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Note

Biological control of *Salvinia molesta*: Population dynamics of *Cyrtobagous salviniae* in Lake B. A. Steinhagen

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INTRODUCTION

The floating fern, *Salvinia molesta* D. S. Mitchell (Salviniaceae) is an important aquatic weed worldwide and is an invasive weed in the tropical and subtropical regions of Africa, Asia, North America, South America, and Oceania (Julien et al. 2009). Biological control of *S. molesta* with the use of *Cyrtobagous salviniae* Calder and Sands (Coleoptera: Curculionidae) provides the most effective and inexpensive control of *S. molesta* (Chikwenhere and Keswani 1997; Julien et al. 2009) in many situations. This semiaquatic weevil was collected from Brazil and was first released in Australia in 1980 (Room et al. 1981). *Cyrtobagous salviniae* has provided spectacular control in Australia (Julien et al. 2012), and to date has been released in 20 countries worldwide, where it achieved successful biological control of *S. molesta* in tropical and subtropical regions (Julien et al. 2009).

Salvinia molesta has been reported from 12 states in the United States and is well established in Texas (Jacono et al. 2001; EDDMaps 2011). Biological control of *S. molesta* in the United States was initiated in 2001 with *C. salviniae* weevils originating from Australia introduced into southeastern Texas and southern Louisiana (Tipping and Center 2003). These introductions successfully managed the *S. molesta* infestations in these areas (Flores and Carlson 2006; Tipping et al. 2008). Following these successes, a facility to mass rear *C. salviniae* was established by the U.S. Army Corps of Engineers in Lewisville, Texas (Nachtrieb 2012). *Cyrtobagous salviniae* has also been mass reared by Louisiana State University in ponds in southern Louisiana and released throughout the state (Sanders 2011). In 2009, an estimated one million weevils were reared and released by this program into several lakes in northeastern Louisiana. However, weevils apparently failed to overwinter at these sites, as none were recovered the following spring. Similarly, efforts to establish *C. salviniae* at Lake Caddo in northeastern Texas have not been successful. Caged populations of *C. salviniae* monitored at Lake Caddo during the winters of 2010 to 2011 and 2013 to 2014 suffered 100% mortality, indicating that overwintering mortality was limiting successful establishment at this site (Obeysekara et al. 2015). These observations suggest a lack of cold tolerance of *C. salviniae* might be an important limiting factor for biological control success in temperate locales of Texas and Louisiana. However, very little is known about the overwintering biology of *C. salviniae* in these temperate regions of the United States. To better understand how cold could influence reproduction and survival, we investigated the seasonal population dynamics of *C. salviniae* from November 2011 to February 2013 at Lake B. A. Steinhagen in east Texas.

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MATERIALS AND METHODS

Population dynamics of *C. salviniae* in the field were examined at Lake B. A. Steinhagen, Jasper, TX (30°52′32′′N, 94°13′15′′W). The experimental site was flooded woodland dominated by cypress trees and an extensive mat of *S. molesta* covered the water surface during most of the summer. Texas Parks and Wildlife had released adult *C. salviniae* at this site from 2010 to 2011 (Wythe 2012). This site was chosen because it holds water throughout the year, remains undisturbed form the boat traffic, have never been sprayed with herbicides and have an overwintering population of *C. salviniae*. Densities of *C. salviniae* eggs, larvae, and adults were determined by collecting 15 samples of *S. molesta* plants at 4-wk intervals from November 2011 to February 2013. Each sample was spaced 5 m apart along a transect, with each sampling site marked by a PVC pipe vertically inserted in the bottom of the lake. During sampling, a 1-m^2 PVC frame was placed on the water surface around the vertical PVC pipe. Following Tipping et al. (2008), the area inside the 1-m^2 frame covered by *S. molesta* mat was visually estimated to nearest 10%. The percent of plants that had developed brown coloration also was visually estimated to nearest 10%. All *S. molesta* plants within two 0.1-m^2 quadrates, placed inside the larger 1-m^2 frame at random, were collected to estimate biomass (Tipping et al. 2008). To estimate *C. salviniae* population density, 1-kg samples of

