J. Aquat. Plant Manage. 49: 83-89

Effect of an herbivorous stem-mining midge on the growth of hydrilla

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ABSTRACT

Hydrilla (*Hydrilla verticillata* [L.f.] Royle: Hydrocharitaceae) is one of the worst invasive aquatic weeds in the United States, with millions of dollars spent annually to control large infestations in all types of water bodies. A major factor contributing to its invasiveness is a pattern of growth that produces characteristic thick surface mats. These dense, thick weed beds impact native plant and animal communities, navigation, flood control, and water quality. An adventive tip-mining midge, *Cricotopus lebetis* Sublette (Diptera: Chironomidae), was discovered attacking hydrilla in Florida's Crystal River watershed. Larvae of *C. lebetis* mine the apical meristems, severely injuring or killing the growing tips. Larval feeding damage changed the plant's architecture by preventing stems from reaching the water surface, and a positive correlation between larval density and frequency of meristem damage was observed. Approximately 200 larvae/m^2, or the equivalent of 1.5 egg masses, damaged about 50% of hydrilla's apical meristems. In the laboratory, 99% of the standing biomass of hydrilla was reduced by larval feeding activity. This type of damage is desirable for hydrilla management because it could eliminate most of the environmental and navigational problems caused by the dense surface mats.

Key words: biological control, *Cricotopus lebetis* Sublette, efficacy, herbivory, *Hydrilla verticillata* (L.f.) Royle

INTRODUCTION

Hydrilla verticillata (L.f.) Royle (hereafter hydrilla) is one of the most invasive aquatic weeds in watersheds along the coastal United States (Langeland 1996, McClellan et al. 2006, USDA, NRCS 2006). Hydrilla grows in water depths from shallow waters to over 6 m. It is widely distributed in warmer regions of the world and occurs as both dioecious and monoecious strains (Holm et al. 1997). The native range of hydrilla is uncertain but reportedly includes Southeast Asia, northern Australia, and East Africa (Cook and Luond 1982, Verkleij et al. 1983).

A dioecious female strain of hydrilla was introduced into Florida from Sri Lanka in the early 1950s through the aquarium trade (Schmitz et al. 1991, Langeland 1996). It was initially propagated by growers of aquarium plants and transplanted into waterbodies in several areas of Florida. It now occurs in every watershed in the state. A monoecious strain of the plant was similarly introduced into the Washington DC area (Steward et al. 1984). Nationally, established populations of the monoecious hydrilla strain occur mainly in the northeastern United States, California, and Washington, whereas the dioecious strain occurs primarily in the southeastern United States, Texas, and California; both strains have been reported from Virginia, the Carolinas, Georgia, and California (USGS NAS 2004).

Hydrilla severely impacts habitat quality, recreational activities, floodwater management, and irrigated agriculture. Due to the diversity of water resource uses (e.g., fishing, recreation, flood control, aquaculture, and crop irrigation), effective hydrilla control is difficult to achieve because environmentally sound options for integrated management are limited (Hoyer et al. 2005). Efforts to control hydrilla currently rely on several Environmental Protection Agency-registered herbicides and nonselective biological control using the grass carp *Ctenopharyngodon idella* Val. (Cassani 1996, Sutton and Vandiver 1998). During the past 30 years, the state of Florida spent more than $210 million controlling more than 162,000 ha (400,000 ac) of hydrilla on public lakes and rivers (Schardt 2010). In addition, Florida's water management and drainage districts as well as numerous counties also spend significant public funds for hydrilla control.

A major factor contributing to hydrilla's invasiveness is its pattern of growth. Hydrilla grows as a sparsely branched, erect rooted plant until it reaches the water surface where it branches profusely. Haller and Sutton (1975) reported that more than 20% of hydrilla's biomass was in the upper 10 cm stratum of the water column. The dense surface mats displace native vegetation, interfere with navigation and flood control; affect native plant, fish and zooplankton communities; and alter water temperature and chemistry (USGS NAS 2004). Furthermore, a fast-growing epiphytic cyanobacterium associated with dense hydrilla mats has been implicated recently in the deaths of bald eagles (*Haliaeetus leucocephalus* [L.]), in the southeastern United States (Wilde et al. 2005). Theoretically, if the surface placement of hydrilla's biomass could be disrupted or redirected, then most of the adverse effects attributed to the plant would be eliminated.

