J. Aquat. Plant Manage. 57: 7–13

Field site analysis of giant salvinia nitrogen content and salvinia weevil density

JULIE G. NACHTRIEB*

ABSTRACT

In 2012, a giant salvinia (*Salvinia molesta* Mitchell) biological control project was initiated in Louisiana. Although similar quantities of salvinia weevils (*Cyrtobagous salviniae* Calder and Sands) were released at all sites, weevil densities were highly variable among sites. In addition, signs of plant nitrogen depletion (yellowing plants) were observed at some sites. Because it is well known that plant nutrition can affect the success of a biocontrol agent because of slowed development and/or reduced fecundity, the correlation between giant salvinia nitrogen content and salvinia weevil density was investigated during the growing seasons of the second (2013) and fourth (2015) years. During 2013, weevils were reintroduced to sites, and the magnitude of adult weevil density increase varied by site. Adult densities at Upper Big Break were 6-fold greater than at all other sites. Larval densities did not change over time, but at Upper Big Break, they were, on average, 10-fold greater than at other sites. Giant salvinia nitrogen content varied among sites and sampling dates, and Upper Big Break plants had greater nitrogen than all other sites during 75% of sampling dates. Additionally, adult and larval densities were significantly correlated to plant nitrogen content. During 2015, trends were less distinct and weevil densities and nitrogen content varied based on the interaction between sampling date and site, but a significant correlation was not detected. Results from 1-yr of a 2-yr study confirmed published reports of the importance of plant nitrogen content to salvinia weevil productivity. Additional studies are warranted to evaluate and understand the role of nitrogen at giant salvinia biocontrol field sites.

Key words: biological control, *Cyrtobagous salviniae*, invasive species management, *Salvinia molesta*.

INTRODUCTION

Sixteen states, Puerto Rico, and the Virgin Island of the United States have recorded infestations of the free-floating aquatic fern giant salvinia (*Salvinia molesta* Mitchell), which is native to South America (U.S. Geological Survey 2016). The negative impacts of giant salvinia have been well documented and include the formation of dense mats, which clog waterways and affect recreational activities, irrigation, drainage, flood mitigation, hydroelectric production, and mosquito control, as well as alter water quality and displace native vegetation (Tipping 2004). Salvinia weevils (*Cyrtobagous salviniae* Calder and Sands) have been successful at managing giant salvinia in at least 13 countries worldwide (Julien and Griffiths 1998), yet success remains low and inconsistent in temperate regions of the United States (Julien et al. 2009, Sullivan and Postle 2010, Mukherjee et al. 2014).

It is well documented that nutrient-poor plant tissues can act as an antiherbivore defense mechanism (Moran and Hamilton 1980). In the case of giant salvinia, considerable research has been conducted in laboratory and field settings to determine the direct effect of plant nitrogen content on salvinia weevil growth and reproduction and the subsequent effect on successful biological control of giant salvinia. Sands et al. (1983) determined that developmental time of weevil larvae was significantly reduced when insects were fed giant salvinia with nitrogen content greater than 1.5% dry wt. Additionally, weevil fecundity was examined for females consuming plants with nitrogen content ranging from 1.5 to 2.5% dry wt (Sands et al. 1986). An increase of 0.1% dry wt nitrogen resulted in a 3.6% increase in eggs laid (Sands et al. 1986). Furthermore, when nitrogen content was increased by 0.5% dry wt, weevil growth rate increased by 15% (Sands et al. 1986).

Research has also been conducted to determine whether nitrogen amendments could reverse a decline in weevil abundance observed during a period of 7 mo at field sites (Room and Thomas 1985). Nitrogen amendments were applied to increase giant salvinia nitrogen content from 1 to 1.75% dry wt, and weevil population sizes increased 5-fold in 11 wk (Room and Thomas 1985). Sites were monitored for a 260-d period, and weevil populations increased 1,000-fold at 1.5% dry wt nitrogen content (Room and Thomas 1985).

Forno and Semple (1987) analyzed the nitrogen content of fronds and submersed fronds (i.e., roots) and identified herbivory-induced nitrogen shifts within giant salvinia. After herbivory, nitrogen content was initially increased in submersed fronds and was then mobilized to new bud and frond tissue, thus producing compensatory plant growth with increased nitrogen content. Consequently, plant response to herbivory may explain the weevil's ability to manage giant salvinia with low-nitrogen content and suggests that initial nitrogen content may not be a limiting factor to a successful giant salvinia biocontrol program. This idea is supported by the documentation of successful management of giant salvinia infestations at sites across Australia, Papua New Guinea, India, and Africa, with giant salvinia nitrogen content less than 1.4% dry wt (Forno 1985, Thomas and Room 1985, Room 1986a, Thomas and Room 1986, Forno 1987).

Research Biologist, U.S. Army Engineer Research and Development Center, Environmental Laboratory, Lewisville, TX 75057. Corresponding author's E-mail: Julie.G.Nachtrieb@usace.army.mil. Received for publication March 23, 2018 and in revised form August 14, 2018.

