Biology and Laboratory Rearing of *Cricotopus lebetis* (Diptera: Chironomidae), a Natural Enemy of the Aquatic Weed Hydrilla (Hydrocharitaceae)

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ABSTRACT

Field and laboratory studies were conducted on *Cricotopus lebetis* Sublette, a midge of unknown origin that causes widespread damage to the aquatic weed hydrilla, *Hydrilla verticillata* (L.f.) Royle, at Crystal River, FL, USA. The larvae of *C. lebetis* burrow into the submerged apical meristems of the hydrilla plant and destroy shoot tips during their development. Abscission of the hydrilla stem tip usually occurs when the last instar excavates a tunnel inside the stem for pupation. Mining damage caused by the feeding larvae induces stunting and basal branching that alters the normal growth pattern of hydrilla. Larval populations of *C. lebetis* and damaged hydrilla shoot tips were highest in the summer (May and June) and fall months (October and November) during 1997 and 1998. In June 1998, more than 70% of the hydrilla shoot tips were damaged by midge larvae. Adults lived in the laboratory, and nuptial flights were not a prerequisite for mating. Females deposited a single egg string containing an average of 154 eggs inside a gelatinous matrix. The eggs took 2 d to complete development and neonate eclosion was synchronized. The larval stage had four instars and required 14 d to develop to the pupal stage, which lasted an additional 2 d. Although most larval chironomids feed on algae or detritus, the larvae of *C. lebetis* fed and developed entirely on the living stem tissue of hydrilla.

KEY WORDS *Hydrilla verticillata*, aquatic weeds, midge bionomics, herbivory, biological control.

HYDRILLA, *Hydrilla verticillata* (L.f.) Royle, is a submerged, multibranched, annual or perennial monocot that is widely distributed in warmer regions of the Old World (Holm et al. 1997, Langeland and Burks 1998). Hydrilla also is considered one of the two most important aquatic weeds worldwide, second only to water hyacinth, *Eichhornia crassipes* (Mart.) Solms (Sorjani 1986). A major factor contributing to the invasiveness of hydrilla is its pattern of growth. Hydrilla grows as a sparsely branched erect rooted stem until it reaches the water surface where it branches profusely (Langeland 1990). The dense surface mats that are produced displace native vegetation, interfere with navigation and flood control, displace native plant, fish and zooplankton communities, and alter water temperature and chemistry (Langeland and Burks 1998).

Two reproductive forms of hydrilla are established in the United States (Langeland and Burks 1998). A dioecious strain of hydrilla was introduced into Florida by the aquarium trade in the 1950s (Schmitz et al. 1991). The monoecious strain was a separate introduction that was discovered in the Potomac River basin in the early 1980s (Steward et al. 1984). Monoecious hydrilla is found in Delaware, Maryland, Washington, and the District of Columbia; whereas dioecious hydrilla occurs in Alabama, California, Connecticut, Georgia, Louisiana, Mississippi, Pennsylvania, South Carolina, Tennessee, and Texas (Netherland 1997, Colangelo 1998). The distributions of both reproductive forms overlap in the Carolinas and Virginia (Netherland 1997). In Florida, the dioecious female strain of hydrilla (herein referred to as hydrilla) causes problems (Langeland 1990, Langeland and Burks 1998). For example, from 1980 to 1993, approximately $39 million were spent managing hydrilla in Florida’s public waters (Schardt 1997). Since 1995, hydrilla control costs using nonbiological methods have increased steadily to over $12 million per year (Schardt and Ludlow 2000).

In 1960, hydrilla was discovered growing on the west coast of Florida in the Crystal River watershed (Langeland 1990). The watershed includes a large spring-fed open area, referred to as Kings Bay, that forms the river’s headwaters. Until the late 1970s, Kings Bay and the residential canals along its eastern shoreline historically supported dense infestations of hydrilla. However, beginning in 1979 the hydrilla populations in Kings Bay and its canal system began to experience recurring declines at 9- to 10-yr intervals (Mataraza et al. 1999). Mataraza et al. (1999) sug-

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gested that tropical storm events are responsible for the periodic hydrilla declines observed in Kings Bay. However, the hydrilla never returned to prestorm levels after a major storm event in 1993 (Mataraza et al. 1999). Indeed, the historical record during the last decade suggests that the growth pattern of hydrilla may be influenced by factors other than storm events. Other factors that may be regulating the abundance of hydrilla in Kings Bay include sediment characteristics (Haller et al. 1983), fungal pathogens (Shearer 1996), and insect herbivory.

In 1992, USDA researchers discovered midge larvae damaging the apical meristems of hydrilla in Kings Bay (G. R. Buckingham, personal communication), and that damaged hydrilla at one site was stunted and unable to grow to the surface. This hydrilla-attacking midge was identified as Cricotopus lebetis Sublette, a species possibly new to Florida (Eppler et al. 2000). Preliminary observations on the biology of C. lebetis as well as photographs of the insect’s life stages were published by Cuda et al. (1999).

