TEMPERATURE DEPENDENT DEVELOPMENT, HOST RANGE, AND DISTRIBUTION OF *CRICOTOPUS LEBETIS* (DIPTERA: CHIRONOMIDAE), A NATURAL EMEMY OF *HYDRILLA VERTICILLATA* (HYDROCHARITACEAE) IN FLORIDA

By

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I would like to thank all who encouraged my intellectual curiosities, especially my advisors Bill Overholt and Jim Cuda. This is also dedicated to my mother and father who have supported my academic interests all throughout my collegiate career. Without these people, this milestone would not have been possible.

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A chironomid midge, *Cricotopus lebetis* Sublette (Diptera: Chironomidae), was discovered feeding on hydrilla in Crystal River, Citrus Co., Florida in 1992, and may be a recent introduction into Florida. Larvae of the midge mine the apical meristems of hydrilla, causing basal branching and stunting of the plant. We investigated the distribution, temperature-dependent development and host range of the midge. The midge was found in a four of six Florida water bodies surveyed, but it was rarely abundant. The relationship of temperature to larval-pupal development revealed that midge survival was highest at temperatures between 20 and 30°C, and the developmental rate increased with increasing temperature. Results of laboratory host range studies showed that the fundamental host range of *C. lebetis* included not only hydrilla, but a variety of aquatic plants in several different families, suggesting that this insect may not be a hydrilla specialist. Dual-choice tests with adult females demonstrated that *C. lebetis* exhibited a preference for certain host plants, and that adults are responsible for choosing suitable sites for larval development. Results from

the survey work indicated that *C. lebetis* is present in several different water bodies throughout Florida, but the factors responsible for its current distribution remain unknown. The results obtained in this thesis provide a better understanding of the abiotic and biotic factors influencing the biology of *C. lebetis* and its potential use as an augmentative biological control agent. This information will be used to determine how *C. lebetis* can be exploited in developing long-term management strategies for hydrilla in Florida.

CHAPTER 1
LITERATURE REVIEW

Introduction

Invasive species have the capability to disrupt natural ecosystems (Dukes and Mooney, 2004). Florida, with a unique island type biogeography, is particularly vulnerable to invasion by non-indigenous plant species. Florida is similar to an island because it is surrounded on three sides by water and the fourth side by a freeze line. Islands are known to have a depauperate flora and fauna, and thus have less biotic resistance to invasion by exotic organisms (Simberloff et al., 1997). It is estimated that 1,400 exotic plant species have been naturalized in south Florida alone. Approximately 70 of these introduced species have become problematic and require extensive management (Rodgers et al., 2011). Invasive species pose ecological threats, and costs associated with invasive weeds in the USA are estimated to be $35 billion annually, with $110 million attributed to aquatic weeds (Pimentel et al., 2005).

Management of invasive plants relies to a large extent on the use of chemical herbicides, which can impact hydrologic systems, recreation, crop irrigation, drinking water, aquatic organisms, and aquaculture (USGS, 2006). Herbicides can be non-selective, cause reoccurring costs to managers, and the use of herbicides often lacks public support. Several common herbicides have been detected in sediments, and fish and mollusk populations in both urban and agricultural streams (USGS, 2006). Moreover, several plant populations have become resistant to chemical herbicides (Holt and Lebaron, 1990; Powles and Yu, 2010).

Biological control may provide an alternative to herbicide application, and can be used as part of an Integrated Pest Management (IPM) plan. Classical biological control

is based on the premise that plant populations are suppressed in their native ranges by the action of natural enemies (Williams, 1954; Keane and Crawley, 2002). When a plant is moved to a new area of the world, it escapes regulation by specialized natural enemies and is able to reach higher densities than found in its aboriginal home (Wolfe, 2002; Torchin et al., 2003). The process of biological control involves searching for host specific natural enemies in the native range of the weed species, and releasing the natural enemies in the invasive range of the weed. When successful, biological control is an economically efficient and sustainable method to manage invasive plants. Biological control often is used as a component in an IPM program along with mechanical and chemical methods.

Hydrilla verticillata (L.f. Royle) has been a problematic aquatic weed since its introduction to the United States in the 1950s (Schmitz et al., 1991), and has been the focus of biological control exploration since the 1970s (Balciunas and Minno, 1985; Buckingham, 1994). Augmentative biological control is mass rearing and releasing insects to reduce pest populations. This project focused specifically on a tip mining midge *Cricotopus lebetis* Sublette (Diptera: Chironomidae) as a potential augmentative biological control agent of hydrilla.

Background

Biological Control

Invasive species pose a threat to the biodiversity of ecosystems. Invasive plants compete directly with native vegetation and cause problems further up the food chain to both invertebrates and vertebrates (Dukes and Mooney, 2004). Classical biological control is regarded as a practical and affordable way to manage invasive weed species in order to reduce the risks that unwanted species pose to natural ecosystems. Other

benefits of biological control are that it can be effective on its own, or can be used in conjunction with other control methods. Biological control may be particularly appropriate for sensitive or conservation areas where mechanical/chemical controls are not permissible or are ineffective (Julien et al., 2007). When proper research methodologies are followed, biological control can be successful, and classical biological control is considered the most successful method of biological control. Of the biological control agents released, 60% have established, and 33% of those have resulted in some degree of control (McFayden, 1998).

Knowledge about a biological control agent can make the difference between successful and unsuccessful establishment in a new environment. The biology and life cycle of the candidate agent must be fully understood before its potential as a biological control agent can be evaluated. Some core questions that must be considered with any weed biological control agent are: 1) Is the species host specific; 2) Will the agent reach densities high enough to have a significant impact on the target plant; 3) Will the agent be able to perform over a wide range of environmental conditions; 4) How will the success of the biological control agent be measured (Julien et al., 2007). Knowing the answers to these questions will increase the success rate of biological control.

Because of increasing concern about the risks associated with biological control, careful risk assessment is essential for obtaining approval for release of a new biological control agent. The historical record indicates that very few biological control agents have had adverse effects, such as negative impacts to human health, non-target effects to native species, enhancing pest species, and becoming pests themselves (Howarth, 1991; Louda et al., 2003). Risk assessment of biological control agents is

difficult because of the complexity of predicting community and ecosystem wide impacts. Developing cost-benefit and risk assessments for every biological control agent would minimize these deleterious effects and improve the overall efficacy of classical biological control (Simberloff and Stiling, 1996).

Proper assessment of host range is an essential component in biological control programs. Host range testing reveals if a potential biological control agent is sufficiently specialized on the target plant to receive approval for release. Host range evaluation involves determining the fundamental host range of the organism and predicting its field host specificity (Sheppard et al., 2005).

Hydrilla verticillata

Description

Hydrilla is a submersed rooted aquatic weed in the family Hydrocharitaceae. Hydrilla can be either monoecious or dioecious. Stems are long and slender with some branching. The leaves are small (20 mm long and 4 mm wide), lanceolate, and occur in whorls of 3-8. The midrib is distinct and sometimes bears small spines. Male flowers are solitary and released underwater as buds, which float and open at the surface of the water. Female flowers are inconspicuous with three transparent petals occasionally with reddish streaks (Cook and Lüönd, 1982).

Distribution

Hydrilla is widely distributed and occurs in Europe, Asia, Australia, New Zealand, Pacific islands, Africa, South America, and North America. Hydrilla occurs in temperate areas, but thrives in tropical regions (Langeland, 1996).

Introduction of hydrilla into the United States

There have been two separate introductions of hydrilla into the USA, with a dioecious form found in the southeast and parts of California, and a monoecious form found in the northeast, California and Washington (Steward et al., 1984; Madeira et al., 2000). Only the dioecious female biotype is known to occur in Florida. The dioecious form was imported into the USA in the late 1950s from Sri Lanka through the aquarium trade and rapidly spread throughout Florida during the 1960s and the rest of the southeastern USA in the 1970s (Schmitz, 1991). The pathway of introduction of the monoecious form into the northeastern USA is unknown. The USA dioecious population is most closely related to a population found in Bangalore, India whereas the USA monoecious population is closely related to plants from Seoul, Korea (Madeira et al., 1997). A major concern is that hydrilla will continue to spread in the northern USA and cause problems similar to those it has created in the southern USA. Ten years after hydrilla's introduction into Florida, it was established in major water bodies of all drainage basins in Florida (Langeland, 1996). Outside of Florida, hydrilla has spread along the Gulf Coast, extending up the Atlantic Coast to Maryland and Delaware, and into the western states of California, Arizona, and Washington (Madeira et al., 2000).

Negative effects

There are contradictory research findings about the impact of hydrilla on native communities (Haller and Sutton, 1975; Hoyer et al., 2008). Haller and Sutton (1975) found that hydrilla displaces native vegetation such as *Vallisneria neotropicalis* Marie-Vict), whereas Hoyer et al. (2008) indicated that it has no significant impact on native plant and animal diversity, species richness, and abundance because it has occupied a vacant niche (Hoyer et al., 2008). Hydrilla has the ability to grow in shallow waters to

depths of 20 feet or greater. It also grows rapidly and can effectively compete for sunlight. Once it reaches the surface, hydrilla branches profusely producing a dense mat that shades out native vegetation (Langeland, 1996).

In highly infested areas, hydrilla can create dense mats of vegetation impeding boat traffic, and it often becomes entangled in boat propellers, thus facilitating its movement to new locations within water bodies, and to new water bodies. Hydrilla can produce a large quantity of propagules and meristems, which gives it a competitive advantage over native plants. The dense mats of hydrilla support filamentous algae and small invertebrates, which further limit light penetration (Haller and Sutton, 1975). Although hydrilla may provide beneficial effects for a select number of aquatic species, deleterious effects may occur to native flora. In drainage canals, hydrilla can greatly reduce flow and cause flooding and canal damage. Negative effects associated with hydrilla invasion include interference with recreational and commercial use of lakes, tourism and sportfishing, reduction of real estate values, reduction of flow in drainage canals, and clogging of intake pipes (Schmitz et al., 1991; Langeland, 1996).

Beneficial effects

While hydrilla continues to plague Florida's waterways, there is some opposition to its management. Hydrilla provides shelter, breeding and oviposition sites for many aquatic organisms. Hydrilla attracts many small invertebrates, but relatively few of these species use living hydrilla tissue as a food source (Balciunas and Minno, 1985). Waterfowl, most commonly coots (*Fulica americana* Gmelin) and ringneck ducks (*Aythya collaris* Donovan), consume the stems, leaves and tubers of hydrilla as a food source (Esler, 1989). Studies have shown that hydrilla supported a high diversity of duck species, and increases in hydrilla were correlated with increases in waterfowl

populations (Esler, 1990). Waterfowl enthusiasts argue that protection of hydrilla is important to the maintenance of healthy waterfowl populations (Johnson and Montalbano, 1984).

Largemouth bass anglers also argue that there is a positive relationship between aquatic vegetation and favorable bass fishing. Dense stands of aquatic vegetation can provide habitats for invertebrates, which are consumed by sportfish (Moxley and Langford, 1982). Among anglers, largemouth bass fishermen are most opposed to vegetation management, and some would prefer to have more aquatic vegetation (Slipke et al., 1998). Highly dense patches of vegetation may negatively impact bass populations, but maintaining moderate levels of vegetation can prove to be beneficial (Brown and Maceina, 2002). Largemouth bass anglers often view aquatic vegetation as beneficial to good quality fishing regardless of the extent of the coverage (Wilde et al., 1992; Slipke et al., 1998).

The everglade snail kite (*Rostrhamus sociabilis plumbeus*) is a federally listed endangered species. Snail kites utilize the exotic apple snail (*Pomacea insularum*) as a food source. Foraging snail kites require snails within 6 inches of the surface, and snails nested in the top of hydrilla plants can be easily captured. The exotic apple snail has invaded Lake Toho, and snail kite populations have increased since its introduction. Since the snail kite has benefited from exotic apple snails nested in hydrilla, treatments of hydrilla in Lake Toho have been reduced (FWS, 2010).

Hydrilla's positive and negative effects give rise to conflicts of interest in creating management solutions for this invasive weed. Finding balance between management solutions that seek to greatly reduce the abundance of hydrilla, and solutions that seek

to manage hydrilla in ways to benefit waterfowl populations, is a difficult task. Those who favor little management of hydrilla to protect waterfowl habitat see the current hydrilla management measures as potentially harmful (Johnson and Montalbano, 1987). Control strategies that allow maintenance of some hydrilla, while reducing its adverse effects, are needed. Biological control may prove to be a solution acceptable to both sides, because biological control does not seek complete eradication of a weed species, but rather a reduction in density.

Hydrilla Control

Mechanical and Chemical Control

Due to the diversity of water uses in Florida, effective control of hydrilla is difficult to achieve because of a very limited number of environmentally sound options (Hoyer et al., 2005). Efforts to control hydrilla rely primarily on the application of synthetic herbicides, the most common of which used to be fluridone. Fluridone received approval by the EPA in 1986 (Arias et al., 2005) and until 2004 was used to control hydrilla with little effect on native vegetation (Doong et al., 1993). Fluridone is a phytoene desaturase (PDS) inhibitor, and under high light intensity causes bleaching of the green photosynthetic tissues (Chamovitz et al., 1993; Boger and Sandman, 1998). With millions of dollars spent annually on herbicide applications, this may not be the most sustainable or economically efficient manner to control hydrilla.

Recently, fluridone resistance has been documented at several locations in central Florida (Michel et al., 2004). Typically, resistance does not occur in plants that do not reproduce sexually (Maxwell and Mortimor, 1994), because recombination of alleles and selection of herbicide resistant genes occurs more frequently in sexually reproducing plants. All known hydrilla in Florida is dioecious female, and reproduces asexually by

subterranean tubers, turions, root crowns, and stem pieces. A mutation in the PDS gene and subsequent selection for resistance is thought to be responsible for the observed herbicide resistance (Chamovitz et al., 1993; Michel et al., 2004). Endoreduplication, which causes variable ploidy levels in hydrilla, can result in gene duplication. The presence of these multiple copies of alleles may enhance the plant's ability to adapt to its environment and may have contributed to the rapid development of herbicide resistance (Puri et al., 2007).

Fluridone resistance has resulted in the inability to control large infestations of hydrilla with the herbicide (Michel et al., 2004). Increased amounts of fluridone must be applied in order to manage resistant populations. Alternative herbicides such as endothall, diquat, and chelated copper have replaced fluridone. Endothall is now the most commonly used herbicide to control hydrilla; however, it is reported to provide only 4-8 months of control compared to 1-2 years of control with fluridone (Hoyer et al., 2005). In order to be effective, endothall must be applied in high concentrations, and requires a long exposure time (Netherland et al., 1991). Heavy reliance on endothall for hydrilla control is cause for concern because over time resistance to endothall may also develop, and increased tolerance to endothall has been recorded in Lake Maitland. (Mike Netherland, personal communication). Moreover, fluridone replacement herbicides are more expensive and treatment can range from $700-1600 per ha, compared to treatment costs ranging from $125-600 per ha for fluridone (Arias et al., 2005).