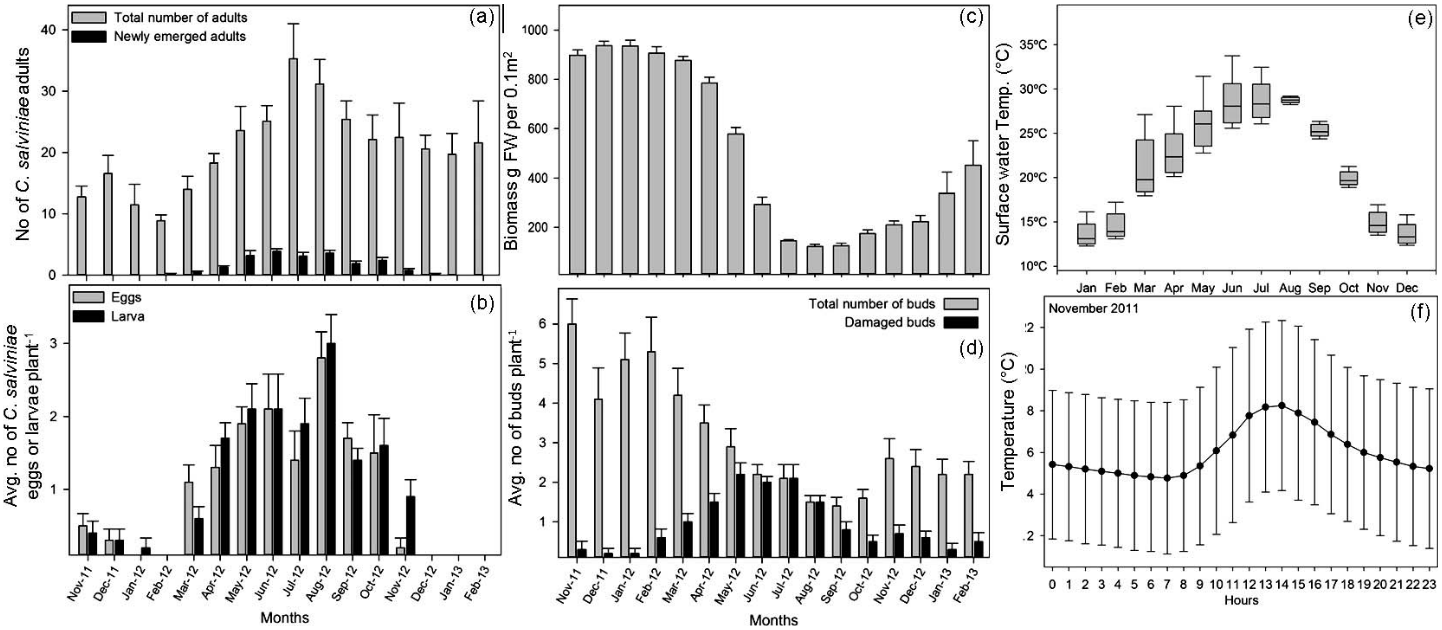

Figure 1. *Cyrtobagous salvinia* population dynamics and surface water temperature observed (mean ± SE) in Lake B. A. Steinhagen from November 2011 through February 2013. (a) Density of *C. salviniae* adults and newly emerged adults kg^{-1} fresh weight of *Salvinia molesta*. (b) Density of *C. salviniae* eggs and larvae plant^{-1} observed. (c) Biomass of *S. molesta* (g fresh weight per 0.1 m^2). (d) Density of healthy buds and buds plant^{-1} damaged by *C. salviniae* feeding. (e) Box plot for each month show average hourly surface water temperature measured across the month. The middle bar = median, box = interquartile range (25th to 75th percentile), whiskers (error bars) above and below the box represent the 90th and 10th percentiles. The reference line shows the threshold oviposition temperature of *C. salviniae* (19 C). (f) Profile of hourly surface water temperature (mean ± SD) recorded during November 2011 in Lake B. A. Steinhagen.

fresh *S. molesta* plants were collected from each sampling frame and placed inside Ziploc brand plastic bags.[1] In addition, 10 *S. molesta* plants (a plant is defined as having one apical bud with preceding five ramets) also were collected from the frame area and placed inside a Ziploc bag. The Ziploc bags were immediately placed inside coolers and brought back to the laboratory and processed within 48 h of collection. Adult *C. salviniae* were extracted from each 1 kg *S. molesta* sample with the use of Berlese funnels and the number of adult weevils and newly emerged adults, having a light brown cuticle color, were counted. Each plant from the sample of 10 plants was dissected under a microscope and total number of buds, number of damaged buds, number of *C. salviniae* eggs and larvae per plant were counted. The daily water temperature profile of the study site was documented by a HOBO® temperature logger[2] floating within 2 cm of the water surface within a *S. molesta* mat. Temperature was measured at 1-h intervals throughout the study period.

RESULTS AND DISCUSSION

Population density of *C. salviniae*

At the start of the experiment in November 2011, adult *C. salviniae* density (mean ± SE) was 12.8 ± 1.7 kg^{-1} fresh weight (FW) of *S. molesta*, and by the following February 2012, weevil density had slightly decreased to 8.9 ± 0.9 adults kg^{-1} FW) (Figure 1a). Adult density then consistently increased from March to July, with highest density (35.3 ± 5.7 adults kg^{-1} FW) observed in July 2012. Adult density then slightly decreased and remained at ca. 20 adults kg^{-1} FW through February 2013. Newly emerged *C. salviniae* adults were first observed in the field during February 2012. No newly emerged adults were recorded during November 2011 and January 2012 and during January and February 2013 (Figure 1a).