In this article, we present a detailed life history of C. lebetis in Florida based on field observations and laboratory rearing studies, and discuss its potential as a biological control agent for hydrilla.

Materials and Methods

Field Studies. Site Description. The Crystal River/Kings Bay watershed (28° 53', 82° 35') is located 115 km north of Tampa in the Big Bend area of Florida’s west coast, and has a surface area of ~54 km² (Mataraza et al. 1999). Originating in Kings Bay in the City of Crystal River, Citrus County, the river flows northwest ~11.3 km to the Gulf of Mexico. Flowing water from a series of springs provides exceptional water clarity and helps maintain a relatively constant temperature of 25°C. Kings Bay is relatively shallow, ranging in depth from 1 to 3 m and has a surface area of ~2 km². The average depth of the watershed is influenced by tidal fluctuations occurring in the Gulf. Under normal climatic conditions, monthly tidal fluctuations vary from 0.7 m at the mouth of the river to 0.3 m in Kings Bay. The climate of the region is humid subtropical with average rainfall between 132 and 142 cm per year. These limnological and climatic conditions are conducive to the growth of the introduced submerged macrophytes Eurasian watermilfoil, Myriophyllum spicatum L. (Haloragaceae) and hydrilla.

Sampling and Seasonal Abundance. Hydrilla samples were collected from a boat in the Plantation Inn Canal system from April to December 1997 and again in 1998. This particular area was selected to conduct our surveys because it was one of the few remaining hydrilla-infested sites in Kings Bay that experienced minimal recreational boat traffic, and that was not subjected to mechanical harvesting operations due to its shallow depth.

A special sampling device was developed to collect only the terminal portions of the hydrilla shoots where the midge larvae and associated damage occur. The sampler was similar to an Ekman grab (Southwood and Henderson 2000), except that it was hand-operated and a sharp cutting blade was attached to one of the opposing jaws. Samples were collected by lowering the open unit over the tops of the hydrilla stems until ~15 cm of the terminal shoots were confined inside the open jaws; the sampler jaws were then shut by spring action, clipping off the hydrilla shoots. Hydrilla and associated water in the sample were placed in a heavy-duty zipper seal plastic bag (64 by 36 cm). Because larvae of C. lebetis are capable of leaving the hydrilla stems when disturbed (G. R. Buckingham, personal communication), the associated water with the plant material was included in the sample to avoid loss of collected larvae.

During 1997, three hydrilla samples (1–2 kg wet weight) were collected along a predetermined transect once a month from April to December. Restricting the number of samples to three per day during the first year of the study facilitated rapid examination of the hydrilla shoots (100 per sample) in the laboratory for tip damage and extraction of live midge larvae for rearing experiments before the hydrilla began to deteriorate.

In 1998, hydrilla was sampled from April to December at 5-m intervals along a 125-m transect established parallel to the concrete seawall lining the south side of the canal. Samples (n = 25) were collected by positioning a 0.25 by 0.25 m PVC (1.27 cm diameter) weighted quadrat fitted with 0.25 m legs over the hydrilla until the legs of the quadrat reached the substrat. All hydrilla in the water column within the quadrat (except for the basal 25 cm) was clipped with the modified Ekman grab described previously. Hydrilla samples were taken at low tide to collect all of the hydrilla growing above the basal 25 cm, the likely zone of midge activity. Hydrilla and associated water samples were processed by straining the samples through two brass sieves (#12 and #14) into a funnel attached to the lid of a pair of 18.9-liter plastic buckets placed one inside the other. The bottom of the inner bucket was replaced with hardware cloth (6 by 6-mm mesh) to allow the strained water to pass into the outer bucket for disposal. The hydrilla samples were placed in a heavy-duty zipper seal plastic bag and stored on ice for transport to the laboratory at Gainesville. Collected water from each hydrilla sample that passed through the funnel received a final straining through a vacuum tube (BioQuip Products, Gardena, CA, USA) modified with organdy cloth (40 by 42 mesh) to trap small midge larvae that otherwise would have escaped during the sampling procedure; the vacuum tubes were changed between samples.