Biological Control

Biological control of hydrilla has been investigated since the 1970s. Several natural enemies have been identified, a few have been released, but none have been

able to provide long-term control. Exploration for natural enemies has been conducted in Asia, Australia, and Africa (Buckingham, 1994; Balciunas et al., 2003; Overholt and Cuda, 2005). Insects from these locations were brought back to Florida for additional research, and four were approved for release, including, two *Bagous* spp. weevils and two ephydrid flies in the genus *Hydrellia,* (Buckingham, 1988; O'Brien and Pajni, 1989; Buckingham and Bennett, 2001).

Bagous affinis Hustache (Coleoptera: Curculionidae) is a weevil from India that was first introduced into Florida in 1987 (Buckingham, 1988). During the dry season, larvae feed on exposed vegetation during low water conditions resulting in reduced sprouting rates of tubers (Buckingham 1988; Godfrey and Anderson, 1994). The larvae burrow into the soil and feed on below ground tubers, and in doing so, they prevent the tubers from sprouting. Due to their unique adaptation to seasonal flooding and drought cycles in their native range, which do not occur in Florida, permanent populations of *B. affinis* failed to establish (Godfrey et al., 1994).

Bagous hydrillae O'Brien (Coleoptera: Curculionidae) is a weevil from Australia that feeds on hydrilla stems. Adults feed on exposed stems and leaves whereas larvae feed and develop only inside submersed stems. During larval development, they fragment the stem and larvae continue to feed on the stem until they reach maturity (Buckingham and Balciunas, 1994). *Bagous hydrillae* was first introduced in Florida in 1991 but failed to establish (Balciunas and Purcell, 1991). Flooding and drought are thought to be major abiotic mortality factors of this biological control agent (Buckingham and Balciunas, 1994).

Of the four insects approved for release, only one ephydrid fly in the genus *Hydrellia* established in Florida, namely *Hydrellia pakistanae* Deonier (Diptera: Ephydridae). Another ephydrid, *Hydrellia balciunasi* Bock (Diptera: Ephydridae), established in Texas, but failed to establish in Florida (Center et al., 1997; Grodowitz et al., 1997). In a laboratory setting, *Hydrellia pakistanae* consumed 60-70% of hydrilla apical leaves. Field studies revealed that *H. pakistanae* populations never reached high enough densities to cause the level of damage observed in the laboratory, and the highest whorl damage in the field was 15%. Field studies also revealed that 100% larval mortality occurred at 36°C, which greatly compromised the effectiveness during warm summer months (Cuda et al., 2008; Wheeler and Center, 2001).

An adventive Asian insect, *Parapoynx diminutalis,* Snellen (Lepidoptera: Pyralidae), was discovered feeding on hydrilla in Fort Lauderdale, Florida in 1976 (Del Fosse et al., 1976). The larvae make cases out of their host plants, attach themselves to the leaf surface and feed on the leaves and stems of hydrilla (Buckingham and Bennett, 1996). Host range testing of this aquatic moth revealed that it fed on a variety of aquatic plants, and therefore was not evaluated further for its potential for augmentative biological control (Buckingham and Bennett, 2001). The moth can still be found in Florida, although it was never purposely released.

In addition to insects, grass carp (*Ctenopharyngodon idella* Val.) have been examined as augmentative biological control agents of hydrilla. Although not host specific, grass carp preferentially feed on hydrilla stems and leaves (Sutton and Vandiver, 1986). Small grass carp can consume quantities as great as their body weight per day, and 8-10% of body weight of larger fish is due to hydrilla consumption

(Shireman and Maceina, 1981). Grass carp are exotic, and after their introduction into the United States, they spread faster than any other introduced fish, and in many cases are considered a nuisance species (Guillory and Gasaway, 1978). Triploid carp, which are functionally sterile, were produced in order to preclude reproduction of individuals released for vegetation control. Stocking water bodies with sterile grass carp can result in the removal of all aquatic vegetation, even in large systems (Klussmann et al., 1988). When properly stocked, grass carp can provide long-term continuous control of aquatic vegetation, but the probability of predicting vegetation changes following a grass carp release is very low due to dynamic processes (Pípalová, 2006).

Although various biological control measures have been investigated, these efforts have met little success in the field, with the possible exception of sterile grass carp. Fluridone resistance has created a renewed interest in searching for new biological control agents of hydrilla (Overholt and Cuda 2005; Cuda et al., 2008). Chemical, physical, and mechanical methods for controlling hydrilla provide only short-term control and are expensive. The discovery of a viable biological control agent may be the key to successful long-term, environmentally sustainable management of hydrilla populations.

Cricotopus lebetis – The Hydrilla Tip Miner

Chironomidae is a dipteran family of non-biting midges. They have a cosmopolitan distribution, and inhabit almost any habitat that is aquatic or wet (Wirth, 1949). The majority of chironomids are microphagous; feeding on algae, animals and detritus, (Oliver, 1971). There are two types of larvae, free living and sedentary. The sedentary types construct and pupate in protective cases. When the pupa is ready to emerge as an adult, it rises to the surface of the water and adult eclosion occurs. This is a critical time in the midge's life because it is susceptible to heavy predation (Oliver, 1971). The

adults live for a short period of time and have reduced non-feeding mouthparts. The main function of the adult life is mating and reproduction.

Water quality is an important variable in determining midge densities and distributions. Due to their sensitivity to water quality, chironomid communities have been used as indicators of water quality and pollution (Saether, 1979). Chironomids also make good water quality indicators because of their widespread distribution in water bodies of varying quality, and their habitat preferences (Paine and Gaufin, 1956). Runoff from surrounding land-uses can contain harmful pesticides that impact water quality. Chironomids have been shown to be very sensitive to the presence of pyrethroid pesticides (Anderson, 1989). Given that certain midge species are sensitive to water quality, this factor should be taken into consideration when developing biological control programs that rely on midges.

A chironomid midge was first discovered in 1992 in Kings Bay attacking the stems of hydrilla (Cuda et al., 2002). The hydrilla was stunted and did not reach the surface as is typical of hydrilla. The origins of this insect are unknown. It could have been introduced with hydrilla, or could be a native species that expanded its host range to utilize hydrilla. The larvae, which can easily be recognized by the diagnostic blue band around the second and third thoracic segments, was identified as *Cricotopus lebetis* Sublette (Epler et al., 2000) (Fig. 1-1) The following description of adults is provided by Epler et al. (2000).

> "Color: In life, pale green with blackish-brown markings; these colors fade to pale brown/stramineous with dark brown to brownish markings in alcohol preserved material. In alcohol preserved material, brown to dark brown on antennae, head, throacic vittae (vittae sometimes jointed posteriorly by diffuse brown area), scutellum, postnotum, median anepisterum II and approximate ventral half of epimeron. Wings clear with light brown veins;

halteres pale. Legs with fore and hind coxae light, mid coxa brown; all trochanters light; fore femur light brown basally, much darker in apical $^{1}/_{3}$ to ½; mid and hind femora basally light with brown apical $^{1}/_{3}$ to ¼; tabiae with brown basal and apical bands, fore tibia slightly darker in middle than mid and hind tabiae; fore tarsi brown, mid and hind tarsi light brown to stramineous. (Fig. 1-2)"

Larvae mine into the apical stem of hydrilla and feed on meristematic tissues. In preparation for pupation, the larvae mine into the stem, and eventually cause abscission of the tip (Cuda et al., 2002) (Fig. 1-3). *Cricotopus lebetis* has only been found attacking hydrilla, except for one specimen collected from a *Potamogeton* sp. (Dana Denson, personal communication) and therefore may have a limited host range, but testing is needed to confirm this hypothesis*. Cricotopus lebetis* may have value as an augmentative biological control agent because it prevents hydrilla from reaching the surface. Preventing hydrilla from 'topping out' would improve boat navigation and water flow, and decrease the likelihood of hydrilla becoming entangled in boat propellers and thereby spreading to new locations (Fig. 1-4). This type of damage has the potential to increase light penetration, thereby increasing the overall biodiversity of the community.

Unlike most other chironomid species, *C. lebetis* feeds on living plant tissues, as opposed to detritus and other substrates. Feeding on living plant tissue is rare, but has been previously observed in *Cricotopus myriophylli* Oliver, a congener that feeds on the invasive aquatic plant Eurasian milfoil (*Myriophyllum spicatum* L.). Studies indicated that *C. myriophylli* had a narrow host range and only fed on two milfoil species (Macrae et al., 1990). Since one species of *Cricotopus* exhibited a degree of host specificity, the host range of *C. lebetis* may also be restricted to one or a few species. The first record of this insect was in 1957 in Louisiana (Sublette, 1964), but hydrilla was not observed in Louisiana until the 1970s (Cook and Lüönd, 1982), which suggests that either hydrilla

arrived in Louisiana earlier than thought, or the midge has hosts other than hydrilla. Water quality variables also may play a key role in determining the distribution of the midge. Conducting surveys in lakes across Florida, and obtaining water quality parameters may help explain the presence or absence of the midge in certain water bodies.

There are various factors affecting the success of an introduction of a new organism. One of the most important factors is temperature. Understanding the thermal requirements of an insect can help in understanding the potential areas for establishment of *C. lebetis.* Among the outcomes of the temperature-dependent development studies are degree-day requirements, which are the degree-days that are required to complete one generation, and the establishment of upper and lower temperature thresholds for development. Integration of temperature developmental data with Geographical Information Systems (GIS) interpolation functions allows for production of maps showing the number of generations of *C. lebetis* may experience in Florida. Cold tolerance studies reveal whether or not an organism is tolerant to cold temperatures, and using this data, isothermal lines can be generated to estimate the limits of northern distributions.

With the exception of basic information on its biology (Cuda et al., 2002) and impact on hydrilla (Schmid, et al., 2010; Cuda et al., 2011), there is little information available on the insect's host range, temperature tolerances, and responses to variation in water quality. Characterization of these factors may help determine whether this midge has the potential to be a successful biological control agent of hydrilla. Once research has been conducted, an IPM program for hydrilla can be developed,

incorporating augmentative release of *C. lebetis* if appropriate. If the midge is shown to be highly host specific, then it may be possible to receive approval for release of the midge in states where it does not occur.

Goals and Hypotheses

The overall goal of this study was to better understand the biology, host specificity, and habitat requirements of *C. lebetis.* A series of experiments was conducted in the laboratory, greenhouse, and under field conditions to assess the following hypotheses:

Hypothesis 1: Water temperature is a determining factor for survival and development of *C. lebetis.*

Objective 1: Determine the influence of temperature on developmental rate and survival of *C. lebetis*

Objective 2: Generate a map predicting the number of generations/year of *C. lebetis* across Florida.

Objective 3: Predict the USA distribution of the midge based on its physiological tolerance to cold.

Hypothesis 2: *Cricotopus lebetis* is a preferential tip miner of *Hydrilla verticillata.*

Objective 1: Determine the host range of *C. lebetis* under no-choice and choice conditions.

Hypothesis 3: Water quality (pesticides, pH, and water hardness and alkalinity) affects the distribution of *C. lebetis* in Florida.

Objective 1: Estimate chironomid diversity in hydrilla tips in Florida lakes and correlate water quality to the presence or absence of *C. lebetis* (Fig. 1-5).

Objective 2: Based on results of field surveys, conduct laboratory studies to determine the influence of selected water quality parameters on *C. lebetis* survival and development.

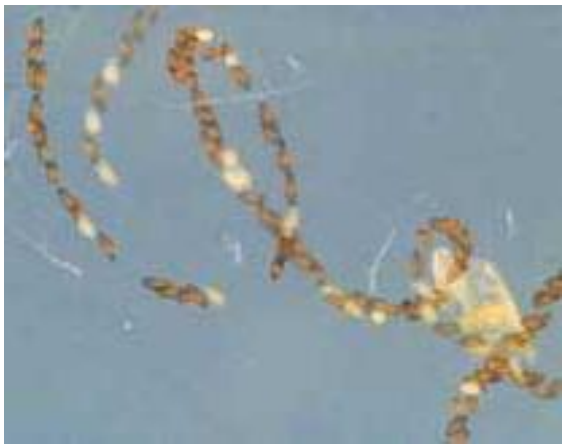
A   B

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Figure 1-1. *Cricotopus lebetis.* A) egg mass B) larva C) pupa D) adult. Photo credit: Jerry F. Butler, University of Florida

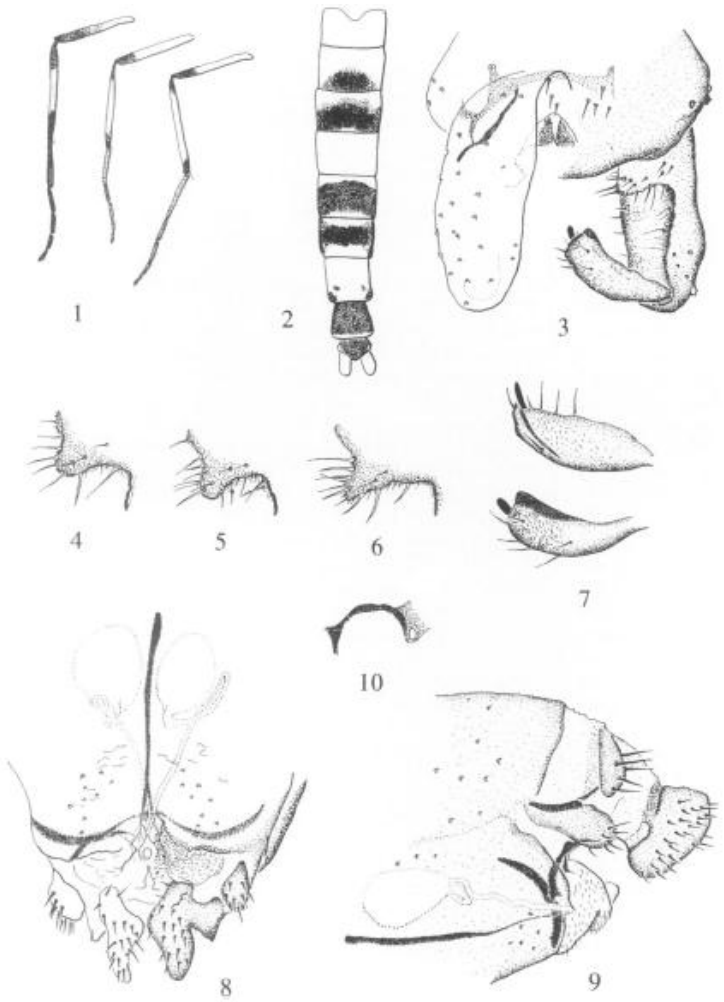

Figure 1-2. *Cricotopus lebetis* adult structures. 1) Male fore, mid and hind legs, 2) Male abdomen 3) Hypopygium 4-5) Inferior olsella variation in Florida material 6) Inferior volsella 7) Variation of gonostylus de to angle observation 8) Female genitalia, ventral 9) Female genetalia, lateral 10) Female coxosternapodeme. Source: Epler et al. 2000.

Figure 1-3. *Cricotopus lebetis* larval feeding damage in hydrilla

Figure 1-4. Hydrilla collected from Lake Rowell exhibiting highly branched stems caused by larval feeding damage, September 2010.