Various mechanical, chemical, and biological methods have been investigated for managing hydrilla infestations in an attempt to control the explosive growth of the weed, but none were as effective as the herbicide fluridone (1-methyl-3-phenyl-5-[3-(trifluoromethyl)phenyl]-4(1*H*)-pyridinone). With the recent discovery of several fluridone-resistant biotypes in Florida (Michel et al. 2004), as well as cross-resis-

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tance to norflurazon (4-chloro-5-(methylamino)-2-[3-(trifluoromethyl)phenyl]-3(2*H*)-pyridazinone; Puri et al. 2009), control efforts will be more costly and less effective. Additionally, the spread of the resistant biotypes to other waterbodies where hydrilla is established is inevitable (Netherland et al. 2005, Puri et al. 2007). The loss of fluridone as a major tool for controlling hydrilla has forced aquatic plant managers and researchers to develop new herbicides (Koschnick et al. 2007), evaluate new use patterns for registered contact herbicides (Getsinger et al. 2008), develop novel biological controls (Cuda et al. 2008), and initiate new surveys for classical biological control organisms in Asia and Africa (Overholt and Cuda 2005, Zhang et al. 2010).

Classical biological control of hydrilla has been investigated since the 1970s (Buckingham 1994). Foreign exploration for insect natural enemies was conducted in Asia, Australia, and to a lesser extent Africa (Balciunas et al. 2002). Surveys were conducted first in India and Pakistan and were later expanded to several other countries in Asia and to Australia. Several natural insect enemies were identified and imported into Florida for final host range testing. Four insects eventually were released, but only 2 ephydrid leaf-mining flies of the genus *Hydrellia* established in the United States (Center et al. 1997, Grodowitz et al. 1997). Unfortunately, these 2 leaf miners have not provided the desired level of control (Forno and Julien 2000, Balciunas et al. 2002). The adventive pyralid moth *Parapoynx diminutalis* (Snellen), which is widely distributed in Asia and Africa, also was discovered in Florida feeding on hydrilla (Delfosse et al. 1976); however, it is not host specific (Buckingham and Bennett 1996) and is ineffective as a biological control agent (Buckingham 1994).

Exploration for natural enemies of hydrilla was conducted in East Africa in the late 1970s and early 1980s by the United States Department of Agriculture (USDA; Pemberton 1980), and by the Commonwealth Institute of Biological Control (CIBC, now CAB International; Markham 1985). Both Pemberton (1980) and Markham (1985) identified the larvae of stem-mining midges of the genus *Polypedilum* (Diptera: Chironomidae) as causing significant stunting of hydrilla in Lake Tanganyika, Tanzania. Regrettably, due to the short duration of the project, these midges were never reared, and their biologies are unknown (Buckingham 1994). However, in the early 1990s an adventive midge was discovered in Crystal River, Citrus County, Florida, causing similar damage to hydrilla (Cuda et al. 1999, 2002). This hydrilla-attacking midge, later identified as *Cricotopus lebetis* Sublette, was not recognized as a distinct species in Florida until 2000 (Epler et al. 2000). It is conceivable that *C. lebetis* was absent from Florida prior to 1985 because it was not listed in faunal surveys of hydrilla in Florida during the early 1980s (Balciunas and Minno 1985). The 1992 discovery of the larvae of *C. lebetis* and heavily damaged hydrilla plants at Crystal River by USDA researchers supports this hypothesis.

Stunting of hydrilla plants observed at Crystal River has been associated with the occurrence of plant-feeding midges *C. lebetis* and an undescribed species belonging to the chironominine genus *Dicrotendipes* Kieffer (G.R. Buckingham, Research Entomologist, USDA Agricultural Research Service, September 1997, pers. comm.). However, reductions in plant growth and biomass or changes in the architecture of hydrilla due to feeding by larval midges have not been demonstrated experimentally. Empirical evidence is needed to determine the effect of midge feeding damage on the growth pattern of hydrilla under controlled conditions.

