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Release and evaluation of *Cyrtobagous salviniae* on common salvinia in southern Louisiana

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ABSTRACT

Common salvinia (*Salvinia minima*) is one of the most widespread, non-native invasive species at the Barataria Preserve of Jean Lafitte National Historical Park and Preserve in southern Louisiana and currently infests more than 3,600 ha and 48 km of navigable waterways. A proven biological control agent, the salvinia weevil (*Cyrtobagous salviniae* Calder and Sands), was field collected on *S. minima* in Florida, checked for pathogens, and then released in the Preserve from June 2002 through June 2005. Few weevils were recovered from heavily shaded sites when compared to full sun sites where weevils were recovered frequently. Plant biomass and percent cover were reduced only in full sun sites. A field nursery site was established at an undisturbed location in Twin Canals in June 2003 and stocked with 2,421 weevils, released periodically until August 2005. Although weevils, including teneral adults, were recovered at all but one release site, population establishment was not confirmed during this period because of multiple perturbations, culminating in Hurricane Katrina, which caused large scale saltwater intrusion which temporarily destroyed all the sites near the end of the project. However, recent weevil detections by other researchers suggest that the weevils did establish in the area. Any future research efforts in this region should focus on identifying full sun, undisturbed sites to expedite establishment and evaluate impact.

Key words: *Salvinia minima*, *Cyrtobagous salviniae*, biological control, aquatic weeds.

INTRODUCTION

Non-native, floating aquatic plants are a significant threat to the wetland habitats that comprise the Barataria Preserve of Jean Lafitte National Historical Park and Preserve in southern Louisiana. The 7,400 ha Preserve, which is predominantly (80%) wetlands, encompasses a myriad of diverse aquatic habitats including globally unique freshwater floating marshes, native bottomland hardwood swamps, and more than 48 km of natural bayous and waterways. Common salvinia, *Salvinia minima* Baker, is an exotic floating fern that colonizes lentic or slow moving, fresh water bodies like ponds, lake, swamps, and marshes and is one of the most widespread exotic plant species found within the Preserve, infesting more than 3,600 ha. The native range of this species encompasses Central and South America (Stoltze 1983, Mickel and Beitel 1988, Palacios-Rios and Cortes 1990) and, besides North America, it is adventive in Bermuda (Weatherby 1937), Puerto Rico (Proctor 1989), and Spain (Lawalree 1964). Jacono et al. (2001) documented multiple introductions of this species in North America where it now occurs in at least 7 states and 69 drainages. Madeira et al. (2003) analyzed the genetic variation of *S. minima* and found relatively close similarity among North American populations with the exception of a recent introduction in Mississippi.

Salvinia minima is present in all Preserve waterways and dominates throughout most of bald cypress-water tupelo swamps and freshwater ponds where it displaces native aquatic species like duckweed (*Lemna* spp.), and may restrict the range of rare species such as the floating antler fern [*Ceratopteris pteridoides* (Hook.) Heiron.]. Persistent floating mats can also degrade water quality by reducing amounts of dissolved oxygen available for fish and aquatic organisms, providing harborages for disease-carrying organisms like mosquitoes, and shading out submerged aquatic vegetation (Thomas and Room 1986). During the long, subtropical growing season in southern Louisiana, mats of *S. minima* often become impenetrable by canoes and small boats, thereby impeding or eliminating recreational opportunities for the visiting public.

Like its larger relative giant salvinia, *Salvinia molesta* Mitchell, *S. minima* shows considerable phenotypic plasticity, mainly because of environmental conditions such as crowding and nutrient availability. Mitchell and Tur (1975) described three basic forms associated with degrees of crowding: 1) a small-leaved, primary form typical of plants invading open water, 2) a slightly larger-leaved secondary form with leaves slightly folded, and 3) a tertiary form typical of mature stands with larger, deeply folded, and densely packed leaves. These growth forms are dynamic and often comprise a continuum of growth forms within a

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population that change temporally and spatially in response to the local environment.