Table 1. Total salvinia weevils released per site and date during field trials in 2013 and 2015.

Waterbody	Site	Weevil Release Date	Total Weevils Released (Larvae and Adults)
Caddo Lake	Big Green Break	30 April 2013	9,800
		28 May 2013	25,700
		27 June 2013	16,400
Lake Bistineau Vicinity	Gravel Pit	30 April 2013	10,800
		28 May 2013	24,100
		27 June 2013	15,200
Saline Lake	Canal Lake Boat Road	30 April 2013	10,100
		28 May 2013	25,100
		27 June 2013	16,400
Turkey Creek Lake	Upper Big Break	30 April 2013	12,500
		28 May 2013	23,200
		27 June 2013	16,400
Caddo Lake	Big Green Break	5 May 2015	7,700
		8 June 2015	4,900
		29 June 2015	5,600
		16 July 2015	5,800
Caddo Lake	Bonham's Arm	5 May 2015	7,700
		8 June 2015	4,750
		29 June 2015	5,200
		16 July 2015	5,200
Saline Lake	Canal Lake Boat Road	5 May 2015	8,700
		8 June 2015	9,050
		29 June 2015	5,000
		16 July 2015	5,800
Turkey Creek Lake	Fence Blind	5 May 2015	7,800
		8 June 2015	7,600
		29 June 2015	4,600
		16 July 2015	6,400

Broad giant salvinia nitrogen content has been recorded at field sites ranging from 0.6 to 4.0% dry wt (Room and Thomas 1986), and it has been estimated that a minimum of 0.9% dry wt plant nitrogen is required for growth of giant salvinia (Room et al. 1985). Room (1986b) estimated the maximum nitrogen uptake by giant salvinia, based on growth rates, to be approximately 0.8 mg g^{-1} dry wt giant salvinia per day.

In 2012, researchers at the U.S. Army Engineer Research and Development Center, Environmental Laboratory, Lewisville, TX, initiated a 6-yr giant salvinia biological control project in select reservoirs in northern and central Louisiana. Although similar weevil quantities were released at all sites in 2012, monitoring revealed that weevil densities were highly variable among sites by the end of the growing season. Because of that high variability, a study was undertaken to determine whether variation in plant nitrogen content among sites could explain differences in adult and larval salvinia weevil density. This article describes the investigation into the correlation between plant nitrogen content and salvinia weevil density.

MATERIALS AND METHODS

Salvinia weevil rearing, harvesting, and field releases were conducted by the U.S. Army Engineer Research and Development Center Lewisville, TX, following methods described in Nachtrieb (2012). Salvinia weevil releases were conducted by collection and release of weevil-laden giant salvinia plants from outdoor rearing containers in Lewisville, TX. Release quantities reflect rearing culture density estimates and are tallied as total salvinia weevils, i.e., adult and larval estimates combined. Weevils were released onto giant salvinia infestations at multiple sites in northern and central Louisiana during the growing seasons of 2013 and 2015 (Table 1; Figure 1). When possible, the same release site was used both years. Four release sites were included each year for a total of six unique sites overall. Weevils were released multiple times each year at each site. In 2013, each site received approximately 50,000 weevils during three releases that occurred from late April through June 2013 (Table 1). In 2015, each site received approximately 23,000 to 29,000 weevils during four release events from early May through July 2015 (Table 1).

Salvinia weevil densities and giant salvinia nitrogen content were monitored approximately every 4 wk from May through August 2013 and every 5 wk from June through October 2015 at each site. At each sampling date, a boat was driven throughout the entire site and five 750 to 1,000-g (wet wt) samples of giant salvinia were randomly collected and placed into 3.78-L (1-gallon nominal size) plastic bags. Samples were returned to the laboratory and placed separately into a Berlese funnel for approximately 48 h for adult and larval weevil extraction into 70% ethanol. Berlese funnels were constructed of galvanized sheet metal (30.5-cm diam) with an inside platform constructed of 0.64 cm (one-quarter inch nominal size) mesh poultry netting located 27 cm from the top. A light fixture with a single 60-W incandescent bulb was placed on top of the funnel and a mason jar (pint or quart capacity) filled with 70% ethanol was attached to the funnel below the platform to collect and preserve extracted salvinia weevils. After 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 wt.