The hydrilla samples were processed at the Center for Aquatic and Invasive Plants, Gainesville, FL, the same day as collected. The temperature in the laboratory was maintained at 25.6 ± 3.6°C (mean ± SD, range 23.9–28.3°C). Larval density estimates were obtained by extracting the midge larvae from the hydrilla samples. Each hydrilla sample was evenly distributed on a plastic grid (13-mm grid opening) that was pressure fitted into a shallow tray (52 by 40 by 7.5 cm). One liter of a preservative (Lysol-No-Rinse Sanitizer for-
merly Rocal], Reckitt and Coleman, Montvale, NJ; active ingredients: alkyl dimethyl benzyl ammonium chloride [10%], ethanol [1.25%]) was added to each tray. The tray was kept inside a screen cage (61 by 61 by 61 cm) during the extraction process to prevent insects from escaping as they emerged from the hydrilla samples. After 48 h, the contents of each tray were strained through organdy cloth, and filtrates rinsed into a corresponding plastic snap cap vial (180 ml) for examination under a dissecting microscope. The percentage of tips damaged by midge larvae was estimated by randomly selecting one fresh apical meristem (2–5 cm in length) from each hydrilla sample (n = 25) at the beginning of the extraction procedure, and examining the individual meristems under a dissecting microscope for evidence of midge damage. The contents of the BioQuip vacuum tubes were examined by rinsing the nylon cloth into a petri dish containing distilled water. The total number of larvae collected in each sample was obtained by adding the contents of each vacuum tube to its corresponding filtrate sample.

**Data Analysis.** The 1997 field data are reported as the number of larvae and pupae observed and percentage of hydrilla shoot tips damaged per sample. For 1998, larval density estimates (number per m$^2$) were obtained by averaging the number of larvae extracted from all the hydrilla samples on each sample day. For both years, the data are presented as (mean ± SEM).

**Laboratory Studies. Plant Culture.** Operating under permits issued by the Florida Department of Environmental Protection (FLDEP) and the United States Department of Agriculture Animal and Plant Health Inspection Service (USDA-APHIS), three dioecious pistillate hydrilla strains to be used as a food source for larvae of *C. lebetis* were propagated in a climate-controlled restricted access greenhouse. Two of the hydrilla strains (Burundi and New Delhi) originated from the USDA Aquatic Plant Management Laboratory’s hydrilla collection in Fort Lauderdale, FL, while a third strain (Florida) was collected locally at Orange Lake, Alachua County, and Crystal River, Citrus County. In addition, in vitro propagated Florida strain hydrilla was cultured as a potential food source. The rationale for testing several hydrilla strains, specifically the Burundi strain from Lake Tanganyika, Africa, was to examine the potential for an East African hydrilla tip midge discovered in earlier surveys (Pemberton 1980, Markham 1986) to accept Florida strain hydrilla as a developmental host if it were introduced to the United States. Demonstrating the reciprocal relationship between *C. lebetis* and the African hydrilla strain would provide experimental evidence that these midges are capable of accepting different hydrilla strains regardless of origin. The Tissue Culture Florida strain was tested because micropropagation of hydrilla can provide a year-round source of vigorous shoot tips that are free of unwanted organisms. The availability of high quality shoot tips on a consistent basis was an important limiting factor for maintaining the midge colony. The New Delhi strain was tested because it grew vigorously year-round under the specific environment in our greenhouse.

The procedure for culturing hydrilla was developed by Goodson (1997). Hydrilla in the current study was propagated in 148 ml plastic cups containing commercially available topsoil mixed with slow-release fertilizer (Osmocote N:P:K, 18:6:12, Scotts-Sierra Horticultural Products, Marysville, OH) at 6 g/ml of soil, and covered with 1 cm of sand. Three hydrilla sprigs of the same strain were planted inside the cups, and transferred by strain into separate 37.9-liter glass aerated aquaria covered with organdy cloth to exclude unwanted organisms. The temperature inside the greenhouse fluctuated between 24 and 32°C, and natural lighting was supplemented with fluorescent lights (Gro-Lights, Osram Sylvania, Danvers, MA) programmed for a 20-h photophase. Upon completion of experiments, all insect-damaged hydrilla was killed by steam autoclaving before disposal.

**Rearing of *C. lebetis.*** Rearing experiments commenced in June 1997, using 20 by 150-mm (35-ml) glass culture tubes containing water (25–30 ml) and a single 5-cm apical hydrilla sprig. Each culture tube was capped with mosquito netting secured with a 9-cm self-locking plastic tie wrap to contain emerging adults. Between June and August, field-collected early and late instars extracted from the field samples were transferred to individual culture tubes containing Crystal River water to obtain a positive association between the larval and adult stages of *C. lebetis*. Crystal River water was used for the initial rearing experiments to minimize mortality resulting from sudden changes in osmotic balance that can occur when field-collected larvae of aquatic insects are transferred to a different water source (Chapman 1975). Subsequent generations of the midge were reared in the same manner except dechlorinated tap water was substituted for the Crystal River water. Culture tubes were maintained in Florida Beach-In environmental chambers (Walker et al. 1993) at a constant temperature of 27 ± 0.1°C, 75 ± 0.1% RH, and a photoperiod of 16:8 (L:D) h. Each culture tube was examined daily under a microscope to monitor the duration (in days) of the larval and pupal stadia and adult emergence.

