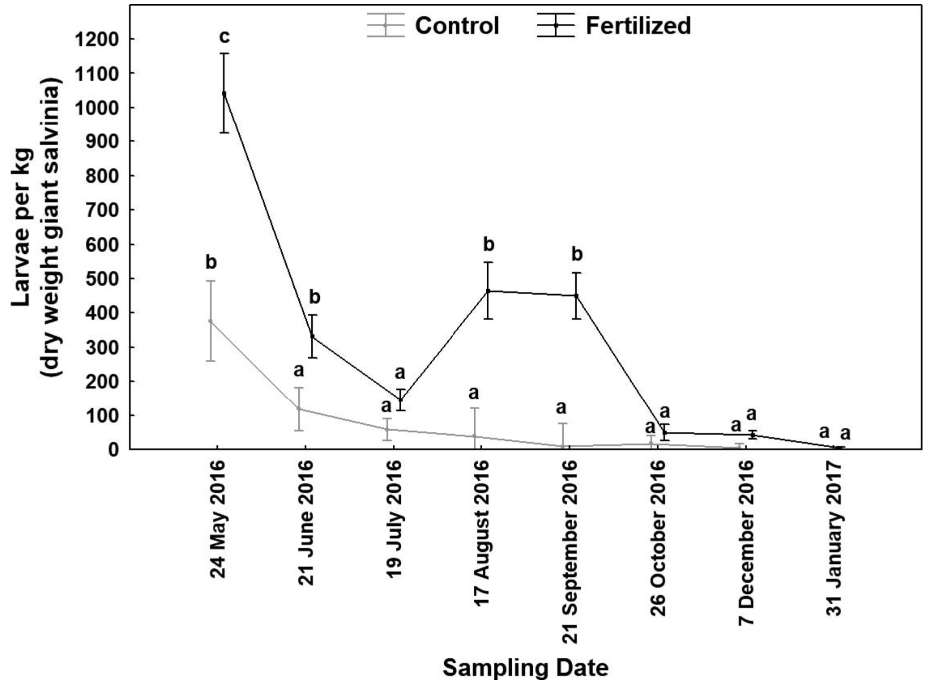

Figure 3. Mean larval salvinia weevil densities (± standard error) in control and fertilized plots in Saline Lake, Louisiana; $n = 10$. Means with the same letter are not significantly different according to the Student-Newman-Keuls test, $\alpha = 0.05$. Repeated measures ANOVA, treatment: $P < 0.001$, $F = 184.43$; sampling date: $P < 0.001$, $F = 24.10$; interaction: $P < 0.001$, $F = 7.10$.

August through September sampling period, maximum water temperatures were reduced to approximately 31 C (Figure 4), and larval densities in fertilized plots increased to 450 larvae per kg (Figure 3). Larval densities in control plots never recovered past July, with densities of 39 and 9 larvae per kg detected in August and September, respectively (Figure 3). By late October, larval densities in fertilized plots were significantly reduced (Figure 3), most likely due to natural reduction in reproduction and larval survival during the onset of winter. By the October through