Although this species has become weedy in areas of the United States, especially Texas and Louisiana, *S. minima* appears less invasive in Florida where it is unable to form persistent mats (Tipping et al. 2012). Its less aggressive nature in Florida may be due to the presence of an ecotype of the specialized herbivore *Cyrtobagous salviniae* Calder and Sands (Coleoptera: Curculionidae) which is widely distributed throughout the state on *S. minima* (Jacono et al. 2001). This smaller-sized weevil was first reported in Florida in 1962 where it was probably introduced unintentionally with *S. minima* (Kissinger 1966). Another ecotype from Brazil, which is larger in size, has been used worldwide as an effective biological control agent for *S. molesta* (Room et al. 1981). Although originally there was a question about the identity of the Florida ecotype because of minor genetic differences with the Brazil ecotype (Goolsby et al. 2000), molecular work has confirmed that the two ecotypes are sufficiently similar so as to be considered one species (Madeira et al. 2006).

Our objectives were to establish a sustainable population of the Florida ecotype of *C. salviniae* within the Barataria Preserve and evaluate the impact of the weevil on areas infested with *S. minima*. The smaller Florida ecotype was chosen because it was readily available in Florida and is more damaging to *S. minima* than the larger Brazil ecotype (Tipping et al. 2010).

MATERIALS AND METHODS

A total of 9,670 *C. salviniae* adults were released into research sites from June 2002 through August 2005. Weevils were field collected on *S. minima* in Florida, checked for pathogens, and then released monthly in the Preserve. Insects were released as adults and the numbers released were dependent upon the supply in Florida, usually ranging from 50 to 100 weevils per site per month. Field study sites included flooded forests, ponds, bayous, canals, and floating marsh locations that were heavily infested with *S. minima*. Sites were the experimental unit and were randomly assigned as either release sites where insects were released, or control sites with no insect releases. A total of five control sites and five release sites were located at least 500 m apart to minimize the likelihood of insect dispersal into control sites (Table 1). The sites located in flooded forests were heavily shaded whereas pond and marsh sites were located in full sun. A separate, non-research site was created as a field nursery in the full-sun Twin Canal area and received multiple releases of weevils totaling 2,421 individuals.

A reference location was created at each field site with a floating square of pvc pipe (7.6 cm diameter) which enclosed 1 m^2 of the resident *S. minima* population. This provided a permanent point for each sampling date and fixed a population of the plant in place for conducting *C. salviniae* releases. The frames were anchored with nylon rope tied to a cinderblock and were accessed by wading, except for the two open marsh sites (Marsh I, Marsh II) which required an airboat for access. The mean biomass of *S. minima* was estimated at each sample date within each site by collecting all live *S. minima* enclosed within four 0.1 m^2 pvc frames (quadrats) that were temporarily placed in a haphazard fashion at four directly adjacent locations in cardinal directions around the reference frame. Twenty-five terminal buds were examined from within each quadrat for damage from *C. salviniae* and rated as damaged or undamaged. Any weevils found during this examination were recorded and categorized as light or dark brown. Teneral adults remain light brown for a few days before becoming fully sclerotized and turning much darker and their presence is indicative of a reproducing population. Plants were lightly compressed to remove excess water and weighed to estimate fresh weight biomass. All plants and insects were then returned to the quadrats. These four samples were averaged to provide a mean for each variable for that site at that sample date.

Table 1. Locations used for establishment and evaluation of *Cyrtobagous salviniae* on *Salvinia minima* at Jean Lafitte National Historical Park and Preserve, June 2002 to June 2005.