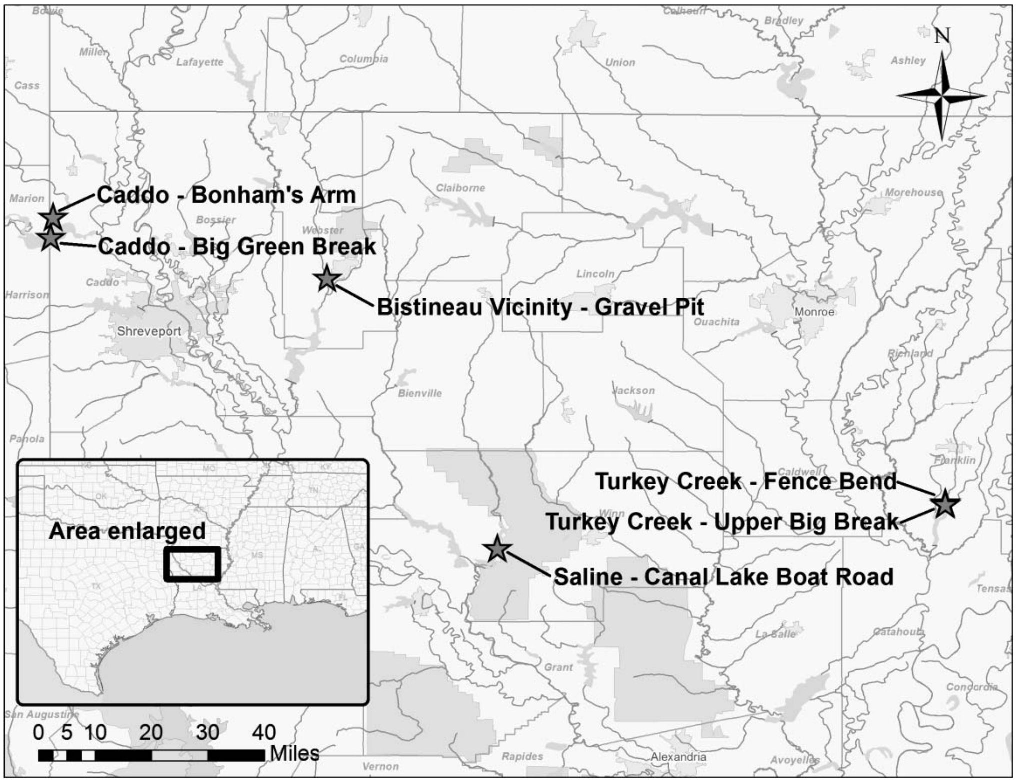

Figure 1. Map of northern and central Louisiana survey sites.

Salvinia weevils extracted into mason jars were sorted and identified through a dissecting microscope to quantify adult and larval weevils kg^{-1} dry wt of giant salvinia. Dried, floating fronds were separated from submersed fronds (i.e., roots), and floating fronds were mailed to the Pennsylvania State University Agricultural Analytical Services Laboratory (University Park, PA) and analyzed by combustion method with an Elementar Vario Max C/N Analyzer[1] to determine the percent of dry wt total nitrogen content.

Statistical analyses

Data were analyzed using Statistica[2] (version 12) software and included ANOVA, Student–Newman–Keuls or Tukey's multiple comparison/range tests, and Pearson product-moment correlation analyses. A separate two-way 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 predictors 1) sampling site, and 2) sampling date. Because not all sites were sampled during both years of data collection, a separate two-way ANOVA was conducted for each year of data collection. Correlation analysis was used to examine the relationship between plant nitrogen and 1) adult weevil densities yr^{-1}, and 2) larval weevil densities yr^{-1} using data averaged by site and sampling date. Statements of significance made throughout text refer to $\alpha < 0.05$. To meet parametric assumptions of normality, nitrogen data were log transformed.

RESULTS AND DISCUSSION

During 2013, significant differences were detected for adult weevil densities among sites ($P < 0.001$) and sampling dates ($P < 0.001$), but no interaction ($P = 0.120$) between sampling site and date was detected (Figures 2a and 2b). Average adult weevil density at Upper Big Break (Turkey Creek Lake) was 1,361 adult weevils kg^{-1}, which was, on average, 6-fold greater than all other sites (Figure 2a). Average adult weevil densities at Canal Lake Boat Road (Saline Lake), Big Green Break (Caddo Lake), and Gravel Pit (Lake Bistineau vicinity) were not significantly different and ranged from 162 to 318 adult weevils kg^{-1} (Figure 2a). Adult weevil densities increased over time, possibly reflecting a combination of multiple weevil releases and reproduction at field sites (Figure 2b). Adult weevil densities collected in July and August were significantly greater than those collected in May and June (Figure 2b).

Larval weevil densities differed significantly among sites ($P < 0.001$), but not sampling dates ($P = 0.264$) in 2013 (Figures 3a and 3b). Differences among sites followed the same trend as adult weevil densities. Upper Big Break had, on average, 10-fold greater larval weevil densities than the remaining three sites (Figure 3a). For the remaining three sites, mean larval weevil densities ranged from 116 to 480 larvae kg^{-1} with no significant difference among sites. It is important to note that each site received approximately the same quantity of weevils during three consecutive releases, yet Upper Big Break had significantly greater adult and larval weevil densities than the other sites had.