After a positive association of the larvae with corresponding adults of *C. lebetis* was obtained, laboratory rearing of the midge was attempted. Three samples of hydrilla were collected from the Plantation Inn Canal site with the aforementioned sampling device on 23 September 1997. Each hydrilla sample (1–2 kg wet weight) was placed inside a heavy-duty zipper seal bag, and stored on ice for transport to Gainesville. In a greenhouse, hydrilla samples were transferred to a large plastic tray (53 by 41 by 15 cm) containing Crystal River water and an aquarium aerator placed inside a screen cage (61 by 61 by 61 cm), and checked twice daily for adult emergence.

Newly emerged adults of the parental generation were aspirated from the screen cage, and allowed to mate. A single mated female was placed in an oviposition container made from an 88 by 50-mm (148-ml) plastic snap cap vial with a ventilation hole (12 mm
appropriate hydrilla strain (single neonate larva into a culture tube containing the larvae and pupae were determined by placing a corded. For each hydrilla strain, development times of adult stages were calculated, and mating and oviposition rates and development times for the immature and capacity for increase, $r_c$ (Laughlin 1965) and cohort emergence were recorded daily for each hydrilla strain. The date of pupation, adult emergence and sex $T_c$ (Bengston 1969) derived from data obtained on survival ($l_n$) and fecundity ($m_x$) of a cohort of females ($n = 13$) reared on the New Delhi strain of hydrilla were also computed.

To mass-rear $C. lebetis$, newly emerged adults were transferred with a mouth aspirator to a 250-ml separatory funnel capped with mosquito netting that was secured with a cylindrical Plexiglas plug. Compounds leached from hydrilla sprigs probably stimulate oviposition (J.P.C., unpublished data), so the separatory funnel was filled with $\approx$100 ml of well water obtained from a 3.8-liter glass jar containing fresh hydrilla sprigs. After the adults mated on the inner walls of the separatory funnel, the females oviposited on the surface of the hydrilla-treated water. The egg masses deposited either sank to the bottom near the stopcock, where they were collected by decanting the water along with the egg masses into a 148 ml plastic vial, or were attached to the inner wall of the separatory funnel at the water line. In the latter case, the egg masses were washed from the inner wall of the funnel with a wash bottle before initiating the decanting procedure.

A single egg mass containing $\approx$150–200 eggs was placed in a 3.8-liter glass jar containing hydrilla (New Delhi strain) and well water. The glass jar was covered with mosquito netting secured with an 84–180-mm adjustable metal clamp to confine emerging adults. The glass jars were held in a Florida Reach-In environmental chamber (Walker et al. 1993) at a constant temperature of $25 \pm 0.1^\circ$C, $75 \pm 0.1$% RH, and a photoperiod of 16:8 (L:D) h until a new generation of adults emerged.

**Biology of C. lebetis.** Observations were made on the egg, larva, pupa, and adult stages of $C. lebetis$. Survival rates and development times for the immature and adult stages were calculated, and mating and oviposition behavior, as well as female fecundity, were recorded. For each hydrilla strain, development times of the larvae and pupae were determined by placing a single neonate larva into a culture tube containing Crystal River water, and a 5-cm apical sprig of the appropriate hydrilla strain ($n = 40$, two replicates per strain). The date of pupation, adult emergence and sex were recorded daily for each hydrilla strain. The capacity for increase, $r_c$ (Laughlin 1965) and cohort generation time, $T_c$ (Bengston 1969) derived from data obtained on survival ($l_n$) and fecundity ($m_x$) of a cohort of females ($n = 13$) reared on the New Delhi strain of hydrilla were also computed.

**Instar Determination.** Larval head capsule width measurements were used to separate instars (McCawley 1974). To obtain a sufficient number of specimens for instar differentiation, larvae were reared on hydrilla according to the aforementioned procedures. Ten or more developing larvae were sacrificed daily during the course of their development to ensure that all instars would be represented in the sample of larvae to be measured ($n = 189$). After larvae were dissected from the plant tissue and preserved in 80% ETOH, they were positioned and examined under a dissecting microscope, and the head capsule widths were measured at the widest point with an ocular micrometer. Measurements of head capsule widths were plotted against the frequency of each measurement, and the resulting groupings were used to separate the instars (Oliver 1971).

Voucher specimens of the adults and immature stages of $C. lebetis$ were preserved in 80% ethanol and deposited in the Florida State Collection of Arthropods. In addition, a series of adults of both sexes, as well as samples of the immature stages, were sent to J. H. Epler (Crawfordville, FL) for identification and description of the immature stages (Epler et al. 2000).