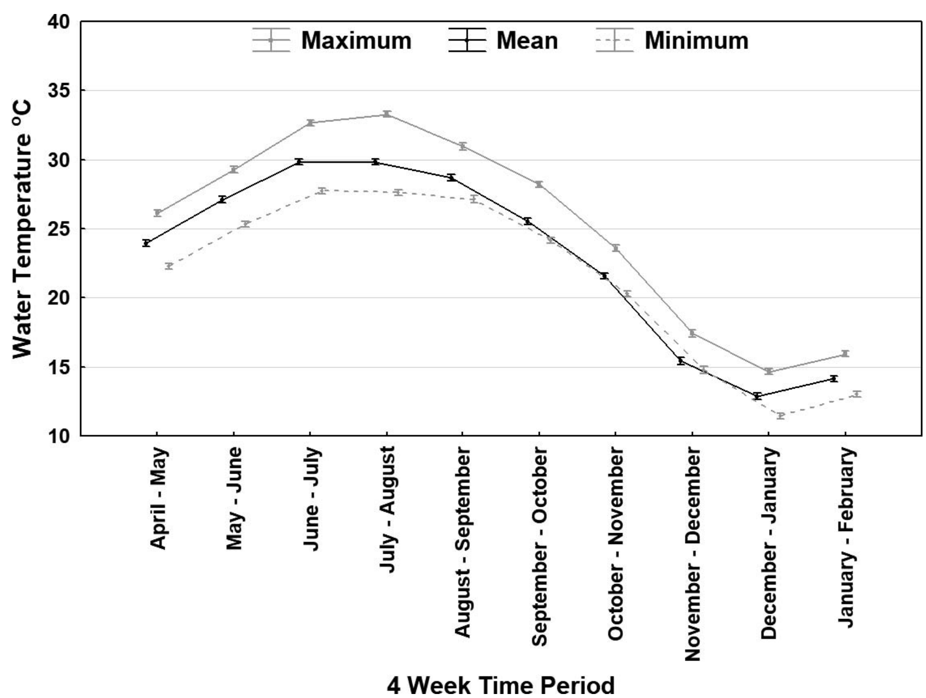

Figure 4. Maximum, minimum, and mean water temperature (C) (± standard error) collected at 5 cm depth beneath giant salvinia mat in three control and three fertilized treatment plots in Saline Lake, Louisiana; $n = 6$. Water temperature was averaged and organized into 4-wk periods.

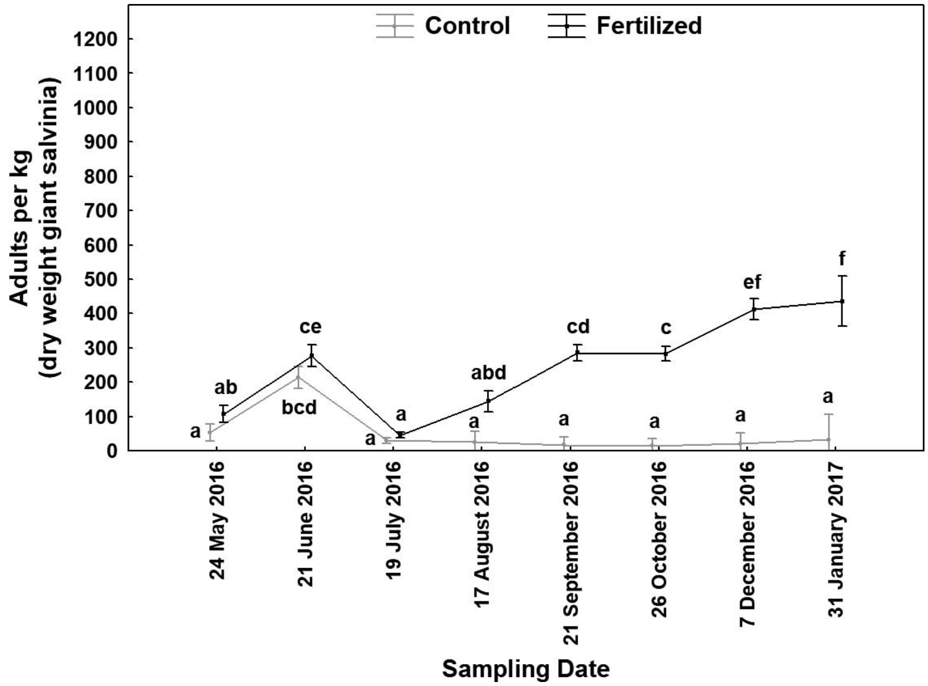

Figure 5. Mean adult salvinia weevil densities (± standard error) in control and fertilized plots in Saline Lake, Louisiana; $n = 10$. Means with the same letter are not significantly different according to the Student-Newman-Keuls test, $\alpha = 0.05$. Repeated measures ANOVA, treatment: $P < 0.001$, $F = 186.87$; sampling date: $P < 0.001$, $F = 9.10$; interaction: $P < 0.001$, $F = 9.06$.

November sampling period, average temperatures were less than 17 C (Figure 4), which is the lower limit for larval survival (Sands et al. 1983). Consequently, due to limited larval survival, there were no statistical differences in larval densities between control and fertilized plots during October, December, and January (Figure 3).