We present the results of field surveys of *C. lebetis* conducted at Crystal River, Florida, to establish whether the observed declines in the occurrence of hydrilla during the 1990s were correlated with the presence of this hydrilla-attacking midge. We also provide for the first time experimental evidence under controlled conditions that feeding damage by *C. lebetis* reduces the biomass of hydrilla.

Study site

The field research site was located in the Crystal River–Kings Bay watershed, about 115 km north of Tampa in the Big Bend area of Florida's west coast. Cuda et al. (2002) provide a detailed description of the site. The headwaters originate in the City of Crystal River, Citrus County. The river flows in a northwest direction approximately 11.3 km where it eventually empties into the Gulf of Mexico. The flowing water produced by a series of natural springs maintains a relatively constant temperature of 25 C and provides exceptional water clarity. The average depth of the Crystal River–Kings Bay watershed is influenced by tidal fluctuations occurring in the Gulf and varies from 0.7 m at the mouth of the river to 0.3 m in Kings Bay. The Kings Bay inlet has a surface area of approximately 2 km^2 and is relatively shallow, ranging from a depth of 1 to 3 m. The region's climate is humid subtropical with annual rainfall between 132 and 142 cm.

MATERIALS AND METHODS

Field surveys

Hydrilla was sampled once a month from April to December 1998 in a protected area of the Plantation Inn Canal system (28°52'49.34"; 82°35'17.50") where a preliminary survey was conducted during the same period in 1997 (Cuda et al. 2002). A transect was established parallel to a concrete seawall along the south side of the canal. Hydrilla was sampled from a jon boat at 5 m intervals according to the procedures described by Cuda et al. (2002). Samples of hydrilla (n = 25) were taken at low tide to collect all hydrilla growing above the basal 25 cm, the zone most likely to experience tip midge damage. The depth and water temperature at the top of the hydrilla mat in the canal were recorded at 25 m intervals along the transect (n = 5) with the aid of an electronic temperature probe mounted on a 2 m PVC pipe fitted with a waterproof tape measure.

In the laboratory, the hydrilla samples were processed, and larval density estimates were obtained following procedures described by Cuda et al. (2002). The percentage of tips damaged by midge larvae was estimated by randomly selecting one fresh apical meristem (2 to 5cm in length) from each hydrilla sample at the beginning of the extraction procedure and examining the individual meristems with a dissecting microscope for evidence of midge feeding damage (n = 25). After 48 h, the air-dried hydrilla samples were trans-

ferred to paper bags, oven dried at 65 C for an additional 48 h, and then weighed to the nearest 0.5 g to obtain an estimate of the average hydrilla biomass/m^2 for each sample date.

Glasshouse Experiments

Two experiments were conducted in an environmentally controlled glasshouse from June 1999 to April 2000 to examine the effect of *C. lebetis* larvae on hydrilla. Both experiments were performed in 127 L plexiglas tanks (76 by 46 by 46 cm) arranged in a randomized block design with 2 treatments and 3 replications. For each experiment, a stand of glasshouse-propagated hydrilla (New Delhi strain) was established in each of the 6 tanks. The New Delhi hydrilla strain was used because previous research showed midge survival was highest on this particular strain (Cuda et al. 2002). Four apical stem sections of hydrilla 20 cm in length were planted to a depth of 5 cm in plastic pots (15 cm dia by 14 cm ht) containing topsoil covered with a 1 cm layer of builder's sand. Two grams of slow release fertilizer (Osmocote®, N:P:K 18:6:12) were mixed into the topsoil as it was added to each pot. The total sediment depth was 12 cm. Nine pots containing the planted hydrilla (36 sprigs total) were placed in each tank. The tanks were filled with well water to a height 72.5 cm and supplied with an aerator. Water temperature was recorded daily in one of the control tanks from a laboratory thermometer suspended below the surface of the water to a depth of 30 cm. Each tank was covered with mosquito netting stapled to a wooden frame to exclude unwanted hydrilla insects (e.g., *Hydrellia pakistanae* Deonier and *P. diminutalis* [Snellen]), and to confine emerging adults of *C. lebetis*. The well water in each tank was recirculated at 7 to 10 d intervals to minimize algae blooms. Ambient light inside the glasshouse was supplemented with banks of fluorescent lights controlled with an electric timer to maintain a 16:8 L:D photoperiod for the duration of the experiments. A hygrothermograph was used to record the maximum and minimum air temperature inside the glasshouse.