Name	Location	Type	Description
V Levee	29°47′37″N 90°06′04″W	Release	Shaded, bayou
Bayou Coquille I	29°47′13″N 90°07′10″W	Release	Shaded, canal
Bayou Coquille II	29°47′31″N 90°07′37″W	Control	Shaded, canal
Wood Duck	29°47′13″N 90°07′10″W	Release	Full sun, pond
Wood Duck II	29°47′16″N 90°06′02″W	Control	Shaded, flooded forest
Palmetto I	29°46′59″N 90°07′09″W	Control	Shaded, flooded forest
Palmetto II	29°47′06″N 90°07′03″W	Control	Shaded, flooded forest
Visitors Center	29°47′00″N 90°06′56″W	Release	Shaded, flooded forest
Marsh I	29°48′48″N 90°07′58″W	Release	Full sun, floating marsh
Marsh II	29°47′53″N 90°08′56″W	Control	Full sun, floating marsh
Nursery Site	29°48′36″N 90°07′33″W	Release	Full sun, Twin Canals south

Two quadrats were also haphazardly placed within the reference frame and plants were processed as above to estimate biomass. Fifty terminal buds were examined in each quadrat for damage and insects. These two samples were averaged to provide a mean as listed above. Dry weight biomass was estimated assuming a plant tissue moisture level of 96% which was calculated in our earlier trials when plants were dried to a constant weight. All density measurements were normalized to 1 m^2 and insect counts and damage assessments were normalized to 100 terminal buds.

Sampling was conducted periodically during the spring, summer, and fall over a 3-year period from June 2002 to June 2005 for a total of 14 sample dates. The results indicated that a mature *S. minima* mat within a site tended to be relatively uniform in terms of biomass, so sampling without apparent bias over time at one general location was sufficient to characterize each site. No sampling was conducted during December, January, and February when biological activity was minimal because of low temperatures. Water conditions were recorded during each sampling event just below the surface near the reference frame using a variety of calibrated automated hand-held meters to measure temperature, dissolved oxygen, and pH. We estimated the percentage, to the nearest 10%, of *S. minima* coverage at the site and inside the reference frame by combining the visual estimates of least two observers. Brown coloration of mats has been associated with insect-damaged and weakened plants with *S. molesta* (Room et al. 1981), so a visual estimate of two observers was also made within the reference frame of the percentage of the *S. minima* mat that appeared green vs. brown, estimated to the nearest 25% within the ranges of 0, 1 to 24, 25 to 49, 50 to 74, 75 to 99, and 100%.

Linking bud damage assessments to only *C. salviniae* was problematic because of the presence of *Samea multiplicalis* (Guenée) (Lepidoptera: Crambidae) and *Synclita obliteralis* (Walker) (Lepidoptera: Crambidae), whose larva feed indiscriminately in the above-water portions of *S. minima* and damaged buds at all sites. We could not consistently distinguish the source of bud damage from among the three herbivores so we elected to tally all damaged buds. Photographic reference points were established and photographs taken to provide visual documentation of the sites for each sample date.

Repeated measures analysis of variance (PROC MIXED) was used to examine the effects of all combinations of year, insect releases, and shading on plant, insect, and water variables averaged over sample dates (SAS Institute 2004). Although the effect of year was significant for pH, the percentage of damaged buds, and plant biomass in the reference frames, there were no trends, no between-subject effect of year, no year interactions with other independent variables (insect releases and shading), nor any within-subject interaction of year with sample date. Sites were characterized as either being in full sun or with some degree of shading. Data were transformed when variances are heterogeneous using square root or arc-sine. Two sample *t*-tests were used to separate means between treatments.

RESULTS AND DISCUSSION

Although the Brazil ecotype of *C. salviniae* has been shown to overwinter in Texas and further north in Louisiana, we were unable to confirm overwintering of the Florida ecotype in this study (Tipping and Center 2003) (Figure 1). However, although we were not able to find weevils on our first trip in the spring of each year, we did recover weevils over multiple dates from the field nursery plus all the release sites site except Bayou Coquille I (Figure 1). Those recoveries were confounded by the possibility that they represented adults or progeny from previous releases in the same year. However, on separate dates we recovered a few teneral adults at the Marsh I and Wood Duck I sites in 2003 only indicating that the weevils had produced at least one new generation after release. Recent communications (July 2012) with other researchers have documented recoveries of weevils at some of the original release sites so the insects appear to have established and persisted, albeit at low levels