**Data Analysis.** Unless indicated otherwise, data are reported as mean ± SEM.

**Results**

Seasonal distribution of $C. lebetis$ and plant damage. The seasonal abundance of larval and pupal stages of $C. lebetis$ and associated hydrilla tip damage during 1997 are shown in Fig. 1. Peak incidence of tip damage occurred in early summer (May and June) and again in the fall (October) when 44.8 ± 8.1% and 57.3 ± 2.1%, respectively, of the examined tips were destroyed by the feeding and mining activity of larvae. The larval population of the midge was highest during the spring and summer months, with a maximum abundance of 14.0 ± 2.1 larvae per sample in June. The extensive tip damage observed in October indicates that larvae were still actively feeding on hydrilla late in the year. The presence of pupae inside the shoot tips

**Fig. 1.** Seasonal abundance of larvae and pupae of *Cricotopus lebetis*, and percent tip damage on *Hydrilla verticillata*, Plantation Inn Canal, Citrus County, FL, 1997.
during each month of sampling except August, September and December suggested that the lifecycle of the midge was completed in 30 d or less. During the entire field season, hydrilla in the Plantation Inn Canal failed to grow to the surface, or "top-out," as expected except in the extremely shallow zone adjacent to the concrete seawall lining both sides of the canal.

Larval populations of *C. lebetis* and associated hydrilla tip damage during 1998 are shown in Fig. 2. Larval density and percentage of damaged hydrilla tips exhibited a bimodal pattern similar to that observed in 1997. The greatest tip damage occurred during June and November when 72% and 60%, respectively, of the examined tips were destroyed by the feeding and mining activity of the larvae. Larval density peaked at 10.9 \( \pm 2.9 \) larvae/\( m^2 \) in May, declined during the summer months, but rebounded in November to a maximum of 34.6 \( \pm 7.8 \) larvae/\( m^2 \). The density of *C. lebetis* larvae was probably higher than reported here because the sampling procedure employed extracted older instars more efficiently than younger ones. The hydrilla may have dried out too quickly during the extraction process, effectively trapping the younger instars before they could leave the plant tissue.

**Mass-Rearing of *C. lebetis***. Between 13 June and 27 August 1997, 17 field-collected early and late instars were transferred to individual culture tubes for adult emergence and subsequent identification. Five larvae (29%) entered the hydrilla sprig provided and completed their development. Two of them were quite small when they were transferred, presumably second instars. Both larvae caused extensive mining damage inside the hydrilla tip, and produced a considerable amount of frass that was pushed outside of the stem by the developing larvae. At this point, it was concluded that the larvae were indeed ingesting and using the living plant tissue of hydrilla as a food source. One of these larvae developed into a female in 9 d, the other into a male in 6 d.

After a positive association between the larval and adult stages of *C. lebetis* was made, a laboratory colony was initiated in September 1997 with a series of adults that emerged from the field-collected hydrilla in the greenhouse. Preliminary observations suggested that adult emergence probably occurs at night because new adults were usually observed in the screen cages in the morning, and only rarely in the afternoon when adult emergence was checked. In total 16 males and 11 females emerged between 26 September and 6 October 1997.

From June 1997 to July 1998, *C. lebetis* was continuously reared in the laboratory for 16 generations using the culture tube procedure (Fig. 3). Overall, the culture tube rearing technique produced 630 adults (343 males and 287 females) with a male:female sex ratio of 1.2:1. Peaks in adult emergence were observed approximately every fourth generation. The highest number of males produced by the culture tube method occurred in generations F2, F6, and F10, whereas generations F3, F6, F11, and F15 produced the highest number of females (Fig. 3). The females deposited a total of 12,113 eggs.

Midge production increased dramatically after the mass-rearing procedure was adopted. In total, 2,872 adults were produced in the laboratory from June to October 1999. During this 3-mo period, 1,669 males and 1,203 females emerged (male:female sex ratio, 1.4:1) with peak emergence occurring in week 8. Females \( (n = 697) \) produced 108,845 eggs, and mean number of eggs per mass was 156.2 \( \pm 3.3 \).

**Adult Stage**. Mating was first observed on 28 September 1997 at 1300 hours when a male and a female, collected from a screen cage, were confined in a vacuum tube after their removal from the mechanical aspirator. The mating process was the same as described by Oliver (1971), and indicated that male swarming behavior, essential for mating in many Chironomidae, was not required for this species. The adults mated on any suitable substrate, including the surface of the water, and copulation occurred in daylight although nocturnal mating cannot be ruled out. Mating adults that were observed in the laboratory remained in copula for 17.5 \( \pm 4.3 \) min (range, 5–68, \( n = 14 \)).