The first experiment was designated the "best case scenario" because it was designed to examine the effect of the midge on unbranched elongating hydrilla, a plant condition that simulates early season growth. In this experiment, the hydrilla plants were allowed to acclimate to the tank environment for only 1 week. Two treatments, presence or absence of midge larvae, were randomly assigned to each pair of tanks. Larvae (80 neonates) of *C. lebetis* were used to inoculate the tanks designated to receive the midge treatment to ensure that all of the 36 hydrilla sprigs would be attacked by at least one larva in the event that natural larval mortality in the tank exceeded 50%. Larvae were added to one of the tanks in each pair on 7, 16, and 26 June for a total of 240 larvae per test tank. The tanks inoculated with *C. lebetis* were monitored daily for adult emergence. Adult midges were removed with an aspirator as they emerged. The adults were sexed and counted and then incorporated into the laboratory colony. The daily removal of the adult midges from the tanks was intended to simulate natural mortality from predators and minimize the opportunity for uncontrolled reproduction that would lead to unnaturally high midge population densities. The other tank in each pair served as an untreated control. The effect of the 2 treatments (midge damaged vs. undamaged hydrilla) was compared by measuring the distance between the top of the hydrilla mat and the water surface based on the premise that larval midge feeding damage induces stunting in hydrilla. When the first experiment was terminated in August 1999, the standing hydrilla stems in each tank were clipped at the substrate level, bagged, and oven dried at 65 C. After 48 h in the drying oven, the biomass samples were weighed to the nearest 0.5 gm.

The second experiment was similar to the first except for slight modifications in the experimental procedure. The second experiment was conducted from October 1999 to April 2000 and was designated as the "worst case scenario." The plexiglas tanks were planted with hydrilla on 9 October, and the sides of the tanks were covered with aluminum foil to prevent light from entering through the sides of the tanks. This modification more closely simulated the light conditions that hydrilla growing in a dense mat would normally experience in a natural waterbody. In addition, the hydrilla stems were allowed to reach the surface and begin branching before the midge larvae were added to the tanks. Also, the number of neonate larvae added to the test tanks was reduced from 80 to 50 neonates with only 2 inoculations occurring on 18 November and 14 December 1999. The rationale for reducing the number of midges added to the test tanks in the second experiment was to simulate midge densities more likely to be encountered under field conditions.

Data analyses

Field time-series data (larval density, damaged meristems, and hydrilla biomass) were analyzed with linear regression using PROC GLM (SAS Institute 1999). Larval densities were log transformed, and the percent damaged meristems were arcsine square root transformed prior to analysis (Zar 1999). For the glasshouse experiments, the effect of larval midge feeding damage on the standing plant biomass was compared between treatments using the TTEST procedure with unequal variances (SAS Institute 1999). For the statistical analyses, a significance level of $\alpha = 0.05$ was used.

RESULTS AND DISCUSSION

Field Surveys

The average water temperature at the top of the submersed hydrilla (the zone where larvae of *C. lebetis* are most active) in the Plantation Inn Canal was 23.8 ± 0.8 C (Figure 1). The constant water temperature observed year-round in the canal is typical of Florida's spring-fed waterbodies (Rosenau et al. 1977). Some larval midges are unable to tolerate high water temperatures. For instance, the minimum and maximum temperature thresholds for *Cricotopus myriophylli* Oliver were 16 and 25 C, respectively (MacRae and Ring 1993), so the potential distribution of *C. lebetis* in Florida could be limited to the cooler spring waters.