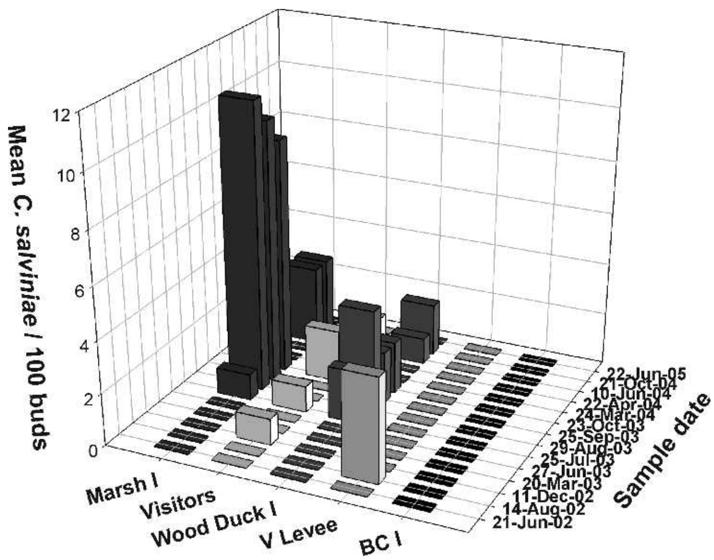

Figure 1. Mean number of *Cyrtobagous salviniae* adults collected at selected release sites from June 2002 through June 2005.

(B. Harris, pers. comm.). No weevils were ever recovered in control sites indicating that only localized dispersal occurred. This was corroborated by the lower grand mean (± SE) number of weevils per meter found within the adjacent quadrats (0.5 ± 0.3) compared to within the reference frames (1.0 ± 0.3) where they were originally released (t_{109} = 2.9, P = 0.004). Fewer weevils were also recovered in shaded sites compared with full sun sites (t_{38} = 2.6, P = 0.01). Overall, a mean (± SE) of 2.1 ± 0.6 weevils m^{-2} were recovered per sample date in full sun release sites while only 0.3 ± 0.1 weevils m^{-2} were recovered in shaded sites.

Air temperatures were predictably lower in shaded versus full sun sites (t_{69} = 1.76, P = 0.07) but water temperatures were unaffected (t_{53} = 0.4, P = 0.69). Dissolved oxygen (T_{63} = 0.74, P = 0.46) and pH (t_{63} = 0.10, P = 0.91) were also unaffected by shading. Most of the shaded sites were in relatively deep shade; for example on 20 March 2003 the mean percent shading in the Visitor's Center, Palmetto I, and Palmetto II sites was 94.5%, 83.3%, and 67.1%, respectively, as measured with a quantum radiometer.

Insect treatments did not directly affect plant biomass in the reference frames but did interact with the presence or absence of shading ($F_{1,99}$ = 6.94, P = 0.0098) (Figure 2). The interaction resulted from a change in magnitude whereby the impact of the weevils was greater in full sun sites compared to shaded sites. A similar effect and interaction occurred with percent cover in the reference frames where coverage decreased with weevil attack in full sun sites (release sites: 57.2% ± 15.9 versus control sites: 82.1% ± 6.0) but not shaded sites (release sites: 87.4% ± 4.3 versus control sites: 83.9% ± 6.8) ($F_{1,112}$, P = 0.04). Although the percentage of brown coloration of the mats was not different between insect treatments, mats in shaded sites were more green (release sites: 84.6% ± 2.6 versus control sites: 84.1% ± 2.1), regardless of whether weevils were released, than mats in full sun sites (release sites: 66.4% ± 4.2 versus control sites: 67.7% ± 5.5) ($F_{1,112}$ = 37.8; P <