CHAPTER 2
TEMPERATURE-DEPENDENT DEVELOPMENT, COLD TOLERANCE, AND POTENTIAL DISTRIBUTION OF *CRICOTOPUS LEBETIS* (DIPTERA: CHIRONOMIDAE), A TIP MINER OF *HYDRILLA VERTICILLATA* (HYDROCHARITACEAE)

Introduction

Hydrilla verticillata (L.f. Royle) is a rooted submersed aquatic macrophyte that can be found throughout Florida, and other parts of the USA. There have been at least two separate introductions of hydrilla into the USA, with a dioecious form found predominately in the southeast, and a monoecious form found predominately in the northeast (Madeira, et al., 2000). The dioecious form of hydrilla was imported into the USA in the late 1950s from Sri Lanka through the aquarium trade. It rapidly spread throughout Florida during the 1960s and into the rest of the southeastern USA in the 1970s (Schmitz, 1991). The pathway of introduction of the monoecious form into the northeastern USA is unknown. Hydrilla has been shown to displace native vegetation such as *Vallisneria neotropicalis* (Haller and Sutton, 1975).

Hydrilla grows rapidly and can effectively compete for sunlight. Once it reaches the surface, dioecious hydrilla branches profusely producing a dense mat of vegetation, thus shading out native flora (Langeland, 1996). Additional negative impacts include interference with recreational and commercial use of lakes, tourism, and sportfishing, reduction of real estate values, reduction of flow in drainage canals, and clogging of intake pipes (Schmitz et al., 1991; Langeland, 1996). In highly infested areas, hydrilla creates dense mats of vegetation impeding boat traffic, and often becomes entangled in boat propellers, thus facilitating its movement to new locations within water bodies, and to new water bodies.

Effective control of hydrilla is difficult to achieve because of a very limited number of environmentally sound options (Hoyer et al., 2005). Efforts to control hydrilla had relied primarily on the application of synthetic herbicides, specifically fluridone. Recently, fluridone resistance has been documented in several locations in central Florida and has resulted in the use of alternative herbicides such as endothall and acetolactate synthase (ALS) inhibitors. Therefore, new management approaches to control hydrilla populations are being investigated (Cuda and Gillett-Kaufman, 2011).

Biological control is one possible management approach, used either alone or integrated with other tactics. In 1992, the chironomid midge *Cricotopus lebetis* Sublette (Diptera: Chironomidae) was discovered damaging apical meristems of hydrilla in Crystal River, Florida (Cuda et al., 2002). Although not certain, the midge was thought to be an adventive species, as it was not discovered in the USA until 1957 in Louisiana (Sublette 1964). Cuda et al. (2002) speculated that the midge might have been introduced into the USA as a contaminant of hydrilla imported through the aquarium trade. Hydrilla damaged by the midge at Crystal River was atypically stunted and did not reach the surface. In preparation for pupation, the larvae mine into the stem, and eventually this causes abscission of the tip (Cuda et al., 2002). This type of damage has the potential to satisfy both those who desire hydrilla stands (e.g. bass fishermen, duck hunters), and those who want to control it. Preventing hydrilla from reaching the water surface would improve boat navigation and water flow, and decrease the likelihood of hydrilla becoming entangled in boat propellers and thereby spreading to new locations. Despite its potential as a biological control agent of hydrilla, additional research to supplement the work completed by Epler et al. (2000), Cuda et al. (2002, 2011), Schmid

et al. (2010) and Cuda and Gillett-Kaufman (2011), is needed to address general biology and impact of *C. lebetis* on hydrilla.

The purpose of this study was to determine the influence of temperature on survival and developmental rate and to use this information to generate maps predicting the average number of generations of the midge in Florida, and the predicted isothermal lines for the northern extent of the distribution.

Materials and Methods

Source and Culturing of *H. verticillata* and *C. lebetis*

Hydrilla was collected from Lake Tohopekaliga (Toho), Osceola Co., FL (28.2° N, 81.4° W), and *C. lebetis* was collected from Lake Rowell, Bradford Co., FL (29.9° N, 82.1° W). Both cultures were maintained at the Biological Control Research and Containment Laboratory (BCRCL), Fort Pierce, FL. *Cricotopus lebetis* was reared by placing hydrilla tips in a large aerated container within a cage constructed from PVC tubing covered with a fine mesh cloth. Containers were filled with well water, *C. lebetis* egg masses were placed in the containers and adults that emerged were collected using a mouth aspirator. Adults were transferred to a 250 ml separatory funnel that had approximately 15 ml of well water as described by Cuda et al. (2002). Females oviposit on the water surface and egg masses were collected by opening the stopcock on the separatory funnel.

Hydrilla was propagated from stems collected at Lake Toho and placed into 10.16 cm pots containing a layer of potting soil covered by sand. The pots were placed into 378 liter tanks in a greenhouse and covered with 60% shade cloth.

Survival and Developmental Time

Temperature-dependent development of *C. lebetis* was investigated in environmental chambers (Model No. E36L, Percival Scientific, Inc., Perry, IA) maintained at 10 constant temperatures (10, 15, 20, 22, 25, 27, 30, 32, 35, 36°C ±1°C). Photoperiod was kept constant at 14:10 (L:D). Healthy, undamaged plant tips, 4-6 cm in length, were placed individually in 35 ml test tubes filled with well water as described by Cuda et al. (2002). Each test tube was placed in a rack that held 40 tubes. Two newly hatched larvae were transferred to each plant tip using a pipette. Once the larvae were introduced into the tubes, a cap with ventilation holes was placed on each tube. Tips were checked daily to ensure that they were fully submerged in order to prevent larval desiccation. Tubes with tips that were completely destroyed received replacement tips to allow complete development to adulthood. Approximately one week after the larvae were introduced, the test tubes were checked daily for adult emergence. The number of days to complete development was recorded in order to calculate the development rate.

Cold Tolerance

Cold tolerance studies were conducted using 2^{nd} - 4^{th} instar larvae. Four insects were placed inside a 35 ml vial containing two hydrilla tips and well water. Insects were acclimated from 20°C to the final temperature in intervals of 5°C every two hours. The larvae were exposed to three constant temperatures (5°C, 7.5°C, 10°C) for 0.5, 1, 2, 4, 8, 16, and 32 days. After each exposure time, insects were placed at room temperature and survival was assessed by observing for movement once the water reached room temperature. The effect of temperature and exposure times on midge survival was analyzed using logistic regression (SAS Institute, 2008). The LT_{50} and LT_{90} (lethal time),

at 5°C and 7.5°C were used to predict isothermal lines delineating regions favorable for *C. lebetis* establishment based on historical weather data. Following methods outlined by Diaz et al. (2008), a model was created in NAPPFAST (Borchert and Magarey, 2007), a database of daily weather information from stations across North America, to record the number of days at or below 5^{o} C and 7.5^{o} C. Probability maps were generated using the last 10 years of weather data to examine the frequency of occurrence of reaching the LT_{50} and LT_{90}. The maps were imported into ArcGIS 9.0 and a line indicating a frequency of occurrence of at least 5 out of the last 10 years was created.

Developmental Rate and Degree-Day Requirement

Survival at different temperatures was analyzed with analysis of variance using the general linear model procedure and means were separated using Student-Newman-Keuls test (PROC GLM; SAS Institute 2008).

Linear Developmental Rate Model

Developmental rate at different temperatures was analyzed using linear regression. The linear portion (15-35°C) of the developmental rate curve [R(T) = a + bT)] was modeled using least squares regression in Excel (Microsoft, Redmond, WA), where T = temperature, a = intercept, and b = slope. The base temperature threshold was estimated by the intersection of the regression line and the x-axis (R(T) = 0). Degree-days were calculated as the inverse slope of the fitted regression line.

Nonlinear Developmental Rate Model

The nonlinear relationship between development rate R(T) and temperature T was analyzed using the Brière-1 model which allows estimation of the upper and lower developmental thresholds (Brière et al., 1999). The model is defined as R(T) = *a* T (T -

T_0) $(T_L - T)^{1/2}$ where R = developmental rate, T = temperature, T_0 = base temperature threshold, T_L = lethal temperature, and *a* = empirical constant. T_0 and T_L were initially set to 6 and 36 °C, respectively, and the equation was then solved iteratively.

Weather Data from Florida

Daily minimum and maximum temperatures from Florida were obtained from 91 weather stations through the Applied Climate Information System (Climate Information for Management and Operational Decisions [CLIMOD], Southeast Regional Climate Center; http://acis.sercc.com). Daily minimum and maximum temperatures were averaged from the last 5-11 years depending the availability of data, which provided 365 values for maximum and minimum temperatures for each station. When there were no missing data points the maximum period of weather data was from 1 January 2002 to 1 January 2012. The minimum period of data available varied, as data points were inconsistently missing from the series.

Calculation of Degree-days and Number of Generations for Geographic Information System (GIS) Analysis

The DegDay program version 1.01, which is an Excel (Microsoft, Redmond, WA) application developed by University of California-Davis (http;//biomet.ucdavis.edu) was used to calculate accumulated degree-days for *C. lebetis*. This application uses the upper and lower temperature threshold for an organism, and daily average of minimum and maximum temperatures to calculate accumulated degree-days by using a single sine method. (Baskerville and Emin,1969). The lower and upper temperature thresholds were estimated from the Briere-1 nonlinear model as 9.52 and 36°C, respectively. The linear regression model was used to calculate the degree days (K) for *C. lebetis* [R(T) =

a bT] as K = 1/b (Campbell et al., 1974). The prediction of the number of generation per year was calculated by dividing the cumulative degree-days per station by K.

Generation of GIS Map for Prediction of *C. lebetis* Generations in Florida

Weather station name, latitude, longitude, and number of *C. lebetis* generations per year were inserted in Microsoft Excel and imported into ArcGIS 9.0 (ESRI Inc., Redlands, CA). The imported file was converted to a shape file using the ADD X-Y DATA function followed by the selection of the State Plane Projection. A shapefile of the border of Florida was obtained from the AWhere Continental database (AWHERE, Inc., Denver, CO) and used to delineate the range of predictions.

The Geostatistical Analysis function in ArcGIS (ESRI, Inc.) was used to generate prediction grids of *C. lebetis* generations across Florida. Values at un-sampled locations were predicted by interpolation of values at sample locations. The inverse distance weighted (IDW) deterministic method was used, where predictions are made by mathematical formulas that generate weighted averages of nearby known values. The IDW model gives more influence to points that are closer than to ones that are farther away. The parameters used in the IDW analysis were as follows:

1. The number of stations used for interpolation was set to a maximum of 15 and minimum of 10.
2. The Power Optimization option was selected generating a Power value of p = 2. This weights weather station values proportional to the inverse distance raised to the power of p.
3. The search neighborhood shape was circular because there were no directional influences on the weighting of number of generations per station. Ellipse parameters were set to: angle, 0 major and minor semiaxis, 1020596.

Climatic Suitability Mapping

Geographic coordinates from sample locations of *C. lebetis* were obtained from voucher specimens, literature and known field collection sites, including my own data

and data provided by Dana Denson (Reddy Creek Improvement District), Doug Strom (Water and Air Associates, Inc.) and Robert Rutter (Florida Department of Environmental Protection, Punta Gorda Branch Office). The predicted distribution of *C. lebetis* in North America was generated from known records and selected climatic variables using the BIOCLIM model (Hijmans et al., 2012), in the freeware program DIVA-GIS. Climatic variables included in the model were maximum temperature in warmest month, minimum temperature in coldest month, and annual mean temperature.

Results

Survival and Developmental Time

Larval survival varied with temperature. Larvae could not complete development at low and high temperature extremes (10 and 36°C). Only a single individual was able to complete development at 35°C. Survival to adulthood was highest at temperatures between 20 and 30°C (Fig. 2-1). The development rate increased with increasing temperature, until reaching 32°C (Fig. 2-2). Degree-day requirements (K) were calculated to be 495.29.

The Brière-1 model estimated the lower and upper developmental thresholds at 9.52 and 36°C, respectively (Fig. 2-3). These values were very similar to those found in the laboratory tests. The model showed that the rate of development increased with temperature until the curve reached an optimum at 30°C, and then decreased rapidly as the temperature approached the upper developmental threshold (Fig. 2-3).

Cold Tolerance

Larval survival when exposed to 5°C for a prolonged period exceeded 4 days in most cases. Only 50% and 10% of insects were able to survive for 8 and 16 days, respectively. No insects were able to survive after 32 days exposure to 5°C (Fig. 2-4)

but larvae were able to survive past 16 days10°C. The isothermal lines showed that at both 5 and 7.5°C, 50% of *C. lebetis* individuals will experience mortality just north of Florida, in northern Louisiana and Georgia, and in north Texas. The lines also predicted survival in California and southwestern Arizona. The lines indicating 90% mortality occur further north into southeast South Carolina, extending through the southeast and stopping in the panhandle of Texas, and then starting again in central Arizona to north California (Fig. 2-5).

GIS Mapping of *C. lebetis* Generations in Florida

Based on degree day requirements, *C. lebetis* is predicted to complete several generations per year in Florida, ranging from 6.8 to 11.7, with most generations in the southern portion of the state, and fewest in the panhandle (Fig. 2-6). Florida counties located south of Palm Beach County had the highest number of generations ranging from 10.2 to 11.7. Counties in the middle portion are predicted to support 8.2 to 10.2 generations per year.

Climatic Suitability Mapping

The predicted distribution indicated that the climate is suitable for the establishment of *C. lebetis* throughout much of the southeastern United States. The highest suitability occurred in Florida, and southern Louisiana. The full extent of the prediction ranged from southern South Carolina to central and east Texas. The map also indicated that areas in California and Arizona were suitable for establishment (Fig. 2-7).

Discussion

Temperature is an important factor that determines community structure and insect distribution. Understanding the thermal requirements for *C. lebetis* is an

important step for predicting where the insect can establish. As expected, the predicted number of generations per year of *C. lebetis* increased as latitude decreased. CLIMOD data only includes air temperatures, but was used to generate the map because comprehensive water temperature data for Florida water bodies are unavailable. Water temperature data collected from sampling water bodies throughout Florida in 2011-2012 indicate that temperatures were below the upper developmental threshold (Chapter 2). *Cricotopus lebetis* develops in the tips of hydrilla, which grow near the water surface during summer months. As water surface temperature is correlated with air temperature (McCombie, 1959), using air temperature data for predictive modeling was a reasonable assumption. Knowing the number of generations that the midge can complete in a year is important, as it will influence population growth in the field. Further research will be required to validate the prediction of number of generations per year and to correlate that with population dynamics in the field.

Host plant quality also can affect the developmental rate of insects. It has been shown that insects developing on suboptimal hosts typically have longer developmental times, and can be more vulnerable to predation due to longer exposure to predators (Häggström and Larsson, 1995). For example, in Chapter 3, I show that the developmental time of *C. lebetis* varies depending on host plant species. Within a species, host plants can vary due to genotype, ontogeny, phenology, and environmental conditions. Van et al. (1977) demonstrated that the growth habits of hydrilla vary depending on water depth and light intensity, and these physiological changes to hydrilla could conceivably influence performance of the midge. Thus, the actual number

of *C. lebetis* generations per year may be lower than what was predicted due to changes in host plant quality.