Topped-out hydrilla was rarely observed at the Plantation Inn Canal site during the course of this study. In addi-

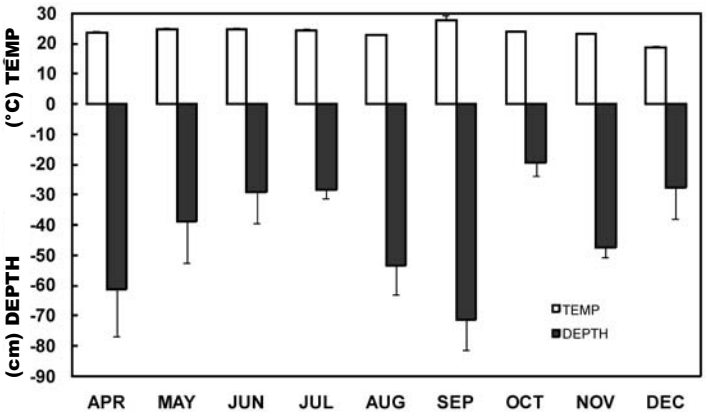

Figure 1. Monthly water temperature and location (depth) of submersed hydrilla recorded at Plantation Inn Canal, Crystal River, FL, 1998.

tion, the majority of the hydrilla plants examined in the field as well as in the laboratory were missing their shoot tips due to midge feeding damage. Throughout the entire sampling period (Apr to Dec 1998), the plants exhibited basal branching and appeared stunted during low tide conditions when the samples were collected. The tops of the hydrilla plants remained well below the water surface, occurring at an average depth of 41.9 ± 5.8 cm (Figure 1). A maximum and minimum depth of 71.2 and 19.4 cm, respectively, was recorded between September and October. A similar growth pattern was observed during preliminary field surveys conducted in 1997 (Cuda et al. 2002). Although it has been reported that hydrilla may remain close to the hydrosoil in spring fed systems where light is not limiting (Spencer and Bowes 1990), the results of the glasshouse experiments (see below) supported the anecdotal evidence that midge feeding damage was affecting the growth habit of hydrilla at this site.

The percentage of hydrilla meristems damaged by *C. lebetis* ranged from 0 to 73%, with a positive correlation between larval density and percent damaged meristems (Figure 2). Meristems damaged by larval feeding activity increased significantly as the density of the larvae increased ($r^2 = 0.56$, $p = 0.0211$). Regression analysis also showed that hydrilla biomass significantly decreased ($r^2 = 0.78$, $p = 0.0494$) as the percentage of damaged hydrilla meristems increased during August to December 1998 (Figure 3).

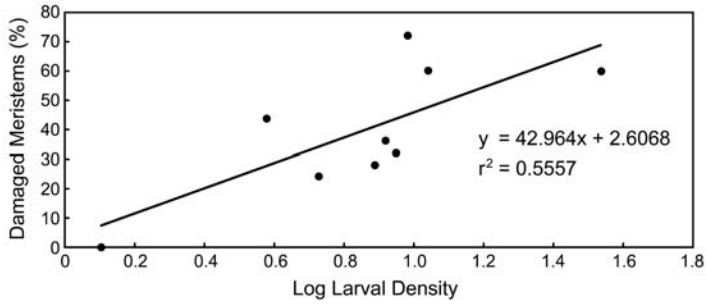

Figure 2. Relationship between the percentage of damaged hydrilla meristems as a function of the larval density of *Cricotopus lebetis* Sublette, Plantation Inn Canal, Crystal River, FL, 1998.

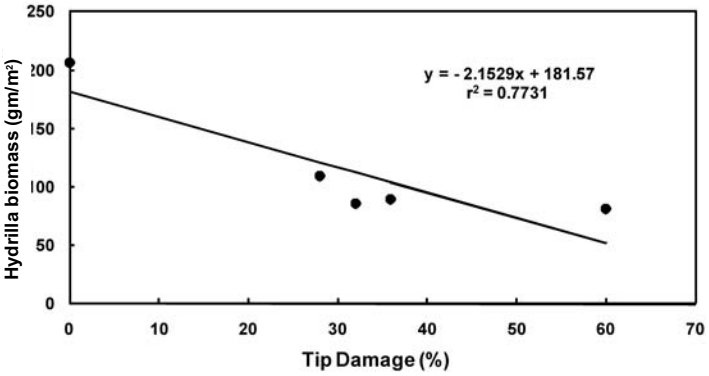

Figure 3. Relationship between the biomass of hydrilla as a function of the percentage of hydrilla meristems damaged by *Cricotopus lebetis* Sublette, Plantation Inn Canal, Crystal River, FL, Aug to Dec 1998.