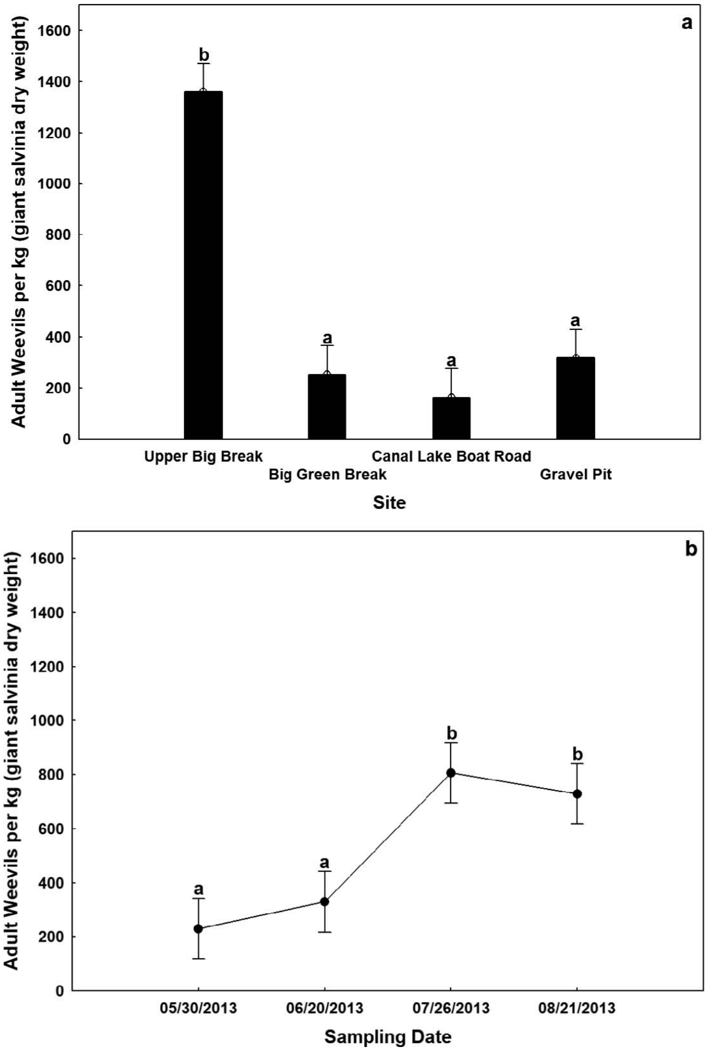

Figure 2. Mean [± standard error (SE)] of adult weevils kg^{-1} (giant salvinia dry wt) per site (A) and per sampling date (B) in 2013. Means with the same letter are not significantly different according to Student–Newman–Keuls test ($\alpha = 0.05$, $n = 5$). Two-way ANOVA, site: $P < 0.001$, $F = 25.212$; sampling date: $P < 0.001$, $F = 6.555$, interaction: $P = 0.120$, $F = 1.649$.

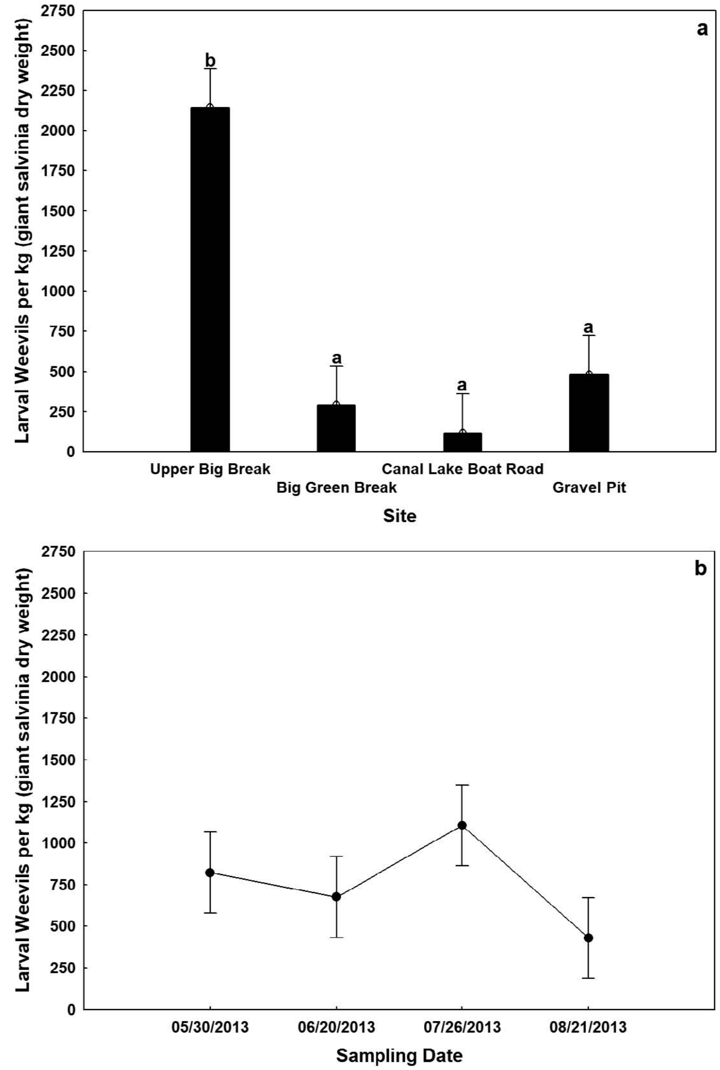

Figure 3. Mean [± standard error (SE)] larval weevils kg^{-1} (giant salvinia dry wt) per site (A) and per sampling date (B) in 2013. Means with the same letter are not significantly different according to Student–Newman–Keuls test ($\alpha = 0.05$, $n = 5$). Two-way ANOVA, site: $P < 0.001$, $F = 14.839$; sampling date: $P = 0.264$, $F = 1.358$, interaction: $P = 0.319$, $F = 1.186$.