Density of *C. salviniae* eggs and larvae

Average density of *C. salviniae* eggs and larvae plant^{-1} was low (< 1 egg or larva plant^{-1}) during November and December 2011 (Figure 1b). No eggs were found during January and February in 2012 and none were found during December through February in 2013. Larvae were not present in samples collected in February 2012 and in December through February 2013. As observed for eggs, larval density was highest during August 2012 (3 ± 0.4 larvae plant^{-1}).

Salvinia molesta biomass

Salvinia molesta biomass declined steadily from March to August, 2012 and in August biomass averaged 122.5 ± 9.9 g FW per 0.1 m^2. Biomass then increased during the fall and winter and averaged 451.9 ± 99.3 g FW per 0.1 m^2 in February 2013 (Figure 1c).

Total and damaged buds per *S. molesta* plants

Average number of buds plant^{-1} was highest at the start of the experiment in November 2011 (6 ± 0.6 buds plant^{-1}) (Figure 1d). Bud density decreased substantially during summer of 2012 with lowest density observed during September 2012 (1.4 ± 0.2 buds plant^{-1}). However, number of buds plant^{-1} damaged by *C. salviniae* consistently increased during July and August 2012, at which time almost all the buds were damaged by *C. salviniae* (Figure 1d).

These results demonstrate that *C. salviniae* overwintered at the study location and that although small numbers of eggs and larvae were present late into the winter, only the adult stage persisted throughout the winter. Also, reproduction ceased during the winter, as no eggs were present during January and February and no recently eclosed adults were present in January. Increasing densities of *C. salviniae* eggs and larvae were associated with an increase in bud damage and decline in *S. molesta* biomass, documenting the efficacy of biological control during the summer. *Salvinia molesta* biomass increased during the winter when eggs and larvae densities were lowest, suggesting that *S. molesta* continued to grow during this winter at this location (Figure 1c). However, it is important to note that no weevils survived in cage studies at Caddo Lake, located ca. 225 km north of Lake B. A. Steinhagen, during winters of 2010 to 2011 and 2013 to 2014. These high rates of adult mortality were attributed to repeated cold exposure because of the multiple cold fronts that occurred during these winters at Caddo Lake (see Table 1 of Obeysekara et al. 2015 for summary of water temperature data). These results have important connotation for our study as well. Our study duration coincided with a relatively mild winter measured in terms of average number of days when water temperature stayed below 4 C (Obeysekara et al. 2015). Although we recorded high weevil population density during our field study, in seasons with colder winter temperature, weevil population could decrease as a result of cold stress. Following these cold winters, inoculative releases of *C. salviniae* must be done to reestablish the insect, which will also increase the resources necessary for the biological control program.

The inability of *C. salviniae* to oviposit below 19 C water temperature is an important limiting factor for biological control of *S. molesta* in temperate locals (Forno et al. 1983). Larvae also fail to develop at water temperatures below 17 C (Sands et al. 1983). Average hourly surface water temperature recorded from Lake B. A. Steinhagen was well below 19 C during 4 mo of winter (Figure 1e). Although no eggs and larvae were collected during winter months (December through February) of 2012 to 2013, some eggs were collected in November and December 2011 and larvae were also collected during November 2011 to January 2012 (Figure 1b). These results suggest that adults oviposit during the short period of time when water temperatures exceed 19 C on warm days in November, as shown for the mean hourly surface water temperature at Lake B. A. Steinhagen during November 2011 (Figure 1f).

To overcome the lack of long-term effectiveness of *C. salviniae* because of high winter mortality and limited oviposition because of cold water, integrating targeted herbicide applications with biological control could potentially achieve greater levels of control of *S. molesta* in the temperate locals of Texas and Louisiana (Mudge and Harms 2012). Numerous studies have demonstrated that such an approach can improve management of invasive weeds (for example, Lym 2005; Paynter 2003). In the case of *S. molesta*, Mudge et al. (2013) reported that flumioxazin (a quick-acting contact herbicides commonly used against *S. molesta*) mixed with nonionic surfactant (with buffering agent blend) showed minimal toxicity on *C. salviniae* adults. They also showed that adult weevils can survive in flumioxazin-treated *S. molesta* plants for more than 4 wk (Mudge et al. 2013). As these authors stated, further field and mesocosm studies will be necessary to understand the suitability of this integrated control strategy better.

SOURCES OF MATERIALS

[1]Ziploc® brand plastic bags, S.C. Johnson & Son, Racine, WI.

[2]Hobo pendent loggers, Onset Computer, Bourne, MA.

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