Glasshouse Experiments

In the best case scenario experiment, hydrilla growth in the tanks inoculated with larvae of *C. lebetis* was significantly reduced ($p < 0.00001$) compared to the control tanks (Figure 4). The control plants followed a normal growth progression by elongating and reaching the surface on day 35. After the plants reached the surface, they branched to form a thick mat typical of topped-out hydrilla. In contrast, the hydrilla plants exposed to the midge larvae began showing the effects of feeding damage by day 13. The hydrilla plants under attack by the midge larvae were stunted and did not produce any new growth. The midge larvae effectively suppressed the growth of hydrilla until day 97 when the midge population eventually collapsed. The average depth to the top of the hydrilla stand attacked by *C. lebetis* was 45.7 ± 8.2 cm, and the water temperature in the tanks was 27.2 ± 1.8 C.

Adult emergence commenced on day 13 (Figure 5), the same day growth inhibition of the hydrilla plants was initially observed. The emergence of adults peaked on day 27 and day 32, when an average of 23.7 ± 17.5 females and 24.3 ± 12.0 males, respectively, was produced in the treatment tanks.

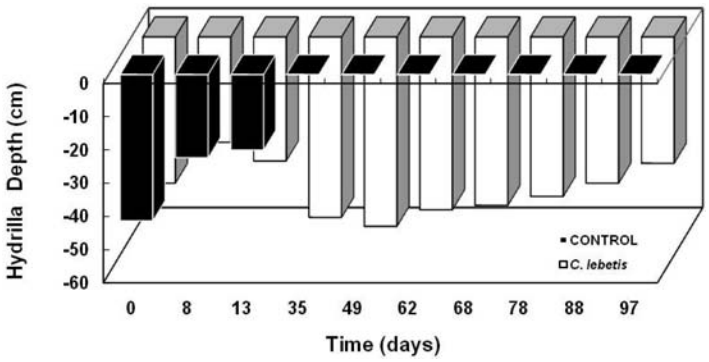

Figure 4. Differences in the growth pattern of hydrilla inoculated (white bars) or not inoculated (black bars) with larvae of *Cricotopus lebetis* Sublette in glasshouse experiment I. Growth indicated by distance from water surface to top of hydrilla plants.

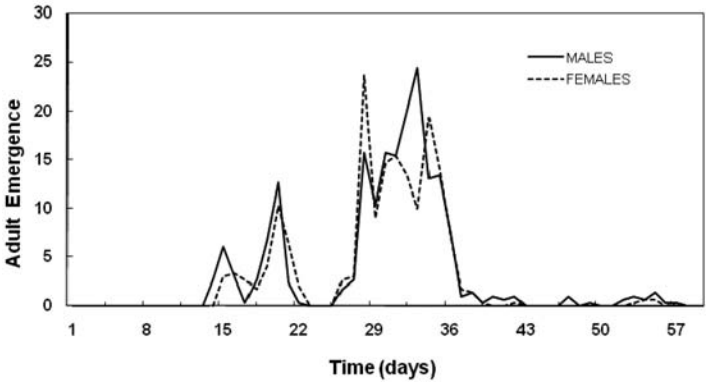

Figure 5. Emergence pattern of adults of *Cricotopus lebetis* Sublette from midge treatment tanks in glasshouse experiment I.