A significant interaction ($P < 0.001$) was detected between site and sampling date for plant nitrogen content in 2013 (Figure 4). Plants collected at Upper Big Break had significantly greater nitrogen content than other sites had during May, June, and July (2.28 to 3.19% dry wt N) and all but one site (Big Green Break) in August (Figure 4). Samples from Canal Lake Boat Road had less nitrogen content than other sites during May and June (1.02 to 1.05% dry wt N) and were significantly less than all sites, except one (Gravel Pit) in July. During May and June, nitrogen content was significantly different among the sampling sites. Differences decreased after the June 2013 sampling date because nitrogen content decreased at Upper Big Break and increased at Canal Lake Boat Road.

When trends were examined from May through August 2013 for each site individually, nitrogen content remained mostly stable and unchanged (Figure 4). Nitrogen content at Big Green Break and Gravel Pit did not change from May through August 2013. At Upper Big Break, other than experiencing a peak in June, nitrogen content did not significantly differ between May, July, and August. Plant nitrogen content increased in July and August at Canal Lake Boat Road, but overall, was valued among the lowest of all sites.

The combined results of adult and larval weevil density and plant nitrogen content suggest a relationship between nitrogen and weevil population growth because plants with higher nitrogen content typically produced increased weevil densities. A correlation analysis was conducted to examine the association between weevil densities and nitrogen content at field sites. Adult and larval weevil density were significantly correlated to plant nitrogen content ($P < 0.05$, adult $r = 0.55$, larval $r = 0.63$). For data collected during the growing season of 2013, it was determined that both adult

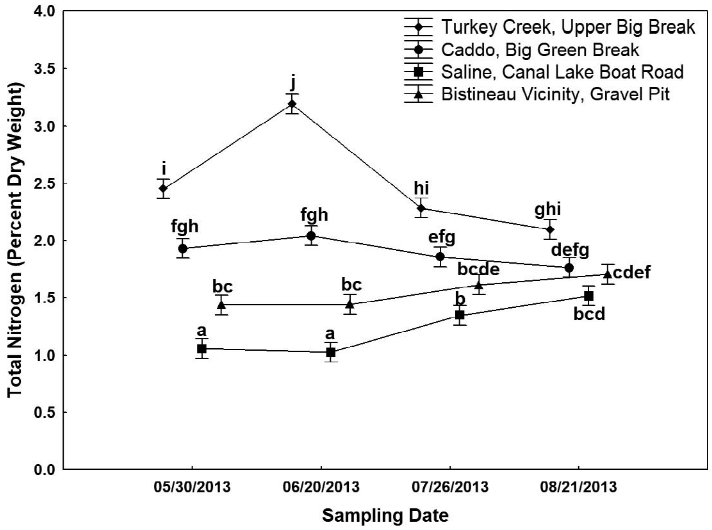

Figure 4. Mean [± standard error (SE)] plant nitrogen content (percentage of dry wt) per sampling date in 2013. Means with the same letter are not significantly different according to Student–Newman–Keuls test ($\alpha = 0.05$, $n = 5$). Two-way ANOVA, site: $P < 0.001$, $F = 174.662$; sampling date: $P = 0.097$, $F = 2.231$, interaction: $P < 0.001$, $F = 12.035$.

and larval weevil densities increased as nitrogen content increased.

During 2015, an ANOVA detected a significant interaction ($P < 0.001$) between site and sampling date for adult weevil densities (Figure 5a). Adult weevil densities were not significantly different among sites or dates during the beginning of the sampling period in June and July. That result was expected because weevil releases had just begun and limited successful salvinia weevil overwintering was detected from salvinia weevil releases during 2014. That trend continued throughout the sampling period for Canal Lake Boat Road, with no changes in adult weevil density and adult densities among the lowest detected from June through October (37 to 54 adults kg^{-1} dry wt). Adult weevil densities at Fence Blind (Turkey Creek Lake) were also low for most of the sampling period and did not significantly increase until the final sample collection in October. Weevil densities at the two Caddo sites (Bonham's Arm and Big Green Break) reacted rapidly to weevil releases and displayed significantly greater adult weevil densities in both August and October (921 to 1492 adult weevils kg^{-1} dry wt). At the last sampling date in October, adult weevil densities at the two Caddo sites were, on average, 1.5- and 36.0-fold greater than at Fence Blind and Canal Lake Boat Road, respectively.