The adults are short-lived and do not feed, which is typical of many chironomids (Pinder 1986).
males and females lived an average of 1.3 ± 0.1 d (males: range 1–2, n = 29; females: range, 1–3, n = 29) (Table 1). The preoviposition period was 0.9 ± 0.3 d (range, 0.3–2, n = 27). Oviposition occurs on the surface of the water. The female inserts the tip of her abdomen beneath the surface and deposits an egg mass. Egg deposition was completed in 9.5 ± 0.5 min (range, 9–10, n = 2). As with most chironomids, females deposited a single egg mass, and died soon afterward (Finder 1986). The number of eggs per mass deposited by the females was 151.5 ± 8.4 (range, 50–250, n = 29), which was similar to the mean number obtained by mass rearing.

**Egg Stage.** The egg mass is linear shaped, and consists of one or two rows of eggs diagonally arranged inside a gelatinous tube that completely surrounds the egg string. The linear configuration of the egg mass is characteristic of other members of the Orthocladiinae (Oliver 1971). Manipulating the egg strings with a camel hair brush or forceps was impossible because the sheath of the gelatinous matrix surrounding the egg string was viscous, causing the egg strings to stick to any object with which they came in contact. The sticky gelatinous matrix probably affords some protection to the egg string and serves to anchor the eggs to hydilla shoots as the egg masses drift through the water column.

The eggs are white when first deposited, but after 24 h only the fertilized eggs turn grayish-brown in color while unfertilized eggs remain white and eventually disintegrate. Red eyespots of the neonate larvae appear immediately before eclosion. The dimensions of the individual eggs were 0.24 ± 0.02 by 0.10 ± 0.01 mm (n = 75). The duration of the egg stage was 2.0 d (Table 1), and the fertility rate 77.0%.

**Larval Stage.** Larval eclosion is synchronous. The neonates are very active but remain inside the gelatinous tube for several hours, crawling from one end of the tube to the other. They exit the gelatinous tube from one of its ends, or occasionally from the middle when exposed to low light conditions or complete darkness. It is unclear whether the first instars chew their way out of the tube or find a weak area in the wall and break through it. The larvae at this stage are nektonic and vulnerable to predation. However, their translucent color, small size, and delayed emergence from the gelatinous tube until stimulated by low light conditions may afford some protection from predators until they can locate and enter the plants’ shoot tips. Once inside the hydilla shoot tip, the larvae mine and feed on the vascular tissues of the apical meristem. The larvae begin to acquire their characteristic green body color with a blue band around the thorax during the second instar. As the larvae mature, their feeding activity creates a 1–2 cm tunnel inside the stems that eventually kills the shoot tips by inducing their abscission. The tunnel created by the feeding larva is not lined with silk but serves as a pupation site (Oliver 1971).

The occurrence of four instars appears to be almost universal in the Chironomidae (Oliver 1971, McCaul 1974, Armitage et al. 1995), and the larvae of C. lebetis are no exception. The first, second, third, and fourth instars are indicated by peak head capsule width values of 0.09, 0.15, 0.20, and 0.30 mm, respectively (Fig. 4). This number is in agreement with other members of the genus (MacRae and Ring 1993) and subfamily Orthocladiinae (Oliver 1971). The duration of the larval stadium ranged from 12.9 to 15.3 d depending upon the strain of hydilla (Table 1).

**Pupal Stage.** Pupation occurs inside the hydilla stem, and the pupa is of the sedentary type (Oliver 1971). Preparation of the pupal case by the mature larva induces shoot tip abscission. Before pupation, the last instar completely severs the tip of the stem to create an escape route for the pharate adult, and caps the opening of the tunnel with cellulose fibers excreted from the wall of the stem. The mature pupa exits the stem by repeatedly undulating its abdomen to break through the fibrous cap, and slowly swims to the surface where adult eclosion occurs aided by an air bubble released inside the pupal exuvium. This is probably the most critical period in the life cycle of the midge because the ascending pupae are no longer afforded the protection of the stem and consequently

**Table 1. Duration (in days) of the immature stages of Cricotopus lebetis reared on hydrilla at 27°C and 16:8 [L:D] hr photoperiod.**

<table>
<thead>
<tr>
<th>Stage</th>
<th>n</th>
<th>Mean (± SEM)</th>
<th>Range</th>
<th>Cumulative mean age</th>
</tr>
</thead>
<tbody>
<tr>
<td>Egg</td>
<td>24</td>
<td>2.0 (0.1)</td>
<td>1–3</td>
<td>20</td>
</tr>
<tr>
<td>Larva</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FL</td>
<td>14</td>
<td>12.9 (0.7)</td>
<td>9–17</td>
<td>14.9</td>
</tr>
<tr>
<td>ND</td>
<td>56</td>
<td>14.2 (0.4)</td>
<td>10–21</td>
<td>16.2</td>
</tr>
<tr>
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<td>4</td>
<td>14.5 (1.0)</td>
<td>12–17</td>
<td>16.5</td>
</tr>
<tr>
<td>TC</td>
<td>7</td>
<td>15.3 (2.0)</td>
<td>10–22</td>
<td>17.3</td>
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<tr>
<td>Pupa</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>1.3 (0.1)</td>
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<tr>
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<td>1.8 (0.2)</td>
<td>1–2</td>
<td>19.1</td>
</tr>
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</table>

* Calculated for each hydilla strain.
* FL = Florida, ND = New Delhi, BD = Burundi, TC = Tissue Culture.