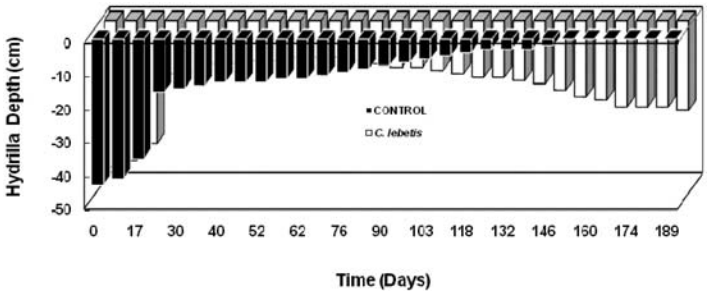

Figure 7. Differences in the growth pattern of normal hydrilla (black bars) and hydrilla attacked by larvae of *Cricotopus lebetis* Sublette (white bars) observed in glasshouse experiment II. Growth indicated by distance from water surface to top of hydrilla plants.

In total, 1075 midges emerged during the course of this experiment, which represented a 4.5-fold increase in the midge population. The observed sex ratio was 1.1:1 based on 562 males and 513 females that emerged during the course of the experiment.

Larval feeding by *C. lebetis* also reduced the standing biomass of hydrilla by more than 99% compared to noninoculated tanks ($p < 0.004$). The standing biomass in the midge treatment tanks was 0.4 gm compared to 90.0 gm in the control tanks (Figure 6).

A similar pattern was observed in the worst case scenario experiment even though fewer midge larvae were used to inoculate the tanks and the hydrilla was allowed to reach the surface and begin branching before the tanks were inoculated. Stunting of hydrilla was delayed due to cessation of growth that normally occurs during the winter, and also because the water temperature was cooler (22.9 ± 0.2 C) in this experiment. Under these conditions, the developmental rate and feeding activity of the midge larvae was apparently retarded until the spring.

Growth suppression in hydrilla as a result of larval midge feeding damage became apparent on day 90 (approximately 2 mo after the tanks were inoculated) and continued until the experiment was terminated (Figure 7). The biomass of hydrilla in the tanks inoculated with midge larvae was significantly lower ($p < 0.002$) compared to the untreated control tanks. The midge larvae caused a steady decline in the growth of the hydrilla over time (Figure 7). The extent of the decline is indicated by the location, or depth, of the hydrilla mat in the water column. On day 189, the top of the hydrilla mat in the midge treatment tank occurred at a depth ~27 cm below the water surface, while the hydrilla plants in the control tanks grew continuously until they reached the surface.

The emergence pattern of the adults produced in this experiment (Figure 8) indicates that the highest adult emergence occurred on 15 January 2000 (day 101), which coincided with the initial decline in hydrilla growth. On average, 63 ± 58.6 males and 41 ± 37.4 females emerged from each inoculated tank on this date. In total, 2434 males and 1771 females (sex ratio 1.4:1) were aspirated from the tanks between December 1999 and April 2000. The adults that emerged (4005 total) represented a 40-fold increase over the initial larval population.

Prior to this study, reports of reductions in hydrilla growth and biomass attributed to feeding damage by midge larvae have been entirely anecdotal (Pemberton 1980, Markham 1985. More recently, Buckingham (1994) made the following observation in reference to the larvae of two African species of *Polypedilum* that purportedly damage the meristems and buds of hydrilla as they mine these plant tissues. ". . . Plants are stunted and multi-branched due to continual attack on the buds which forces new shoots to grow which are then attacked . . . ".

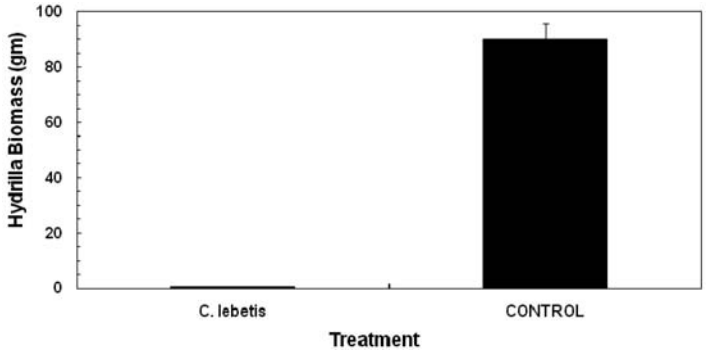

Figure 6. Comparison of hydrilla biomass produced in tanks with and without larvae of *Cricotopus lebetis* Sublette in glasshouse experiment I.