ANOVA also detected a significant interaction ($P < 0.001$) between site and sampling date for larval weevil densities in 2015 (Figure 5b). At the first sample in June, all sites contained low larval densities and were not significantly different. Larval densities at Canal Lake Boat Road followed the same trend as adult weevil densities, and despite multiple weevil releases, there was no statistical change in larval densities from June to October (5 to 138 larvae kg^{-1} dry wt). For all other sites (Big Green Break, Bonham's Arm, and Fence Blind), larval weevil densities increased from June to July at similar rates and were not

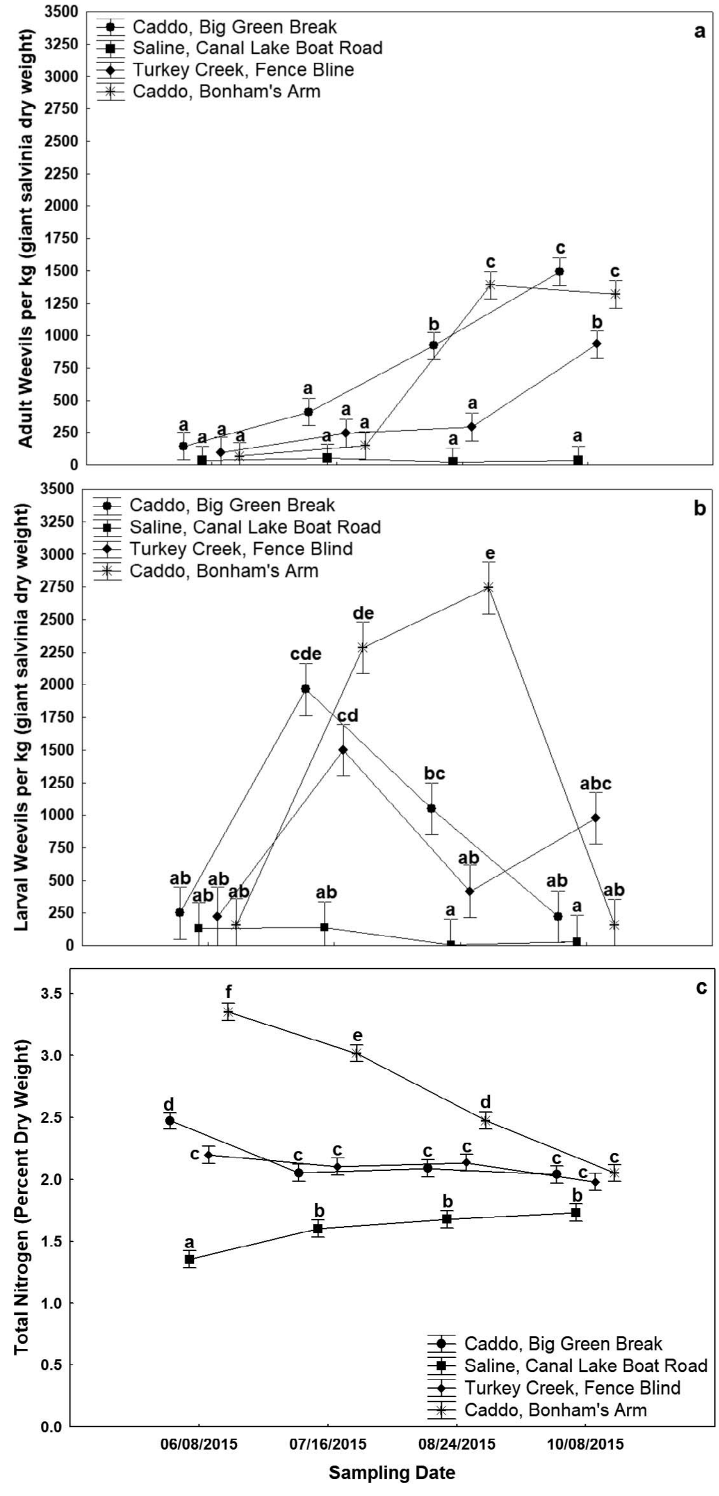

Figure 5. Mean [± standard error (SE)] adult weevils kg^{-1} (giant salvinia dry wt) (A), larval weevils kg^{-1} (giant salvinia dry wt) (B), and plant nitrogen content (percentage of dry wt) (C) per sampling date in 2015. Means with the same letter are not significantly different ($\alpha = 0.05$, $n = 5$, adult weevil density and plant nitrogen content by Student–Newman–Keuls; larval weevil density by Tukey's range test). Two-way ANOVA adult weevil density (A), site: $P < 0.001$, $F = 39.285$; sampling date: $P < 0.001$, $F = 54.422$, interaction: $P < 0.001$, $F = 11.074$. Two-way ANOVA larval weevil density (B), site: $P < 0.001$, $F = 27.235$; sampling date: $P < 0.001$, $F = 35.846$, interaction: $P < 0.001$, $F = 12.213$. Two-way ANOVA plant nitrogen content (C), site: $P < 0.001$, $F = 194.689$; sampling date: $P < 0.001$, $F = 13.628$, interaction: $P < 0.001$, $F = 19.286$.

different. Larval weevil densities decreased from July to August at both Fence Blind and Big Green Break, and consequently, Fence Blind was not significantly different than larval densities recorded at the site with the lowest weevil densities (Canal Lake Boat Road). Larval densities at Bonham's Arm continued to increase from July to August and peaked at 2,742 larvae kg^{-1}. When sampled in October, larval weevil densities were not different among any sites.