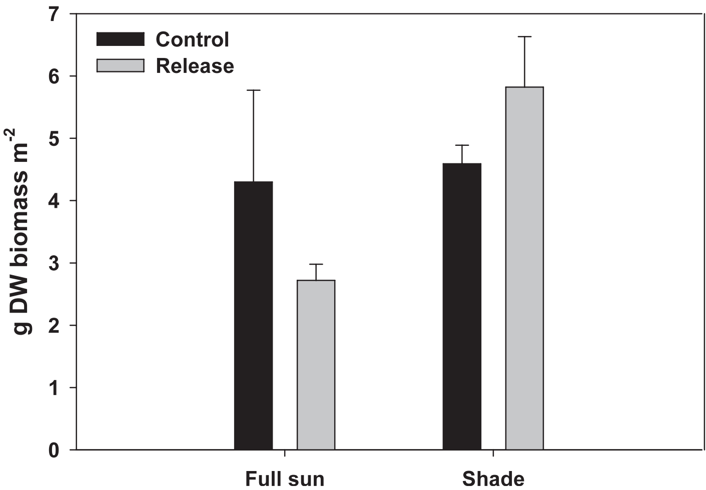

Figure 2. Grand mean (± SE) biomass of *Salvinia minima* for all sample dates in control and release sites under full sun or shaded conditions.

0.0001), indicating that this metric was confounded by abiotic factors.

There was a trend of less *S. minima* biomass within the reference frames compared to the adjacent quadrats at release sites ($T_{46} = 1.15$, P = 0.13 [full sun]; $T_{55} = 1.46$, P = 0.07 [shaded]), but not control sites ($T_{11} = 0.65$, P = 0.26 [full sun]; $T_{82} = 0.35$, P = 0.63 [shaded]), indicating again the localized effect of *C. salviniae* (Figure 2). A larger mean (± SE) percentage of buds of common salvinia were damaged in release sites (17.7 ± 2.3%) than control sites (7.5 ± 0.9%), regardless of the presence of shading ($F_{1,104} = 12.18$, $P < 0.0007$). The number of weevils recovered was positively correlated with the percentage of damaged buds ($r = 0.39$, $n = 105$, $P < 0.0001$).

Plant morphology was influenced at times by the insect treatment or the shade treatment or both. For example, a larger percentage of the primary growth form of *S. minima* was present in both release sites ($F_{1,112} = 8.2$; P = 0.005) and shaded sites ($F_{1,112} = 8.3$; P = 0.004) with no interaction (Figure 3A). This phenomenon has been observed with *S. molesta* following attack by *C. salviniae* whereby plants within mats compensate for herbivore damage by producing new ramets which are initially in the primary growth form (PWT, unpublished data). There was a smaller percentage of the secondary growth form in release sites ($F_{1,112} = 6.1$; P = 0.01) (Figure 3B) while the percentage of the tertiary growth form was equivalent between sites ($F_{1,112} = 1.2$; P = 0.26) (Figure 3C). However, a higher percentage of the plant populations in full sun sites consisted of the tertiary growth form ($F_{1,112} = 15.0$; P = 0.0002). Weevils may have preferred full sun sites because of higher temperatures or larger percentages of tertiary growth forms in *S. minima* populations. Tipping and Center (2005) found the Florida *C. salviniae* adults preferred larger, tertiary growth stages of *S. minima* over smaller, primary growth stages.