Field sampling has shown that *C. lebetis* occurrence is sporadic (Chapter 4). In summer months, the water temperature in Florida lakes can range from 30-35°C (Beaver, et al., 1981) which is approaching the upper developmental threshold. Water temperatures in hydrilla mats near the water surface tend to be higher than water temperatures at a one-meter depth in the hydrilla mat, or in open water (Bowes, et al., 1979; Cuda et al., 2008). In some portions of Florida, water temperatures in hydrilla mats may be too high for *C. lebetis* development, and result in high mortality. Vegetation mats reaching 45°C have been reported (Wheeler and Center, 2001), which is well above the upper lethal threshold of *C. lebetis*. Wheeler and Center (2001) partially attributed the poor performance of the ephydrid fly, *Hydrellia pakistanae*, to high temperatures in hydrilla mats. Likewise, *C. lebetis* may suffer high mortality during the peak of the summer months, resulting in local extirpation. Thus, temperature extremes could play a critical role in determining the persistence of *C. lebetis* in Florida water bodies. If permanent populations of *C. lebetis* cannot be sustained in water bodies experiencing temperature extremes, then restocking efforts would have to be made periodically.

The BIOCLIM model predicted that suitable locations for the establishment of *C. lebetis* exist throughout the southeastern USA. However, the data available to generate this map was limited, as extensive field sampling for *C. lebetis* has not been conducted. Although data was limited, this map resembles the distribution based on the isothermal cold tolerance lines, suggesting that the model prediction may be reasonable. Further

sampling, both within and outside the predicted distribution, would be useful for validation and refinement of the midge's distribution.

The results from the cold tolerance study indicate that *C. lebetis* can tolerate exposure to 5°C for 0.5 – 4 days. High mortality did not occur until exposure time exceeded 8 days. This may indicate that *C. lebetis* has a broader lower temperature threshold compared to the upper threshold. At high temperatures approaching the upper developmental threshold, nearly 100% mortality occurred. High rates of survival were recorded at 30°C, but there was a steep decline in survival once temperatures reached 35°C. Wider ranges of lower temperature thresholds are common among insects (Bayoh and Lindsay, 2004). The lower developmental threshold, the temperature at which the developmental rate is estimated to be zero, was 9.52°C.

Water temperature data are useful to determine sites where *C. lebetis* can survive and establish. Water temperatures during the winter months throughout Florida range from 8-15°C (Beaver et al., 1981), and these temperatures are mostly within the range of the developmental threshold. Water bodies that experience cold temperatures (≤ 5°C) for prolonged periods of time will be unsuitable for establishment of *C. lebetis.* The isothermal lines support this hypothesis, as 50 and 90% mortality was predicted just north of Florida and north of southeastern South Carolina respectively. The isothermal lines also show that establishment may be possible in California and in southern Arizona, but no records of *C. lebetis* exist in those states. In North America, *Cricotopus lebetis* has been found only in Louisiana and Florida (Cuda et al., 2002), although it would seem likely that it also occurs in Mississippi and Alabama as well. The insect could be native to North America and was able to expand its host range to include

hydrilla after the weed's introduction, or may be an adventive species (Cuda et al., 2002).

The overwintering strategy of *C. lebetis* is unknown. Chironomids have been shown to exhibit a variety of methods to deal with cold temperatures including supercooling, freeze tolerance, and dispersal to new areas (Danks, 2007). It is also unknown what stage of this insect overwinters, although nearly all chironomids overwinter as larvae (Danks, 1971). Chironomids have been shown to survive short periods of extreme cold temperature by freeze tolerance (Bouchard et al., 2006). If *C. lebetis* exhibits any of these characteristics, then the predicted range may extend further north than what was shown in the prediction maps. Further studies investigating the freeze tolerance of this insect would aid in determining whether or not *C. lebetis* possesses overwintering adaptations.

The global distribution and native range of this insect are unknown. Cuda et al. (2002) speculate that the midge may have been introduced with hydrilla through the aquarium plant trade. If so, this does not provide much help in narrowing the source population since hydrilla is so widely distributed in the Old World. In order to determine the origin of the midge, foreign exploration should be conducted to search for the midge in locations where hydrilla occurs. Then genetic work should be conducted on different geographical populations to determine the geographic origin.

Temperature-dependent development and cold tolerance studies provide basic information that can be used to develop or improve rearing methods, and to predict field colonization and establishment. Temperature experiments revealed that optimal temperature conditions for *C. lebetis* are from 20 - 30°C, and explain why this insect is

widely established throughout Florida. Ongoing studies of population dynamics and field impact studies will reveal the potential value of *C. lebetis* as an augmentative biological control agent.

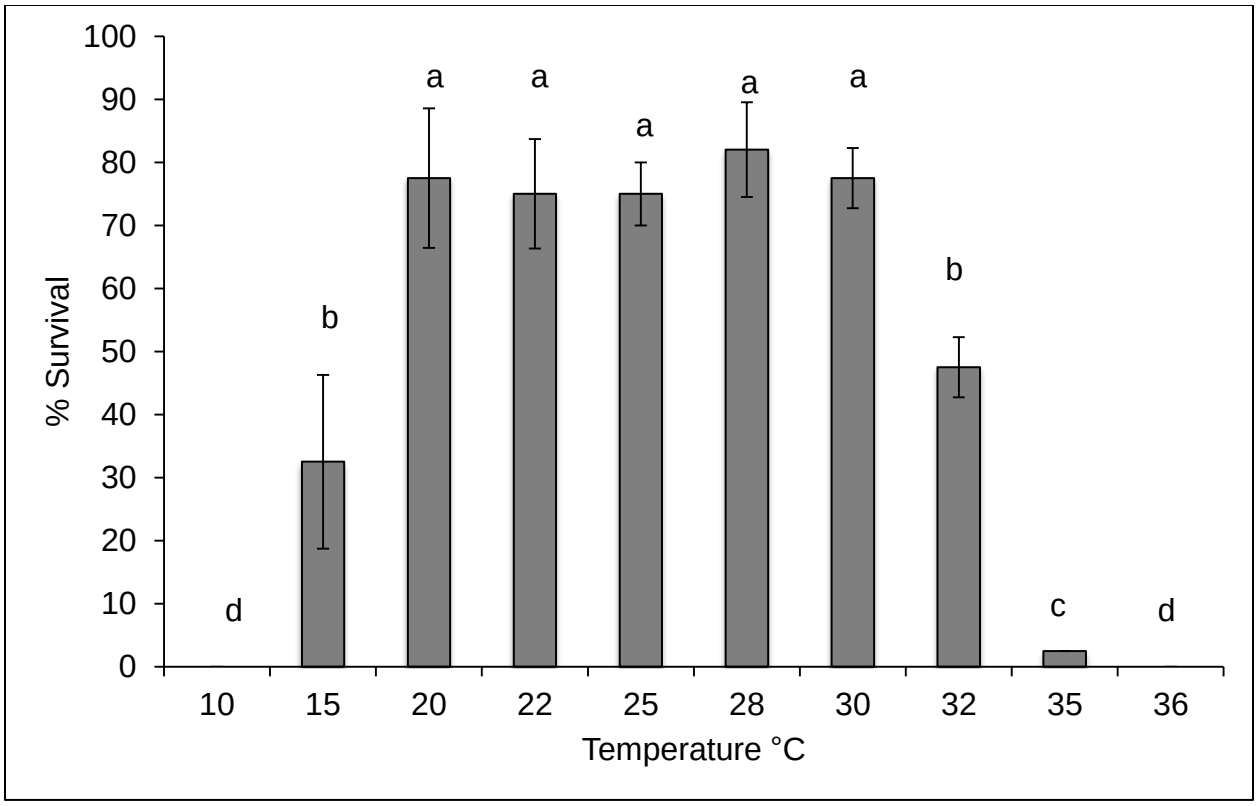

Figure 2-1. Percent survival of *C. lebetis* larvae at various temperatures during the temperature-dependent study. Letter groupings represent statistically different means.

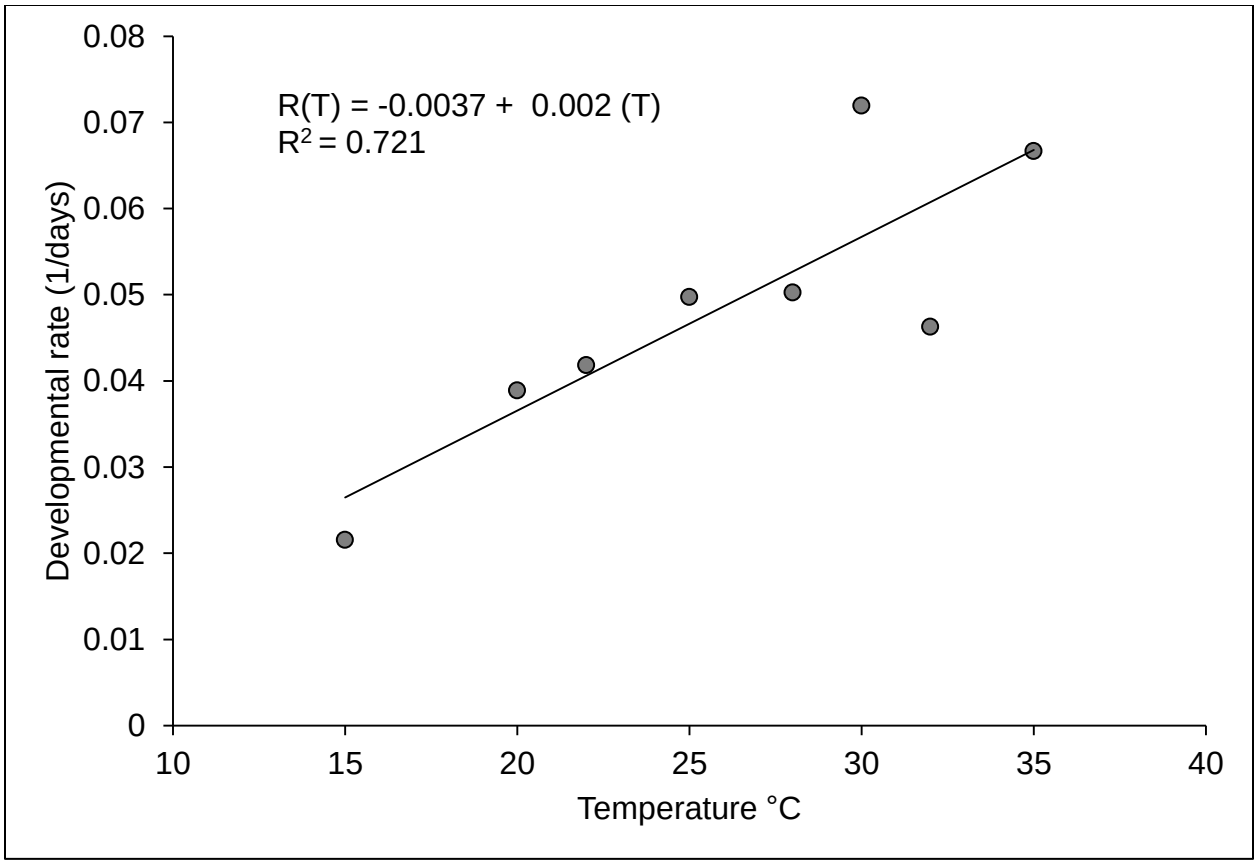

Figure 2-2. Developmental rate of *C. lebetis* larvae exposed to various temperatures during temperature-dependent development. Dots are observed values the line is the expected linear regression. R = rate; T = temperature.

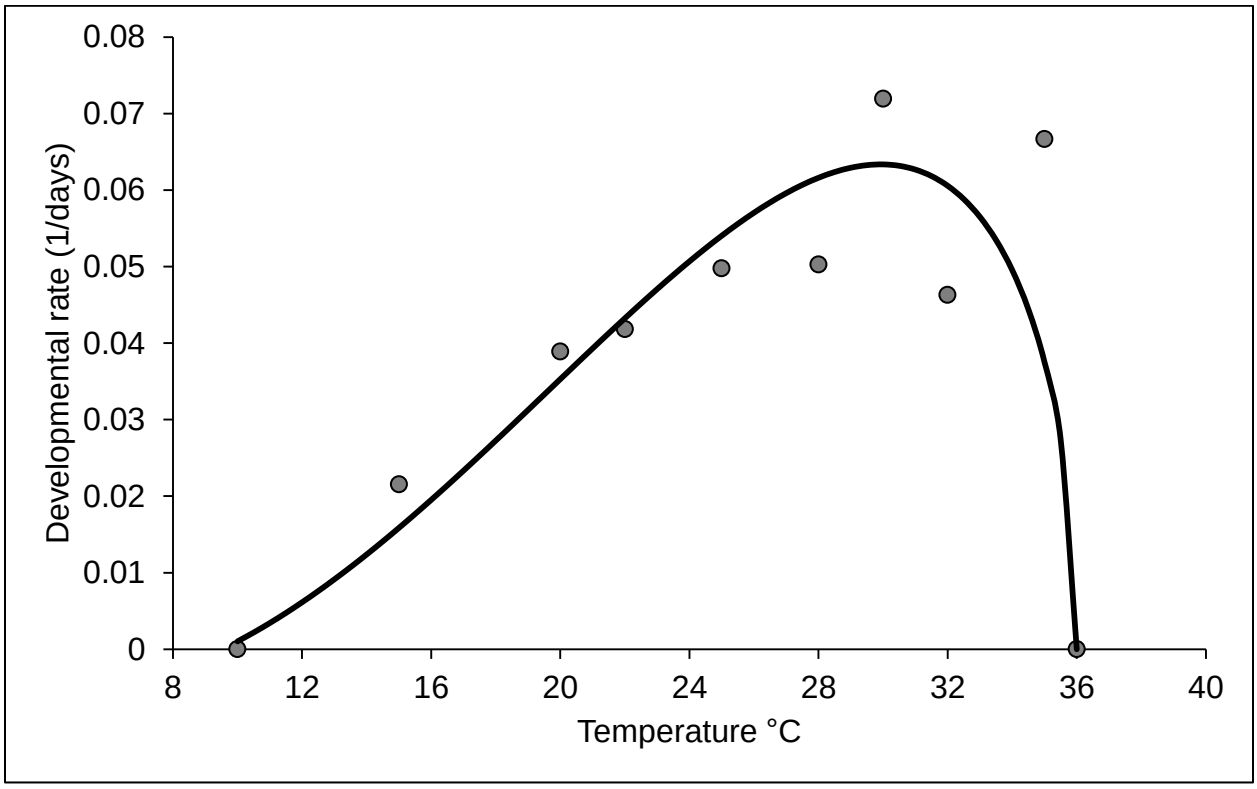

Figure 2-3. Brière-1 model estimating upper and lower temperature thresholds. Dots represent observed values. Upper threshold: 36 °C, Lower threshold: 9.52°C.