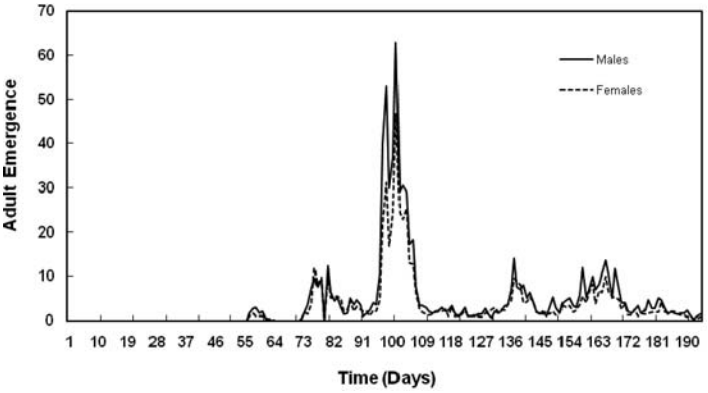

Figure 8. Emergence pattern of adults of *Cricotopus lebetis* Sublette from midge treatment tanks in glasshouse experiment II.

Our research provides the first empirical evidence that feeding damage by the midge *C. lebetis* stunts the growth and reduces the biomass of hydrilla under both field and laboratory conditions. Larvae of this herbivorous midge mine the apical meristems of the hydrilla plant and in the process disrupt new shoot growth. By severely injuring or killing the plant's growing tips, the developing larvae prevent new hydrilla stems from reaching the surface of the water column, or "topping out." This type of damage is desirable for managing hydrilla infestations because it would eliminate most of the adverse effects caused by the formation of the dense surface mats, such as changes in biodiversity, water chemistry, circulation, and temperature.

Recently, T.D. Center identified several characteristics of the "ideal" hydrilla biological control agent. To be effective, the insect should (a) damage vital plant tissues, (b) be reproductively prolific, (c) complete its life cycle entirely on submersed hydrilla, (d) exhibit endophagy to avoid predation, (e) adapt to local climatic and habitat conditions, (f) thrive on established hydrilla biotypes, and (g) exhibit a narrow host range. The stem mining midge *C. lebetis* satisfies nearly all of these criteria. The larvae damage hydrilla's apical meristems, preventing the shoots from increasing in length and producing primary tissues. Females deposit over 150 eggs, complete a generation in <2 weeks, and exhibit a high net reproductive rate, R_o = 28.8 (Cuda et al. 2002). Except for the nonfeeding adult stage, the life cycle of *C. lebetis* occurs entirely on submersed hydrilla (Cuda et al. 2002). The recent discovery of *C. lebetis* in Lake Rowell, Bradford County, Florida (29°55'16.57"; 82°09'32.75") suggests the midge is capable of adapting to different habitat conditions (J.P. Cuda, Associate Professor, University of Florida, September 2010, pers. observ.), and development of the insect on fluridone resistant hydrilla also has been documented (Schmid et al. 2010).

These results confirm that further evaluation of *C. lebetis* as a potential biological control agent for hydrilla is warranted. Additional research should focus on the insect's ability to adapt to different environmental conditions, especially maximum and minimum temperature tolerances. Manipulative field trials at multiple sites throughout Florida also are needed to corroborate the apparent localized control of hydrilla observed at the Crystal River site. Finally, the host specificity of *C. lebetis* will need to be demonstrated before the insect can be considered for widespread adoption as a hydrilla management tool. However, if host range testing shows the midge is not a hydrilla specialist, it still could be mass-reared and used in an augmentation biological control program against dense hydrilla infestations within the state boundaries of Florida and Louisiana, where the midge is naturalized.

ACKNOWLEDGEMENTS

Special thanks are extended to Gary Buckingham for his encouragement and guidance during the early stages of the project. We also are grateful to Kathy Kraft, Judy Gillmore, Rob Lowen, and Jennifer Possley for their technical assistance, and William Overholt and Robert Copeland for reviewing an earlier draft of the manuscript. This research was supported by USDA/ARS—University of Florida/IFAS Cooperative Agreement No. 58-66299-7-010.

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