![Fig. 4. Head capsule width measurements of larvae of Cricotopus lebetis Sublette. Peaks within groupings indicate four instars (20 ocular units = 0.6 mm).](https://academic.oup.com/aesa/article-abstract/95/5/587/28698)
are vulnerable to predation (Oliver 1971). The duration of the pupal stadium ranged from 1.0 to 1.8 d (Table 1).

**Host Suitability.** Data on the suitability of three different hydrilla strains (Florida, New Delhi, Burundi), as well as the tissue cultured Florida strain, to support complete development of the midge, are presented in Figs. 5 and 6. Larvae completed their development on each of the four types of hydrilla tested. Survival of *C. lebetis* was higher on the New Delhi strain despite the fact this strain of hydrilla presumably does not occur in the United States. On day 13, a survival rate of 60% was observed on the New Delhi strain compared to 30% for the other types of hydrilla (Fig. 5). The suitability of the New Delhi strain of hydrilla as a host for the midge also is reflected in the greater number of adults produced on this strain (males, 13.0 ± 2.0; females, 13.0 ± 1.0) (Fig. 6). Complete development of the midge was lowest on the Burundi strain; only a few males were produced.

**Generation Time and Rate of Increase.** The capacity for increase, r<sub>c</sub>, was computed for *C. lebetis* to determine how fast the midge would multiply following its release into a new environment unchecked by abiotic and biotic mortality factors. The capacity for increase was calculated using laboratory data obtained on duration and survival of the immature stages (larvae and pupae), and oviposition and survival of females reared on the New Delhi strain of hydrilla (Fig. 7). The rate of increase for *C. lebetis* was calculated only for the New Delhi strain because of the higher survival on this particular strain. The net reproductive rate (R<sub>o</sub>), or the number of times each female midge multiplies per generation, was 28.8. The cohort generation time (T<sub>c</sub>), or the mean age of the females at the birth of female offspring, was 14.4 d. Therefore, the capacity for *C. lebetis* to increase each generation (r<sub>c</sub>) was calculated to be 0.23. The number of times the midge would multiply per day (the finite rate of increase, l) was 1.26 and its population would double every 3.01 d.

**Discussion**

Larvae of most chironomids are microphagous, feeding on algae and detritus. However, a few genera in the subfamilies Orthocladiinae and Chironominae include species that are 'obligate phytophages' as defined by Armitage et al. (1995), mining the soft tissues of submersed macrophytes and using the living plant material as a food source (Oliver 1971, Pinder 1986). Recently, this feeding strategy has been studied in some detail in the Orthocladiine genus *Cricotopus* because of the possibility that it could be exploited for the biological control of Eurasian watermilfoil (Kangasniemi and Oliver 1983; MacRae et al. 1989, 1990; MacRae and Ring 1993).

The 1992 discovery at Crystal River, FL, of another species of *Cricotopus* whose larvae mine the shoot tips of hydrilla is significant not only because feeding on
living tissue is rare among the Chironomidae (Armitage et al. 1995), but this finding has important implications for biological control of hydrilla. Previous research implicated midge larvae as causal agents of damaged stem tips on stunted hydrilla plants in Africa (Pemberton 1980, Markham 1986). Therefore, it is conceivable C. lebetis may be an important biotic factor contributing to the declines of hydrilla periodically observed in Kings Bay. Hydrilla mats were rarely observed at the Plantation Inn Canal study site during the course of this study. In addition, the majority of the hydrilla plants examined in the field as well as in the laboratory were missing their shoot tips as a result of midge-feeding damage. During the 2-yr field study, the hydrilla plants at Crystal River consistently exhibited basal branching, and appeared stunted during low-tide conditions when the samples were collected.

Three hypotheses are offered to explain the rather sudden appearance and successful establishment of this hydrilla-attacking midge in Florida: (1) C. lebetis was not detected in earlier surveys of the insects associated with hydrilla in the southeastern United States conducted by Balciunas and Minno (1985). However, the unique color pattern of the larvae and obvious feeding damage do not support this hypothesis. (2) The midge is a native species that developed a ‘new association’ with hydrilla. To reject this hypothesis, extensive field surveys on resident submerged macrophytes or host-range testing that were beyond the scope of this study would be required to determine whether the midge is a generalist on other native aquatic macrophytes or a specialist on hydrilla. (3) C. lebetis is an adventive species that arrived in Florida subsequent to the surveys of Balciunas and Minno (1985).