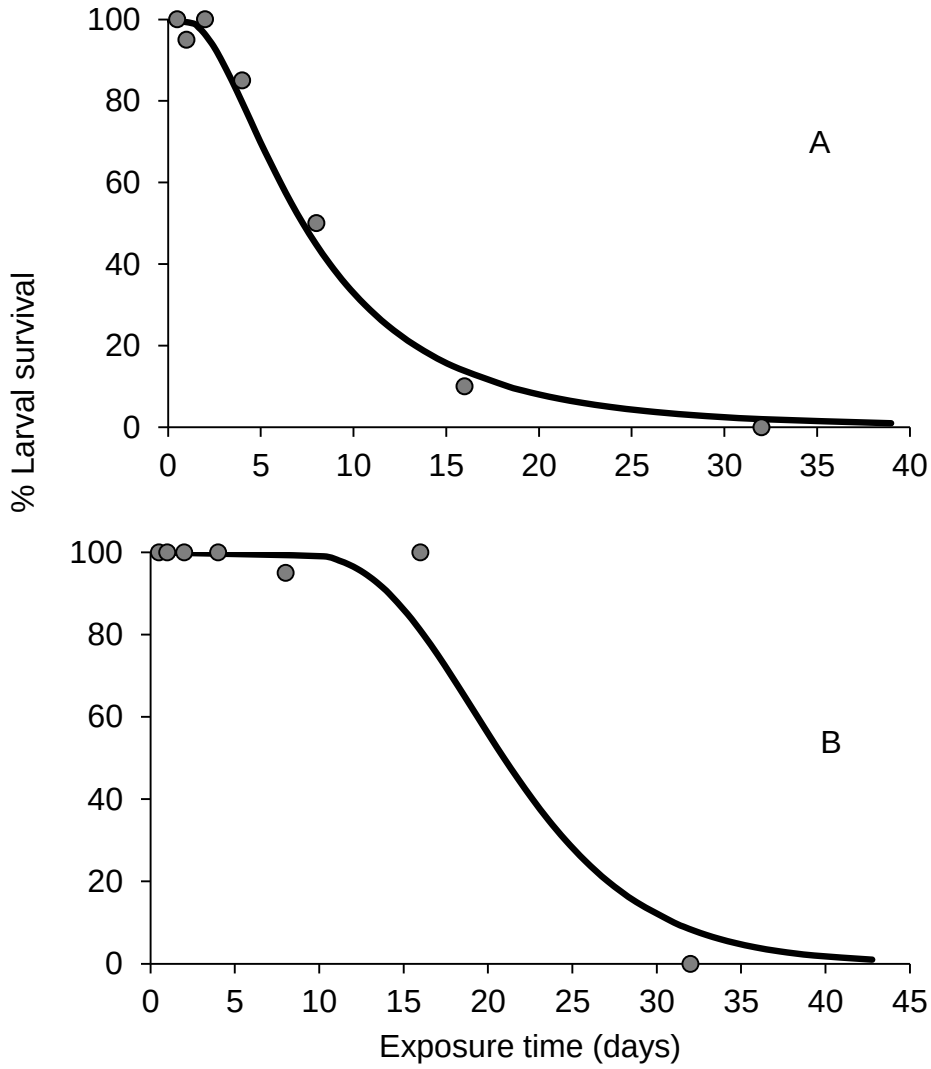

Figure 2-4. Larval survival of *C. lebetis* at different exposure times at A) 5°C B) 7.5°C. Single dots are observed values and lines are expected value of the logistic regression. Maximum survival occurred at 7.5°C at exposure time of 16 days.

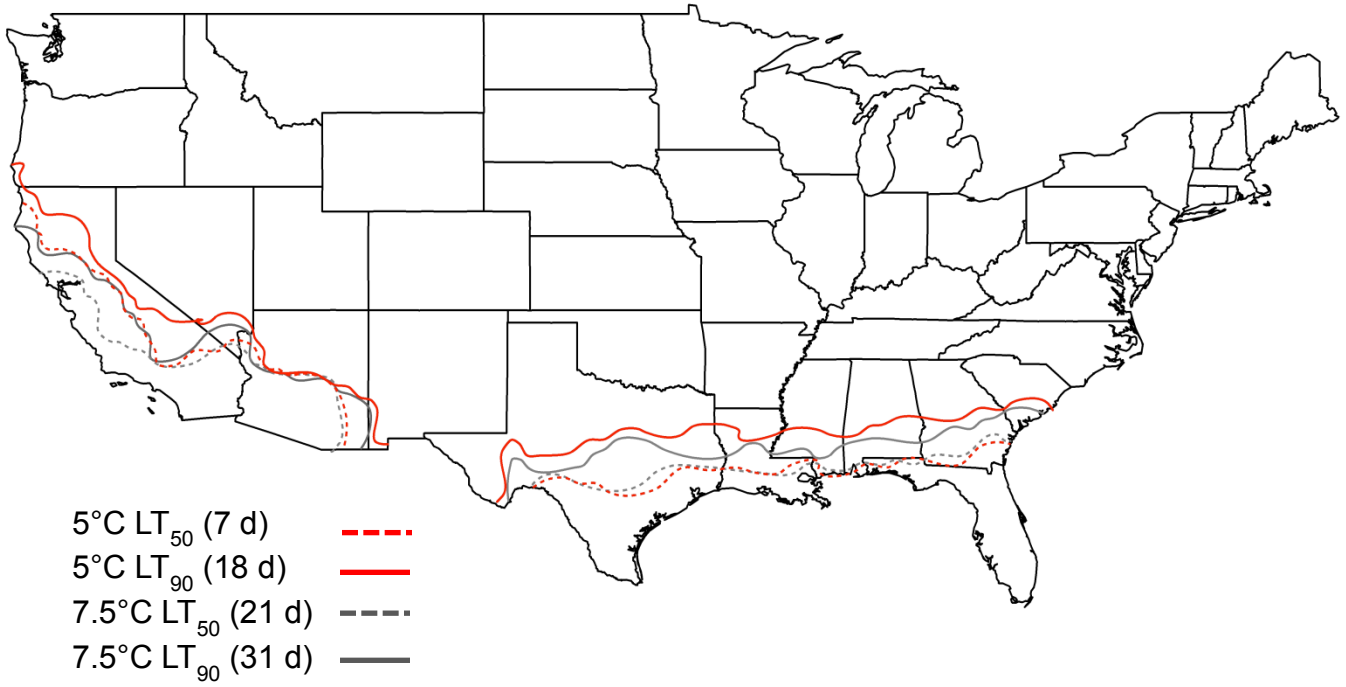

Figure 2-5. Map showing the isothermal lines (LT_{50} and LT_{90}) at 5 and 7.5°C for *C. lebetis.* Lethal times at each exposure indicate after how many days in which 50 and 90% of the population will experience mortality. Maximum survival occurred at 18 days and 5°C.

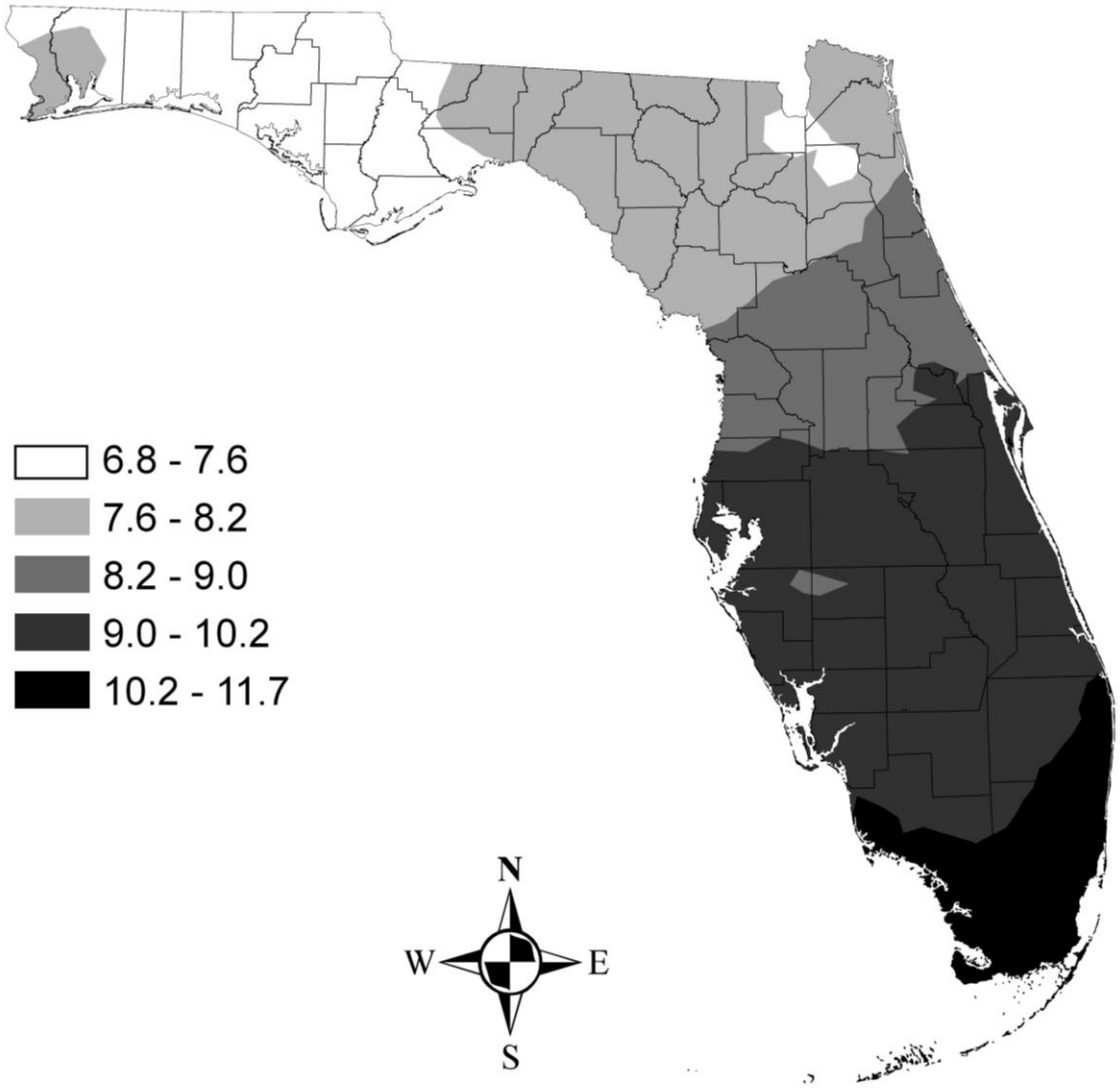

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Figure 2-6. Geographical information system map showing the predicted number of generations of *C. lebetis* in Florida per year.

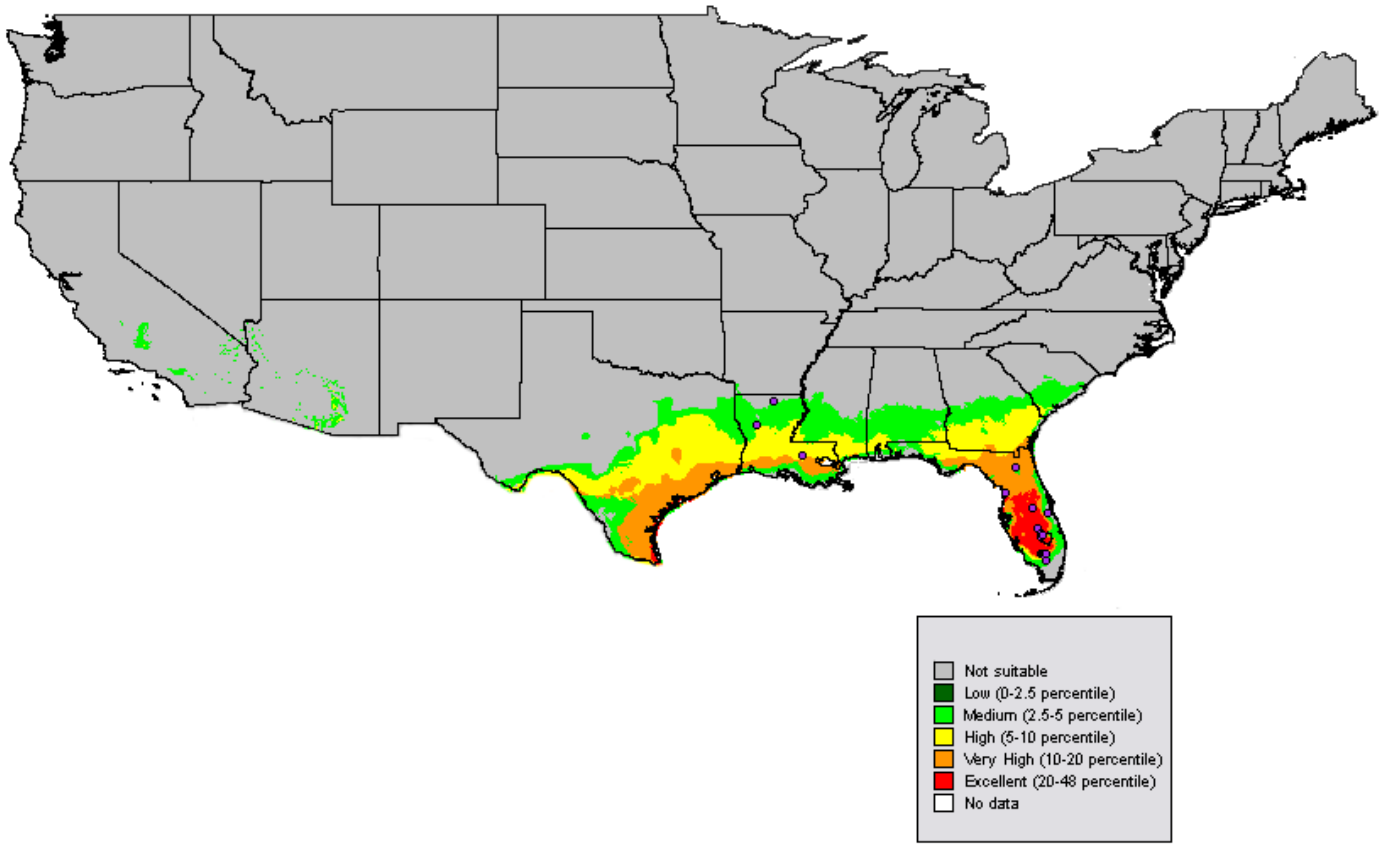


Figure 2-7. Model prediction of climate suitability for *C. lebetis* using known sampling locations and climate records. Purple dots represent locations where *C. lebetis* has been recovered from this study, published literature, and voucher specimens. Map indicates suitability percentiles for establishment locations for *C. lebetis.*

CHAPTER 3
HOST RANGE OF *CRICOTOPUS LEBETIS* (DIPTERA: CHIRONOMIDAE), A TIP MINER OF *HYDRILLA VERTICILLATA* (HYDROCHARITACEAE)

Introduction

Hydrilla verticillata (L.f. Royle) is a submersed aquatic weed that has been the target of biological control programs since the 1970s. Hydrilla was imported into the United States through the aquarium trade in the late 1950s, and rapidly spread throughout Florida and the rest of the southeastern USA within 20 years (Schmitz et al., 1991). Shortly after its introduction, hydrilla was present in all major water bodies of all drainage basins in Florida (Langeland, 1996). Hydrilla causes many negative impacts including displacement of native vegetation (Haller and Sutton, 1975), impediment of boat traffic, recreational and commercial losses, clogging of intake pipes and canals, and reductions in tourism and real estate values (Langeland, 1996; Schmitz et al., 1991). Managing hydrilla is both time consuming as expensive, and there are few effective management options (Hoyer et al., 2005). The long-term use of the herbicide fluridone has resulted in the selection of several populations of fluridone resistant hydrilla in Florida, and has limited management options further, resulting in the inability to control large infestations (Michel et al., 2004).