A significant interaction ($P < 0.001$) between site and sampling date was also detected for plant nitrogen content in 2015 (Figure 5c). In general, Fence Blind, Canal Lake Boat Road, and Big Green Break followed trends similar to 2013, with nitrogen content not changing much at individual sites throughout the sampling period (Figure 4 and 5c). In contrast to that trend, nitrogen content at Bonham's Arm significantly decreased throughout the sampling period (Figure 5c). Nitrogen content at Canal Lake Boat Road was significantly lower than all other sites and ranged from 1.35% to 1.73% dry wt in June and October, respectively. Nitrogen content at Fence Blind did not change throughout the sampling period and remained at a midrange of 1.98 to 2.20% dry wt. Big Green Break also contained midrange nitrogen content throughout the sampling period of 2.04 to 2.47% dry wt, which was significantly greater than that at Fence Blind only in June. Nitrogen content at Bonham's Arm significantly decreased throughout the sampling period from 3.35% to 2.05% dry wt but was significantly greater than all other sites from June through August.

Despite some similarities between data collected in 2013 and 2015, a significant correlation was not obtained for adult or larval weevil densities and nitrogen content in 2015 ($P < 0.05$, adult $r = 0.10$, larval $r = 0.41$). Several data trends could have contributed to the inability to detect a significant correlation. At Bonham's Arm, nitrogen content decreased throughout the season as weevil density increased (Figures 5a–c). Additionally, although nitrogen content was statistically different among high (Bonham's Arm), low (Canal Lake Boat Road), and midrange (Big Green Break and Fence Blind) sites for most of the sampling period, adult and larval weevil densities greatly overlapped among sites and were not statistically different regardless of nitrogen content (Figures 5a and 5b).

Despite the absence of consistent results between the 2 yr of this study, general trends in giant salvinia nitrogen content and its affect on salvinia weevil density can be identified. During 2013, giant salvinia at Upper Big Break contained greater nitrogen content than all other sites during May, June, and July and more than all but one site during August. Differences among sites were the greatest in June, with Upper Big Break containing 2.17% more dry wt nitrogen than the lowest site, Canal Lake Boat Road. Consequently, Upper Big Break had 6-fold greater adult and 10-fold greater larval salvinia weevil densities. That cause and effect of increased plant nitrogen content resulting in greater weevil densities was consistent with the findings of Sands et al. (1986), in which a 0.5 increase in the percentage of dry wt nitrogen increased salvinia weevil growth rate by 15%.

Results at Canal Lake Boat Road provide evidence of the opposite effect of reduced plant nitrogen content resulting in low salvinia weevil densities. At Canal Lake Boat Road, plant nitrogen content was lower than all other sites during all sampling dates during 2015. Increased nitrogen contents at Big Green Break, Fence Blind, and Bonham's Arm ranged from 0.31 to 2.0% more dry wt nitrogen than found at Canal Lake Boat Road. Furthermore, despite repeated salvinia weevil introductions, adult and larval weevil densities did not change over time at that site. At all other sites, adult weevil densities increased over time, and larval densities experienced an increase from June to July. Although various other unknown factors could have led to reduced salvinia weevil density at Canal Lake Boat Road, results suggest nitrogen may have been a limiting factor.

Finally, at most individual sites, nitrogen content varied little over time. Field sites typically contained plants in the tertiary growth stage that covered 100% of the water surface for the duration of the study. Available nitrogen was likely used by the dense plant stand to maintain plant growth and replenish used nitrogen, thus limited changes in plant nitrogen content were detected. If that trend is typical in water bodies with vast giant salvinia infestations, future plant nitrogen sampling methods can be substantially reduced in frequency.

Several research projects are currently being undertaken to increase understanding of the effects of nitrogen and temperature on salvinia weevil density at field sites. At Saline Lake, LA, a controlled and replicated study was initiated to monitor the effect of nitrogen amendments to salvinia weevil density after a single salvinia weevil release occurrence at enclosed field plots (J. G. Nachtrieb et al., unpub. data). By standardizing salvinia weevil density and manipulating plant nitrogen, this study aimed at specifically determining the direct effect of plant nitrogen content on salvinia weevil success at field sites. An additional study is being conducted at 13 enclosed field plots across Louisiana to monitor plant nitrogen, salvinia weevil density, and temperature year-round. That study will monitor the relationship between plant nutrition and weevil density and will evaluate the weevil's ability to overwinter. Through these projects, we expect a better understanding of the environmental parameters affecting weevil establishment in the southern United States.

SOURCES OF MATERIALS

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

[2]Statistica software, version 12, StatSoft, Inc., 2300 E. 14th Street, Tulsa, OK 74104.