Adult weevils were not detected in any plots before introduction in late April 2016. After introduction, adult weevil densities in both control and fertilized plots increased gradually at similar rates from April to June (Figure 5). Between June and July, there was an unexplained sharp decline in adult weevil densities in both control and fertilizer treatments (Figure 5), which was similar to the larval density decline during the same time period and, likewise, could potentially be attributed to high water temperatures. Subsequently, adult densities in fertilized plots were able to recover and were significantly greater ($P < 0.001$) than control adult densities from September 2016 through January 2017 (Figure 5). Adult densities in fertilized plots peaked in January at 436 adults per kg (Figure 5). In contrast, adult densities in control plots were unable to recover past July, and densities ranged between 15 and 32 adults per kg (Figure 5). The lack of adult recovery was most likely due to reduced nitrogen availability that ultimately limited fecundity.

As previously mentioned, percent coverage of giant salvinia and Cuban bulrush varied substantially between control and fertilized plots (Figure 1). In the control plots where nitrogen levels were low and minimal salvinia weevils established, giant salvinia was present at all sampling dates at 96 to 100%, and Cuban bulrush peaked at approximately 25% coverage in September (Figure 1). In contrast, adult and larval salvinia weevils successfully established in large densities in fertilized plots, and giant salvinia was reduced to approximately 20% coverage from September 2016 through January 2017, while Cuban bulrush covered 100% of the plots from August through October (Figure 1). In the fertilized plots, the reduction of giant salvinia was likely the result of salvinia weevil herbivory and being outcompeted by the dense infestation of overlying Cuban bulrush. It should also be noted that the substantial reduction in giant salvinia coverage in fertilized plots most likely positively skewed salvinia weevil adult densities. Due to the reduction in suitable habitat, both older and newly emerged adults were forced to condense onto the limited plant material, thus potentially artificially increasing adult weevil densities.

This study demonstrates that applying minimal fertilizer to field populations of giant salvinia can provide season-long effects for giant salvinia nitrogen content and salvinia weevil establishment and confirms the importance of plant nitrogen content during initial introduction of salvinia weevils. Following a decline in recently introduced adult and larval weevil densities, salvinia weevil population growth recovered in plots in which nitrogen was supplemented, and plant nitrogen content ranged from 1.28 to 2.24% dry weight nitrogen. In contrast, plant nitrogen content in control plots ranged from 0.94 to 1.38% dry weight nitrogen, and although salvinia weevil presence was detected, population growth was arrested. These results are similar to those found by Room and Thomas (1985) in which initial salvinia weevil introductions declined for a period of 7 mo, yet following fertilizer amendments, weevil densities increased fivefold in 11 wk. Research conducted by Nachtrieb (in press) during 2013 and 2015 additionally confirmed reduced giant salvinia nitrogen content (values ranging from 1.02 to 1.73% dry weight nitrogen) on Saline Lake and limited salvinia weevil establishment. Low plant nitrogen content and the subsequent inability of the salvinia weevil to increase population densities most likely substantially contributed to the prior lack of weevil establishment success in Saline Lake. Although it is not feasible to fertilize large areas within a given waterbody, short-term fertilization of the weevil introduction site could expedite salvinia weevil establishment. It is suggested that natural resource managers and biocontrol practitioners take note of plant nitrogen content when selecting field release sites.

SOURCES OF MATERIALS

[1]SiltMax 1 DOT Barrier, Elastec, 1309 W. Main St., Carmi, IL 62821.

[2]Miracle-Gro® Water Soluble All Purpose Plant Food (24-8-16), Scotts Miracle-Gro Company, 14111 Scottslawn Rd, Marysville, OH 43041.

[3]NeverPump™ Bak-Pak® DC Sprayer, H. D. Hudson Manufacturing Company, 1000 Foreman St. SE, Lowell, MI 49331.

[4]HOBO®, Onset, 470 MacArthur Blvd., Bourne, MA 02532.

[5]Elementar Vario Max C/N Analyzer, Elementar, Elementar Strasse 1, 63505 Langenselbold, Germany.

[6]STATISTICA™ version 12, formally of StatSoft, Inc., Tulsa, OK, currently owned by TIBCO Software Inc., 3307 Hillview Ave., Palo Alto, CA 94304.

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