Biological control is one possible management approach, either alone or integrated with other tactics. There are three main types of biological control; classical, augmentation, and conservation (Cuda et al., 2008). Classical biological control is based on the premise that plant populations are suppressed in their native ranges by the action of natural enemies (Williams, 1954; Keane and Crawley, 2002). When a plant is moved to a new area of the world, it escapes regulation by specialized natural enemies and is able to reach higher densities than occur in its aboriginal home (Wolfe,

2002; Torchin et al., 2003). The process of biological control involves searching for host specific natural enemies in the native range of the weed species, and releasing the natural enemies in the invasive range of the weed. Classical biological control has been the most successful method of biological control. Augmentative biological control involves the release of natural enemies to supplement natural populations, and relies on mass-rearing and continual release efforts. This method is used when natural enemies are unlikely to reach high enough densities on their own to achieve control of the pest species. Conservation biological control involves identifying factors that limit the effectiveness of a natural enemy, and modifying them to increase the effectiveness of the beneficial species. This can involve either reducing the factors that limit effectiveness or providing resources that the natural enemy needs in the environment.

Host specificity testing is an essential component of any biological control program (Sheppard et al., 2005), and is used to determine whether a potential agent is suitable to be released in the field. The centrifugal phylogenetic method developed by Wapshere (1974) is followed to define the host range of an insect. Predicting non-target effects of a potential agent is critical to assess ecological risks associated with releasing biological control agents (Louda and Arnett, 2000). Biological control has advantages over other management options including diminished health concerns because there is less use of herbicides, (Pimental and Andow, 1984; Pimental et al., 1984). Moreover, decreased herbicide use will result in decreased selection pressure for plants to develop resistance to herbicides (Holt and Hochberg, 1997), and costs over time are less. An advantage of augmentative biological control over classical biological control is that release permits are not required to make field releases in areas where the agent already occurs.

Various insects from around the globe have been investigated as potential biological control agents of hydrilla, and a four have been released (Buckingham, 1988; O'Brien and Pajni, 1989; Buckingham, 1994; Buckingham and Bennett, 2001; Overholt and Cuda, 2005). Unfortunately, none of the insects released have had significant impacts in the field (Buckingham and Balcionas, 1994; Wheeler and Center, 2001), and efforts to identify classical biological control agents are still ongoing.

In 1992, the chironomid midge *Cricotopus lebetis* Sublette (Diptera: Chironomidae) was discovered damaging apical meristems of hydrilla in Crystal River, Florida (Cuda et al., 2002). Although not certain, the midge was thought to be an adventive species, as it was not discovered in the USA until after the introduction of hydrilla (Epler et al., 2000). Moreover, *C. lebetis* has only been collected from hydrilla, except for one specimen recovered from a *Potamogeton* sp. (Dana Denson, personal communication), suggesting that it may be monophagous. The feeding habits and behavior of the insect also suggest that it may be specific to hydrilla, as it utilizes the apical stems to complete development (Cuda et al., 2002). This insect may have use as an augmentative biological control agent since it prevents hydrilla from reaching the water surface, and therefore diminishes many of the negative effects of the weed (Cuda et al., 2011). Because *C. lebetis* already occurs in Florida waterways, permits are not required to release it within the state, but host specificity testing is required if the insect were to be released in other areas of the USA, or in other areas of the world, where it does not occur. The purpose of this study was to investigate the host range of *C. lebetis* and use this information to gauge its potential as a biological control agent of hydrilla.

Materials and Methods

Source and Culturing of *H. verticillata* and *C. lebetis*

Hydrilla was collected from Lake Tohopekaliga (Toho), Osceola Co., FL (28.2° N, 81.4° W), and *C. lebetis* was collected from Lake Rowell, Bradford Co., FL (29.9° N, 82.1° W). Both cultures were maintained at the Biological Control Research and Containment Laboratory (BCRCL), Fort Pierce, FL. *Cricotopus lebetis* was reared by placing hydrilla tips in a large aerated container within a mesh cage constructed from PVC pipes. Containers were filled with well water, and *C. lebetis* masses were placed in the containers. Emergent adults were collected using a mouth aspirator, and transferred to a 250mL separatory funnel with approximately 15 mL of well water. Females oviposit on the water surface, and egg masses were collected by opening the stopcock on the separatory funnel (Cuda et al., 2002). Hydrilla was propagated from stems collected at the field site and placed into 10.16 cm pots containing a layer of potting soil covered by sand. The pots were placed into 378 liter tanks and covered with 60% shade cloth inside a greenhouse.

No-Choice Larval Development Tests

Healthy, undamaged plant tips, 4-6 cm in length, were placed individually in 35 ml test tubes filled with well water. Each test included three non-target plants and hydrilla as the control (Table 3-1). There were 10 test tubes per plant species and the tubes were placed randomly in a rack that held 40 tubes (Cuda et al., 2002). The experiment was replicated three times. Each tip was exposed to two newly hatched *C. lebetis* larvae by using a pipette to transfer the larvae to each test tube. Once the larvae were introduced into the tubes, a cap with ventilation holes was placed on each tube. All racks were placed in an environmental growth chamber maintained at 25°C and 14:10

(L:D) photoperiod (Model No. E36L, Percival Scientific, Inc., Perry, IA). Tips were checked daily to ensure that they were fully submerged in order to prevent larval desiccation. Tubes with tips that were completely destroyed received replacement tips to allow complete development to adulthood. Approximately one week after the larvae were introduced, the test tubes were checked daily for adult emergence. Plant species that supported development to the pupal stage were considered to be suitable hosts. The number of days to develop was recorded in order to calculate the development rate on each plant species.

Paired-Choice Test

Plant tips of hydrilla and other species that supported complete development (*Elodea canadensis* Michx., *Egeria densa* Planch., and *Najas guadalupensis* (Spreng.) Magnus.) were used in a dual choice experiment. Plant tips were placed in a container that was divided into two sections using wire screen mesh with holes approximately 1 cm^2. Each side of the container had 40 plant tips, one side with hydrilla and the other side with a selected test plant. The container was placed into a small mesh cage, and aerated with an aquarium pump placed in the center of the container. In total, 100 neonates were placed in the center of the container and released. Larvae were left in the container to develop and after 10 days and plant tips were dissected under a microscope to determine the presence or absence of larvae. Damage to tips was rated on a scale of 0-5 with 0 – no damage, 1 – minimal damage, not visible to naked eye, 2 – light damage 10-20%, 3 – moderate damage – 20-50%, 4 – heavy damage >50%, 5 – tip abscission. This process was replicated 3 times with each plant species.

Paired-Choice Olfactometer Test

An aquatic olfactometer was constructed using a Y-tube and a low flow peristaltic pump (Model No. 13-876-1, Fisher Scientific, Waltham, MA). The Y-tube was glass, with an inside diameter of 4mm, having two arms and a stem 5.3 cm and 5.5 cm in length, respectively. Distilled water was pumped at a rate of 0.575 ml/min into each arm of the Y-tube, and was measured by collecting water from each arm in a graduated cylinder for 20 minutes and dividing the volume of water from each arm by 10. A small plant tip was placed in one arm of the Y-tube and the other arm remained empty (Fig. 3-1). The Y-tube was placed on a flat translucent surface that was lighted from the back. A camera attached to a microscope was used to project the image onto a computer screen, and screen recording software was used to record all trials. A neonate was released into the stem of the Y-tube and given a maximum of 10 minutes to move towards one of the olfactometer arms. Larvae that had not moved into one of the arms after 10 minutes were recorded as no response. A larva was recorded as having made a decision once it entered an arm of the Y-tube. This process was replicated 10 times using hydrilla, *E. canadensis*, and distilled water only.

Host Finding Behavioral Test

A small Petri dish, 3.5 cm in diameter, was divided into quadrants using an indelible marker, and a small circle was drawn in the middle of the dish to serve as the starting point. Two holes were drilled into opposite ends of the Petri dish and plastic tubing, 0.20 cm in inside diameter was inserted into each hole. Distilled water was pumped through the Petri dish using a peristaltic pump at a rate of 0.25 ml/min to create a constant flow. A single plant tip of either hydrilla or *E. canadensis* was placed in quadrant 4 so that the water current was flowing across the plant tip to the other side of

the dish. A pipette was used to place a neonate into the center of the dish, and the larva was given 60 minutes to locate the plant tip. Video imagery was captured through a dissecting microscope and recorded on a computer. The path of the larva was traced on an acrylic sheet, and the time spent in each quadrant was recorded. This process was replicated 10 times using hydrilla, *E. canadensis,* and a control with no plant material. The position of the insect was also determined every three minutes to examine their movement pathways (Fig. 3-2).

Paired-Choice Adult Oviposition

A plastic divider 2.6 cm. in height was glued to the base of a cubic cage (29.8 cm on each side) to divide the base of the container in two. The cages were translucent plastic with mesh screen on three sides with a plastic top and bottom. Each side of the container was filled with 300 mL of distilled water, and 20 plant tips (*H. verticillata, N. guadalupensis, or E. canadensis)* approximately 5-8 cm in length were placed into one side of the cage. The experiment also was conducted using pieces of artificial aquarium plants resembling hydrilla (Walmart, Bentonville, AR). Four *C. lebetis* pairs were released into the cage, where they had equal access to each container. Adults were left in the cages for 48 hours, after which the containers were examined for the presence of egg masses. The number and location of egg masses were recorded.

Data Analysis

Means from the no-choice larval development, the paired-choice test, and the time spent in each quadrant from the host finding behavior test were compared with analysis of variance and means separated with Student-Newman-Keuls test when ANOVAs were significant (SAS Institute, 2008). The relationship between survival and development rate from the no-choice larval test was analyzed in Excel (Microsoft,

Redmond, WA) using a least squared regression [R(S) = a + bS)], where T was the survival rate, R was the developmental rate, a was the intercept, and b was the slope, using all data points except zero values. Data from the aquatic olfactometer bioassay were analyzed using a G test of independence (Sokal and Rohlf, 1995). The movement patterns of the host finding behavior test were examined by fitting the data to a correlated random walk model (CRW) as described in Brouwers and Newton (2009). The equation was as follows: $R^2_n = nL_2 + (2L^2_1)\ (c / (1\text{-}c))\ (n - (1c^{(n\text{-}1)/2}) / (1 - c))$ where L^1 = mean move length (here in cm); L^2 = mean squared move length (here in cm^2); n = number of consecutive moves; c = mean cosine of the turn angle. The mean square distances travelled in each phase (every 3 minutes) were compared to model predicted values with linear regression and the regression slopes were tested for equality to one (PROC REG, SAS Institute 2008). The results from the paired-choice adult oviposition tested were analyzed using a G test of independence (Sokal and Rohlf, 1995).

Results

No-Choice Larval Development

Larvae were able to complete development on the majority of plants tested (Fig. 3-3). The plants that supported the best development were in the same family as hydrilla (Hydrocharitaceae) or the closely related family, Najadaceae (Fig. 3-4). High survival rates on some of the plants indicate that they may be better hosts for *C. lebetis* than dioecious hydrilla. Survival was higher on monoecious hydrilla (100%) and *E. canadensis* (96.7%) than dioecious hydrilla (56.6%), Developmental rate also varied between hosts, and was higher on *E. canadensis* (13.9 days) than either of the hydrilla types. Interestingly, development was fast on *Vallisneria americana* Michx. (15.2 days), a member of the Hydrocharitaceae family, although it was a very poor host for survival

(6.7%). Plants in families more distantly related to hydrilla, such as Potamogetonaceae, Ceratophyllaceae, and Cyperaceae, were generally poorer hosts than the more closely related plants. Survival on *Chara vulgaris* L., a green algae only distantly related to vascular plants, was 43.3%. *Utricularia macrorhiza* Leconte, a carnivorous bladderwort, supported larval feeding, but was the only plant tested that did not allow complete development to adulthood. Dioecious hydrilla, the target plant, supported moderate performance of *C. lebetis* in comparison to the other plants tested with an average survival rate of 56.6% and a development time of 19.73 days. There was a significant positive linear relationship between survival and development time ($F = 12.92$, $df = 39$, $P < 0.001$) (Fig. 3-5).

Paired-Choice Test

The proportion of plant tips infested with larvae was higher for *E. canadensis* (76.4%) compared to hydrilla (26.8%) ($F = 15.46$, $df = 5$, $P = 0.017$) (Fig. 3-6). Moreover, damage to *E. canadensis* was higher than damage to hydrilla with an average score of 3.77 for *E. canadensis* compared to 1.88 for hydrilla ($F = 13.73$, $df = 5$, $P = 0.021$). When hydrilla was compared with *N. guadalupensis*, the percent of plants with larvae was significantly higher in *N. guadalupensis* ($F = 6.59$, $df = 5$, $P = 0.043$), but there was no difference in the damage score ($F = 2.89$, $df = 5$, $P = 0.14$) (Fig. 3-7). Hydrilla and *E. densa* did not differ in either the damage score ($F = 0.02$, $df = 5$, $P = 0.88$) or the percent of plant tips infested with larvae ($F = 0.50$, $df = 5$, $P = 0.52$).

Paired-Choice Olfactometer Test

For the majority of replications, larvae did not respond. In the test with hydrilla versus distilled water, one larva entered the olfactometer arm with distilled water. Larvae did not respond in all the other replications with hydrilla. In the test with *E.*

canadensis versus distilled water, only one larva responded and chose *E. canadensis* (Fig. 3-8).

Host Finding Behavior Test

Regressions of observed mean square distance traversed on the CRW model predicted values were significant for hydrilla, *E. canadensis,* and distilled water only (hydrilla: P = 0.004, *E. canadensis:* P = 0.0005, control: P = 0.007) and the slopes of the regressions were not different from one for *E. canadensis* (F = 3.28, df = 18, P = 0.08) and the control (no plant material) (F = 0.53, df = 18, P = 0.48). This suggests that in arenas with *E. canadensis* or only water, the movement pattern conformed to a theoretical model of correlated random walk, in which the direction of movement is correlated with the direction taken in the previous step (i.e., organisms tend to continue moving in the same direction) (Fig. 3-9). However, with hydrilla, the slope of the regression of observed values on model predicted values was less than one (F = 4.9, df = 18, P = 0.04), indicating that movement did not conform to the CRW model.

The times spent in the four quadrants of the Petri dish were not different for hydrilla, *E. canadensis* or the control, but there appeared to be a slight trend towards spending more time in quadrant 4, which had the plant piece, or nothing in the case of the control (Fig. 3-10). The trace patterns indicate that larvae may swim very close to the host plant, but not locate the host (Fig. 3-11). In one replication with a hydrilla tip, the larvae was able to locate the plant in 10.4 minutes, but in the remainder of replications, the larvae had not settled on the host plant after 60 minutes. Larvae did not locate the plant tip in the trials with *E. canadensis.*

Paired-Choice Adult Oviposition

Females preferred to lay eggs on or near hydrilla when given a choice between hydrilla and artificial hydrilla (G_{adj} = 13.01, df = 1, P < 0.001), and hydrilla and distilled water (G_{adj} = 16.76, df = 1, P < 0.001) (Fig. 3-11). There was no difference when given a choice between hydrilla and *N. guadalupensis* (G_{adj} = 0.517, df = 1, P > 0.3). Females preferred to lay eggs on *E. canadensis* over hydrilla (G_{adj} = 5.1, df = 1, P < 0.05). There was also preference to lay eggs on artificial hydrilla versus distilled water (G_{adj} = 4.69, df = 1, P < 0.05) (Fig. 3-12).