In summary, despite a concerted effort to establish and evaluate the Florida ecotype of *C. salviniae*, insect density was generally low and damage to *S. minima* was both minimal and localized in time and space. This stands in stark contrast to conditions in Central and South Florida where populations of *S. minima* are cyclically suppressed by higher densities of *C. salviniae* (Tipping et al. 2012). Weevils did better in full-sun sites which were not subjected to perturbations like floods, droughts, or boat traffic. Hurri-

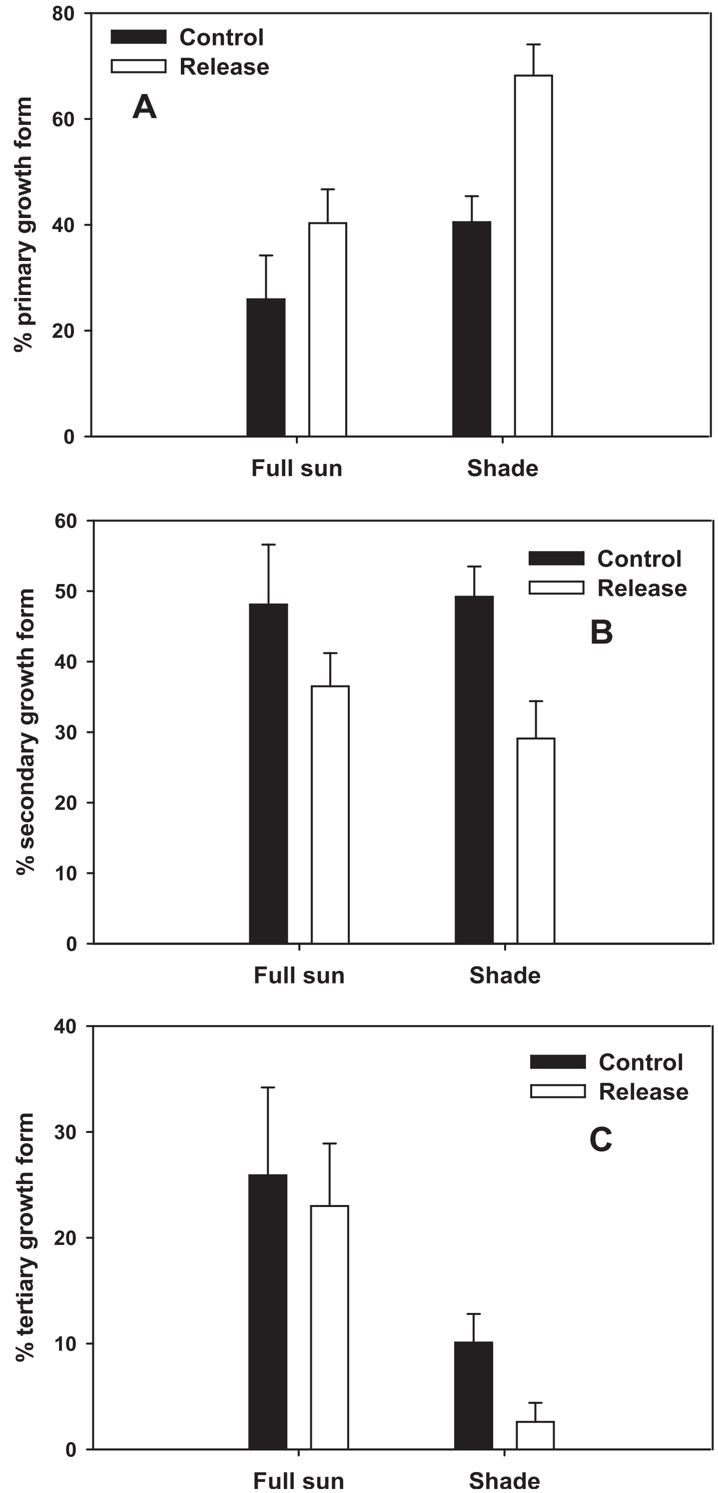

Figure 3. Grand mean (± SE) percentage of (A) primary, (B) secondary, and (C) tertiary plant growth forms in *Salvinia minima* populations for all sample dates in full sun and shaded sites with and without releases of *Cyrtobagous salviniae*.

cane Katrina effectively ended our efforts by destroying all the plots from large scale saltwater intrusion and damaging many of the buildings in the Preserve. Any future efforts to further establish and evaluate this insect should focus on releasing *C. salviniae* in protected, full-sun areas only.

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