ACKNOWLEDGEMENTS

This research was conducted as a joint effort among the Louisiana Department of Wildlife and Fisheries and the U.S. Army Corps of Enginners Aquatic Plant Control Research Program. Permission to publish was granted by the Chief of Engineers. The author thanks Dr. Gary Dick and Nathan Harms for review of the paper and Chetta Owens, Hunter Zmolik, Jonathon Ward, Daniel Markbreiter, William Finkbeiner, Wesley Maddox, Alexander Perret, Jonathan Winslow, and Blake LeBlanc for technical assistance.

LITERATURE CITED

Forno IW. 1985. How quickly can insects control salvinia in the tropics? In: Proceedings of the 10th Asian-Pacific Weed Science Society Conference. Chiangmai, Thailand, November 1985. Mass & Media Co., Bangkok, Thailand

Forno IW. 1987. Biological control of the floating fern Salvinia molesta on north-eastern Australia: plant herbivore interactions. Bull. Entomol. Res. 77:9–17.

Forno IW, Semple JL. 1987. Response of *Salvinia molesta* to insect damage: changes in nitrogen, phosphorus and potassium content. Oecologia (Berlin) 73:71–74.

Julien MH, Griffiths MW. 1998. Biological control of weeds. A world catalogue of agents and their target weeds. 4th ed. CABI Publishing, Wallingford, UK.

Julien MH, Hill MP, Tipping PW. 2009. *Salvinia molesta* D.S. Mitchell (Salviniaceae), pp 378–407 In: R. Muniappan, G. V. P. Reddy, and A. Raman (*eds.*). Biological control of tropical weeds using arthropods. Cambridge University Press, Cambridge, UK.

Moran N, Hamilton WD. 1980. Low nutritive quality as defense against herbivores. J. Theor. Biol. 86:247–254.

Mukherjee A, Knutson A, Hahn DA, Heinz KM. 2014. Biological control of giant salvinia (*Salvinia molesta*) in temperature region: cold tolerance and low temperature oviposition of *Cyrtobagous salviniae*. Biocontrol 59:781–790.

Nachtrieb JG. 2012. Rearing the salvinia weevil for biological control of giant salvinia at the US Army Corps of Engineers Lewisville Aquatic Ecosystem Research Facility, pp.13–24. In: A. Knutson and J. G. Nachtrieb (*eds.*). A guide to mass rearing the salvinia weevil for biological control of giant salvinia. ESP-475, Texas A&M AgriLife Extension Service Special Publication, College Station, TX.

Room PM. 1986a. Biological control is solving the world's *Salvinia molesta* problems, pp. 271–276 In: Proceedings of the 7th European Weed Research Society/Association of Applied Biologists Symposium on Aquatic Weeds, Loughborough, UK. Doorwerth, Netherlands.

Room P M. 1986b. Equations relating growth and uptake of nitrogen by *Salvinia molesta* to temperature and the availability of nitrogen. Aquat. Bot. 24:43–59.

Room PM, Sands DPA, Forno IW, Taylor MFJ, Julien MH. 1985. A summary of research into biological control of salvinia in Australia. Proc. VI Int. Symp. Biol. Contr. Weeds 19-25 August 1984, Vancouver, Canada, Delfosse, ES (ed.) Agric. Can. pp. 543–9 (1985).

Room PM, Thomas PA. 1985. Nitrogen and establishment of a beetle for biological control of the floating weed salvinia in Papua New Guinea. J. Appl. Ecol. 22:139–156.

Room PM, Thomas PA. 1986. Nitrogen, phosphorus and potassium in *Salvinia molesta* Mitchell in the field: effects of weather, insect damage, fertilizers and age. Aquat. Bot. 24:213–232.

Sands, DPA, Schotz M, Bourne AS. 1983. The feeding characteristics and development of larvae of a salvinia weevil *Cyrtobagous sp*. Entomol. Exp. Appl. 34:291–296.

Sands, DPA, Schotz M, Bourne AS. 1986. A comparative study on the intrinsic rates of increase of *Cyrtobagous singularis* and *C. salviniae* on the water weed *Salvinia molesta*. Entomol. Exp. Appl. 42: 231–237.

Sullivan PR, Postle LA. 2010. Low temperature reproduction of *Cyrtobagous salviniae*: Good news for biological control of salvinia in temperate climate. J. Aquat. Plant Manage. 48:92–96.

Thomas, PA, Room PM. 1985. Towards biological control of salvinia in Papua New Guinea, pp. 567–574. In: E. S. Delfosse (*ed.*). Proceedings of the VI International Symposium on the Biological Control of Weeds, Vancouver, Canada, 19–25 August 1984. University of British Columbia Press, Vancouver, Canada.

Thomas PA, Room PM. 1986. Taxonomy and control of *Salvinia molesta*. Nature (London) 320:581–584.

Tipping, PW. 2004. Giant salvinia, pp. 174–177. In: E. M. Coombs, J. K. Clark, G. L. Piper, A. and F. Cofrancesco Jr. (*eds.*). Biological control of invasive plants in the United States. Oregon State University Press, Corvallis, OR.

U.S. Geological Survey. 2016. Nonindigenous aquatic species database. Gainesville, FL. Accessed August 10, 2018.