Discussion

Host range testing is an essential component of biological control programs. Accurate information about the host range helps to quantify potential risks to native and economically important plant species (Briese, 2005). Hydrilla is a highly damaging invasive species in Florida's water bodies and is therefore actively managed, primarily with the use of herbicides. (Langeland, 1996) The presence of fluridone resistance throughout the state has increased concerns about the availability of tools to manage hydrilla (Hoyer et al., 2005). Biological control may be an appropriate management strategy if effective host specific agents can be identified. Determining the fundamental host range of *C. lebetis* is a first step toward assessing its potential as a biological control agent. The observed mining activity (Cuda et al., 2002) was an indication that *C. lebetis* may be host specific to hydrilla, as leaf mining insects tend to have narrow host ranges (Hespenheide, 1991). Therefore, host range testing was pursued to test this hypothesis. The feeding and oviposition preferences were analyzed and behavioral tests were conducted in order to understand the host range of this insect.

The results of the no-choice larval developmental study showed that *C. lebetis* has a broad host range and can feed on a variety of host plants belonging to several different families. Moreover, *C. lebetis* was able to complete development in a taxonomically diverse group of plants including a non-vascular plant, *C. vulgaris*, although survival tended to be highest on plants in the family Hydrocharitaceae, and in the closely related Najadaceae. However, in contrast to the hypothesized narrow host range, these findings suggest that *C. lebetis* is a generalist. This information provides new insight into the biology of *C. lebetis* and its potential as an augmentative biological control agent.

Interestingly, two test plants, *E. canadensis* and monoecious hydrilla, were more suitable for the development of *C. lebetis* than dioecious hydrilla. These plant species occur further north in the USA than Florida. *Elodea canadensis* ranges from Quebec south to North Carolina, Alabama and Arkansas, and westward to Manitoba, British Columbia, Colorado and California (Nichols and Shaw, 1986). It was reported in Jackson County in the panhandle of Florida in 1937; however, recent surveys suggest that it may no longer occur there (Raymond Hix, personal communication). Monoecious hydrilla occurs in northeastern USA and the Atlantic coastal area as far south as South Carolina (Madeira, et al., 2000) and originated from Korea (Madeira et al., 1997; Steward et al., 1984). There are concerns that monoecious hydrilla may cause problems as severe in the northern USA as dioecious hydrilla causes in the south (Langeland, 1996). If field tests reveal that *C. lebetis* prefers hydrilla in the field, it may still be worthwhile exploring the use of *C. lebetis* to control monoecious hydrilla

populations, but use of the midge may be restricted to the southeast based on temperature studies (Chapter 1).

The results of the paired-choice tests confirm the findings of the no-choice larval development test. In the no-choice larval development test, *E. canadensis* was one of the best hosts, and that finding is supported by the paired-choice test where the damage score and percent of infested tips of *E. canadensis* were significantly higher than those of hydrilla. There were no differences between hydrilla and *E. densa,* and this is consistent with the no-choice tests because the performance on those two plants was very similar.

The olfactometer and host finding behavior tests were conducted to investigate the foraging behavior of *C. lebetis*. The low response rate, and random movement in locating a host plant, may be a reflection of the generalist feeding niche of *C. lebetis*. The host finding behavior test confirmed the results of the olfactometer test, as the larvae were unable to locate the host in all but one trial. The arena of the host finding behavior experiment may have affected the insect's ability to locate the host; under normal conditions in water bodies, the insect would be exposed to light only from above, whereas the Petri dish arena was lighted from below. Lighting from the underside, which was necessary to follow the insect's movement, could conceivably have affected host-finding behavior. In addition, the paired-choice larval host finding experiment was conducted over a much longer period than the Petri dish experiment (10 days compared to 60 minutes), which could have influenced results. Moreover, *C. lebetis* may be a nocturnal forager (J. P. Cuda, personal communication), and thus may not have

displayed typical foraging behavior under lighted conditions, regardless of whether the light source was above or below the insect.

Cricotopus lebetis seems to lack the ability to locate host plants using olfactory cues. Although there appeared to be a trend towards spending more time in the quadrant where the host plant was located, the same trend was seen when no plant material was in the Petri dish. This suggests that the insect may have exhibited a tendency to move upstream against the water flow as quadrant 4 was where the water entered the dish.

The pathway the insect followed during the 60 minute appeared to be random based on the fit of the fit of the data to the CRW model. The observed and predicted values were not different with *E. canadensis* or the control, indicating that larval movement did not appear to be directed. Interestingly, when hydrilla was in the arena, movement did not conform to the model. Examination of the data suggests that when hydrilla was present, displacement was less than predicted. According to Brouwers and Newton (2009), when observed values fall below model predicted values (i.e., when the slope < 1), more random movement is suggested with less directional persistence.

In some cases, the insects were observed swimming in very close proximity to the host plant, but they did not make contact. Therefore, it is hypothesized that *C. lebetis* larvae must make physical contact with a plant to be able to determine if the plant is a suitable host. It has been shown that many generalist insects lack receptors to detect acute olfactory signals (Schoonhoven et al., 2005); whereas specialist herbivores often have receptors to detect host specific plant volatiles (Anderson et al., 1995; Hansson et

al., 1999; and Stranden et al., 2003). *Cricotopus lebetis* may not be able to detect plant derived chemicals that have diffused into the water.

The oviposition test suggests that females are responsible for choosing an adequate site for larval development and survival. Females overwhelmingly choose to oviposit in the vicinity of plant material, whether it was a real or artificial. This finding suggests that females may respond to both visual and olfactory cues. Egg masses were laid on artificial plants when it was the only material available, but when given a choice between real and artificial plants, the real plants were chosen. Studies have shown that the visual cue of light polarization affects oviposition behavior of some chironomid species (Lerner et al., 2008). The initial step in selecting oviposition sites could be based on visual cues, which stimulate landing in the vicinity of plant material. Once females land, additional cues could indicate whether or not that plant is a suitable host. Moreover, the gelatinous egg masses of chironomid species are moved by water currents, and adhere to substrates (Williams, 1982). Therefore, the survival strategy of *C. lebetis* appears to be to oviposit egg masses in areas with plants that can support larval development. In this strategy, larvae do not search for a host, which is consistent with the findings of the larval host finding experiments. Field abundances may be low because of biotic (predation, competition) and abiotic (weather, pesticides, water quality) factors.

Laboratory bioassays to delineate the fundamental host range of *C. lebetis* suggest that this insect is a generalist feeder of aquatic plants. However, the ecological host range may differ from the fundamental host range (Haye et al., 2005). A next step in assessing the potential value of *C. lebetis* as an augmentative biological control in

Florida (or a classical biological control agent for release in other states or countries) would be to evaluate the host range in the field. A potential shortfall of laboratory based host range tests is that they are unnatural in comparison to nature. If field choice-tests reveal that *C. lebetis* prefers hydrilla over other aquatic plants, *C. lebetis* may have use as an augmentative biological control agent. With the exception of one specimen recovered from a *Potamogeton* sp. (Dana Denson, personal communication), all field records to date of *C. lebetis* that are associated with host plants have been from hydrilla. This could be due to a sampling bias, or reflect an actual field preference for hydrilla. If the latter is true, then releases of *C. lebetis* may result in minimal damage to non-target plants. Moreover, *C. lebetis* does not kill hydrilla plants, but rather stunts their vertical growth and results in profuse branching below the surface (Cuda et al., 2011). The effects of *C. lebetis* on other plant species has not been investigated, but are likely sub-lethal. Impact studies to determine the densities at which this insect can create a significant reduction in hydrilla biomass need to be conducted. Since *C. lebetis* is already found widely in Florida and does not appear to have much impact on hydrilla, insect densities may have to be significantly augmented to achieve acceptable levels of control.

Table 3-1 List of plants tested in no-choice larval development

Family	Species	Origin	Common Name
Hydrocharitaceae	*Elodea canadensis* Michx.	Native	Canadian Waterweed
	Egeria densa Planch.	Exotic	Brazilian elodea
	Vallisneria americana Michx.	Native	American Eelgrass, tapegrass
	Hydrilla verticillata (monoecious) (L.f. Royle)	Exotic	Hydrilla
Najadaceae	*Najas guadalupensis* (Spreng.) Magnus	Native	Southern Naiad
Potamogetonaceae	*Potamogeton illinoensis* Morong	Native	Illinois Pondweed
Ceratophyllaceae	*Ceratophyllum demersum* L.	Native	Coontail
Alismataceae	*Sagittaria kurziana* Glück	Native	Strap-leaf Sagittaria
Cyperaceae	*Eleocharis baldwinii* (Torr.) Chapm	Native	Road-grass
Lentibulariaceae	*Utricularia macrorhiza* Leconte	Native	Bladderwort
Characeae	*Chara vulgaris* L.	Native	Muskgrass

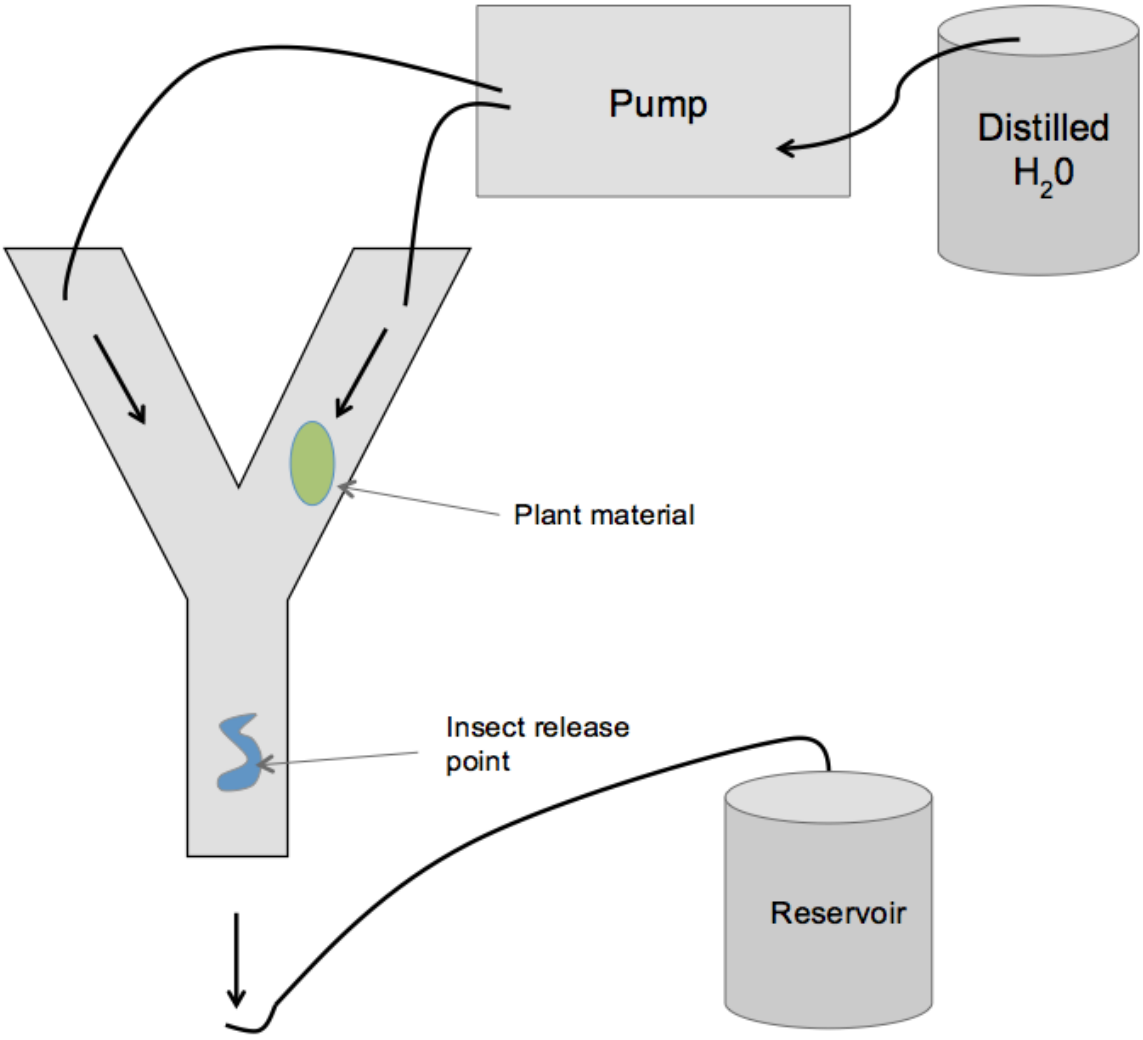


Figure 3-1. Aquatic olfactometer setup used in the dual-choice test

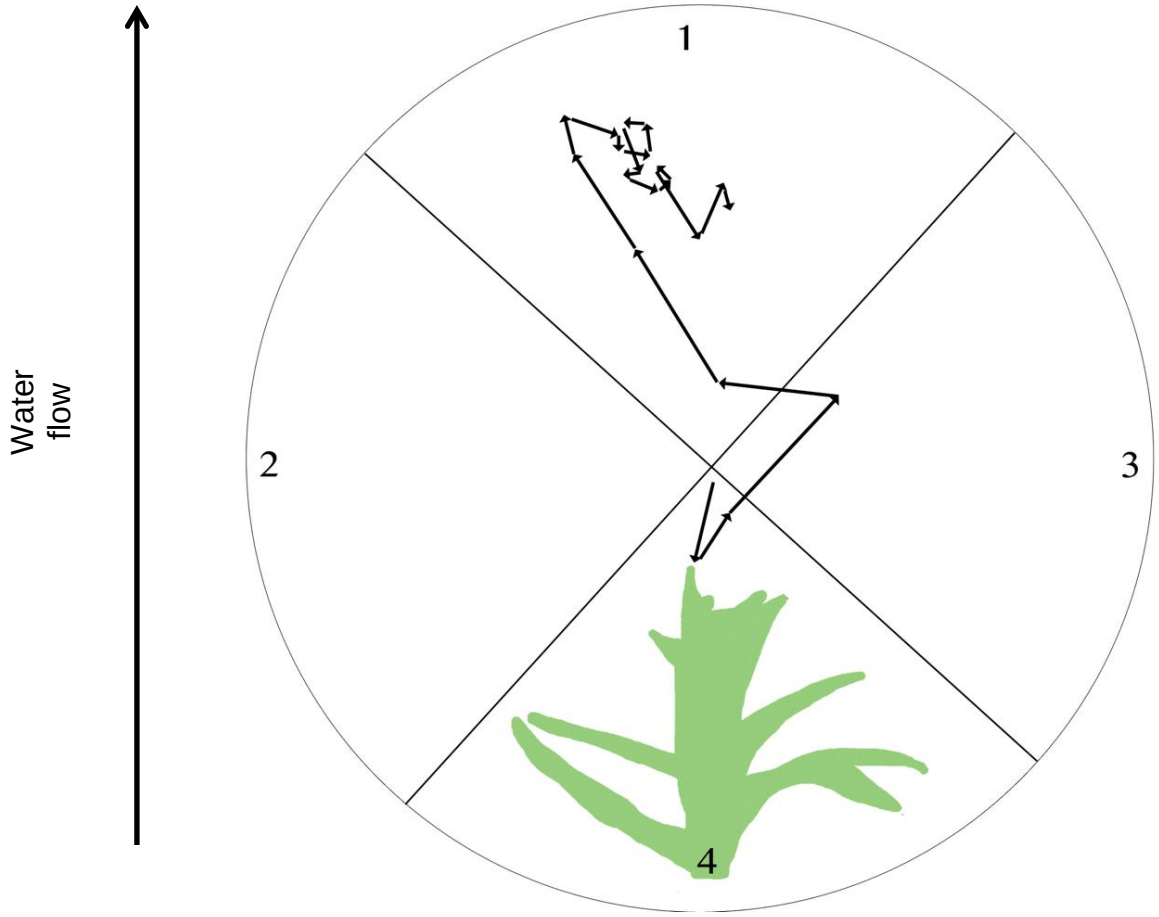


Figure 3-2. Example of movement analysis for correlated random walk (CRW) model. Insect was given 60 minutes to locate plant tip in quadrant 4. Arrows indicate each step insect made every 3 minutes.