The anecdotal evidence supports the adventive species hypothesis. Cricotopus lebetis could have immigrated to Florida in the 1980s as a contaminant of imported aquarium plants. In the 1970s, another hydrilla-attacking insect [Paraponyx diminutalis (Snellen) (Lepidoptera: Crambidae)] successfully hitchhiked to Florida in this manner (Delfosse et al. 1976, Buckingham and Bennett 1989). There also appears to be some uncertainty about the origin of the Nearctic Cricotopus taxa. According to Epler et al. (2000), the chironomid genus Cricotopus contains many undescribed species, particularly in the Nearctic Region. Many of the Nearctic taxa are poorly known, and some may actually be conspecific to originally described Palearctic species (Epler et al. 2000). For example, C. myriophylli Oliver, a congener of C. lebetis, was discovered attacking Eurasian watermilfoil in British Columbia, Canada (Kangasniemi and Oliver 1983, Oliver 1984). Cricotopus myriophylli was thought to be an indigenous species (Newman et al. 1996, 1998) although its limited distribution in North America and the results of host range tests suggested otherwise (MacRae et al. 1990, MacRae and Ring 1993, Buckingham 1994). Buckingham (1998) discovered the native range of this midge species when he collected larvae on Eurasian watermilfoil in the People’s Republic of China that were later identified as C. myriophylli.

Perhaps the most compelling evidence to support the adventive species hypothesis involves the circumstances surrounding the appearance of the midge and hydrilla in the state of Louisiana. Cricotopus lebetis was originally described in 1964 from adult specimens collected in 1957–1959 from Natchitoches, LA (Epler et al. 2000). Hydrilla, unknown as host plant of C. lebetis until this study, was first detected in Louisiana in 1973 in Sibley Lake, a municipal reservoir for the City of Natchitoches (Johnson and Manning 1974). This coincidence suggests that the midge might have entered the United States as a contaminant of hydrilla.

The relatively constant water temperature of 25°C maintained year-round in Kings Bay (Mataraza et al. 1999) is a unique feature of Florida’s spring-fed water bodies. According to MacRae and Ring (1993), some larval midges are unable to tolerate high-water temperatures. If C. lebetis is one of these temperature-sensitive species, elevated water temperatures may limit its distribution in Florida. Most water bodies infested with hydrilla in Florida that are not spring fed usually exhibit extreme temperatures during the summer months, often exceeding 40°C in the hydrilla mat (J.P.C., unpublished data). Eutrophic conditions in many of Florida’s hydrilla-infested waterbodies may also limit its establishment and spread (A. Ali, personal communication).

The discovery that adults of C. lebetis were capable of mating without swarming was a critical behavioral aspect that contributed to the success of the laboratory rearing and colonization phase of this study. In many species of chironomids, including the closely related milfoil midge C. myriophilli, male swarming is essential for successful reproduction (MacRae et al. 1989). Identifying and duplicating the proper conditions for inducing male swarming behavior in a confined laboratory environment would have been a daunting, if not impossible, task if C. lebetis exhibited this behavior.

The ability to successfully rear the midge in the laboratory also contributed to its identification. Cricotopus lebetis could not have been positively identified to species without using the characters associated with the larval and pupal stages provided by laboratory rearing (Epler et al. 2000). Two different techniques were developed for rearing the hydrilla tip midge C. lebetis. The culture tube procedure proved useful for studying the life cycle and biology of C. lebetis in the laboratory but was labor-intensive. The development of a mass-rearing technique increased rearing efficiency by eliminating the necessity of pairing adults to induce mating, and reduced the handling time for collecting the egg strings deposited by the females.

Four insects from the native range of hydrilla have been introduced into the United States for classical biological control of this highly invasive aquatic plant (Buckingham 1994). To date, none of these insects has effectively controlled hydrilla (Grodowitz et al. 1997, Forno and Julien 2000, Wheeler and Center 2001), although one insect [Hydrellia pakistanae Deonier
(Diptera: Ephydridae) is widely established (Center et al. 1997). Wheeler and Center (2001) suggested that future biological control candidates not only should be host specific but capable of developing completely on submersed hydrilla. Cricotopus lebetis is an herbivorous midge possibly new to Florida (Epler et al. 2000) whose larval and pupal stages develop entirely on submersed hydrilla shoot tips. If it can be demonstrated experimentally that C. lebetis is capable of suppressing hydrilla shoot growth and has a narrow host range, then this insect could be mass-reared and used in an augmentation program against hydrilla in other spring-fed water bodies in the United States, or become a candidate for classical biological control in other countries where hydrilla is considered an invasive weed and the limnological conditions are conducive to its establishment.

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References Cited


Markham, R. H. 1986. Biological control agents of Hydrilla verticillata, final report on surveys in East Africa. Miscel-


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