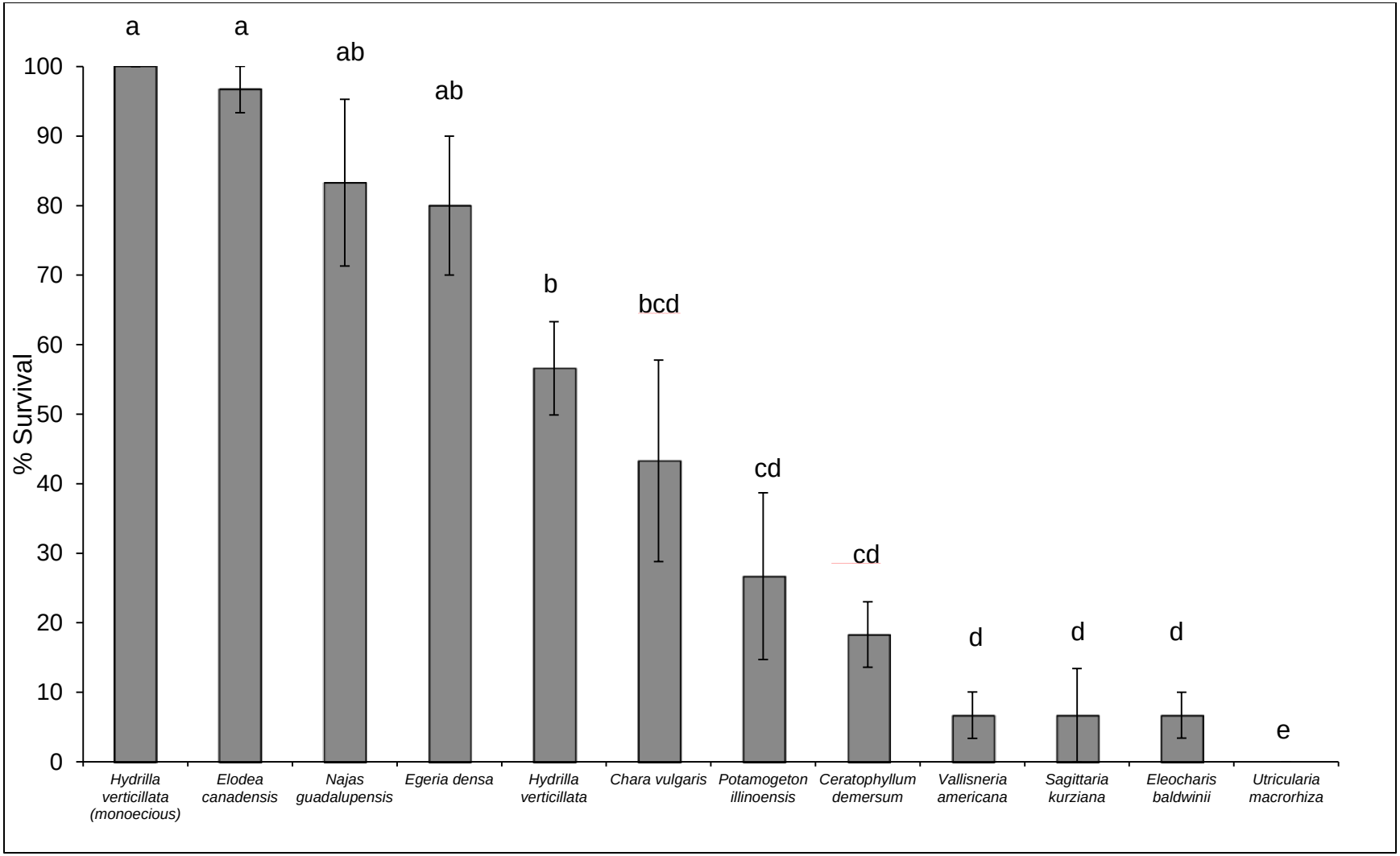


Figure 3-3. Survival of *C. lebetis* larvae on various aquatic plants under no-choice conditions. Survival occurred on 3 non-native plants. Survival was higher on monoecious hydrilla, *E. canadensis,* than on dioecious hydrilla.

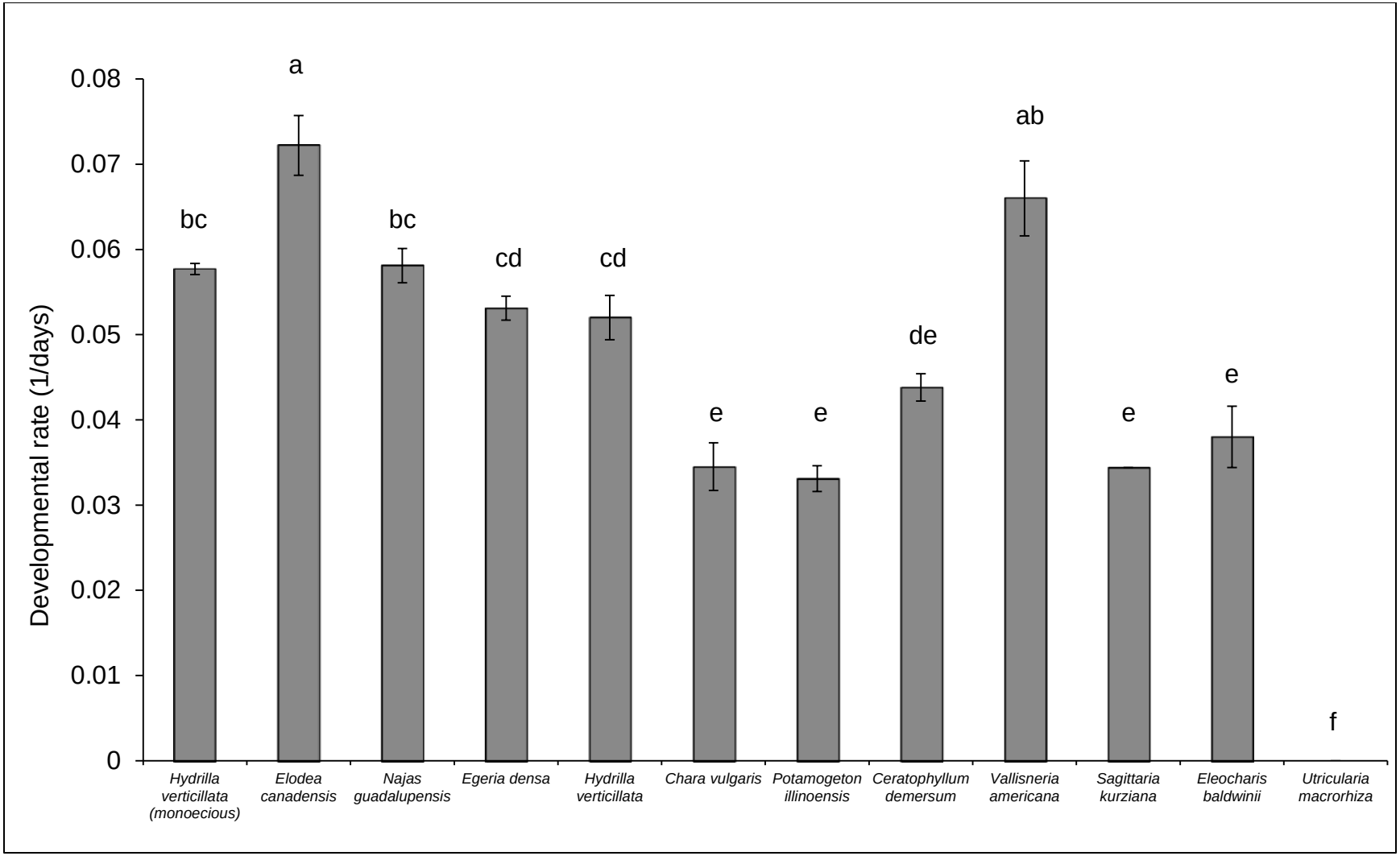


Figure 3-4. Developmental rate of *C. lebetis* larvae on various aquatic plants under no-choice conditions. Development was faster on *E. canadensis* and *V. americana* than on dioecious hydrilla.

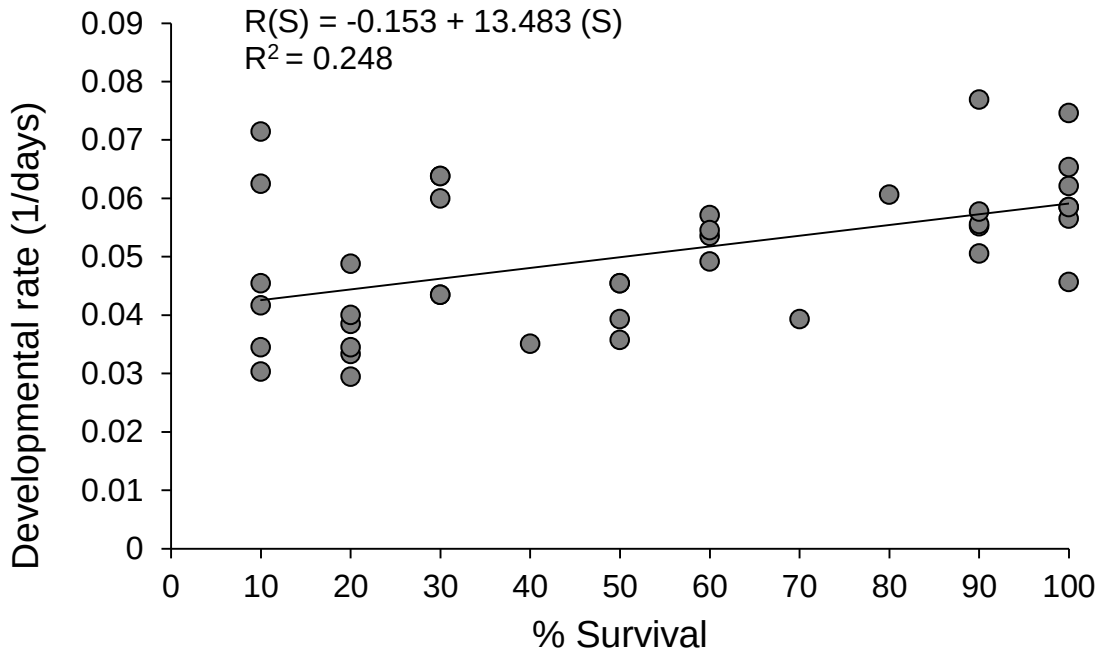


Figure 3-5. Percent survival and development rate of *C. lebetis* larvae on host plants tested. As developmental rate increased survival on host plant increased. Developmental rate was slowest on *C. vulgaris, P. illinoensis, and S. kurziana* and highest on *E. canadensis.*

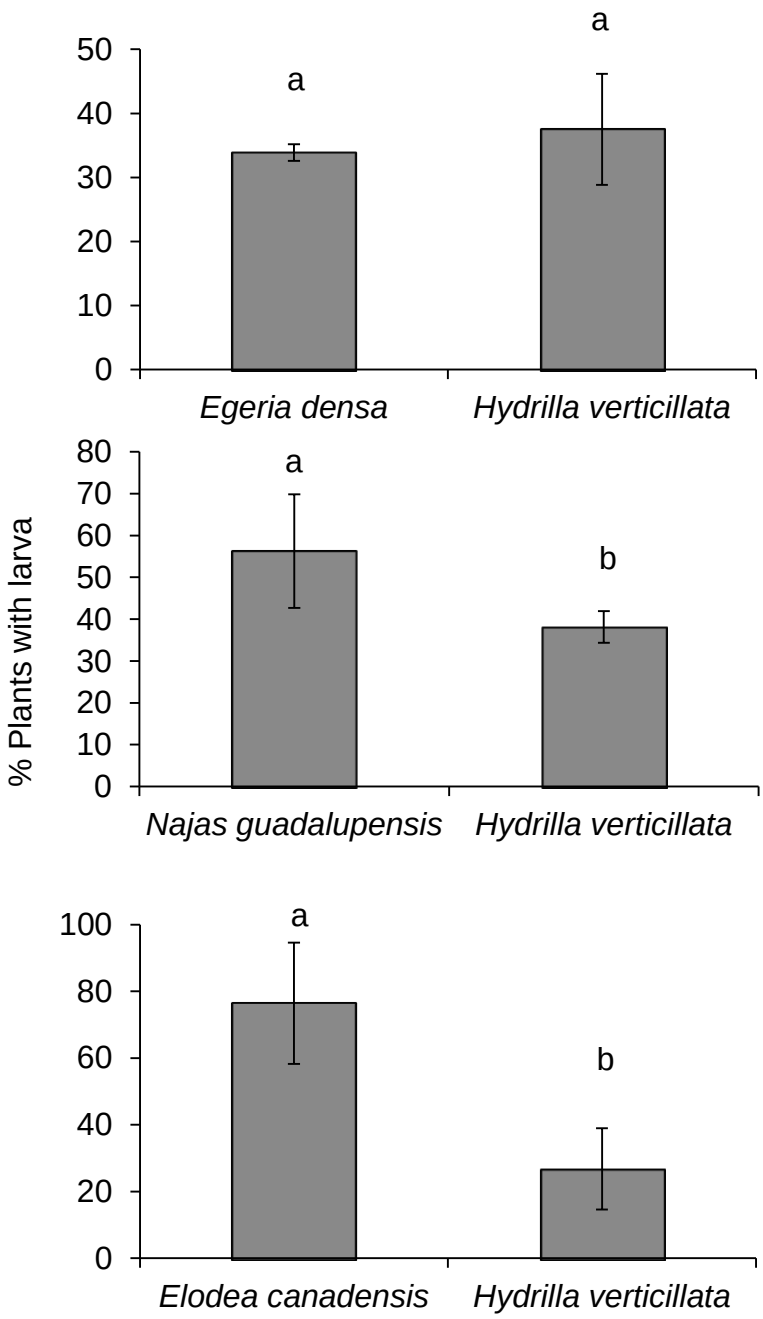


Figure 3-6. Percent of plant tips infested with *C. lebetis* larvae under paired-choice conditions

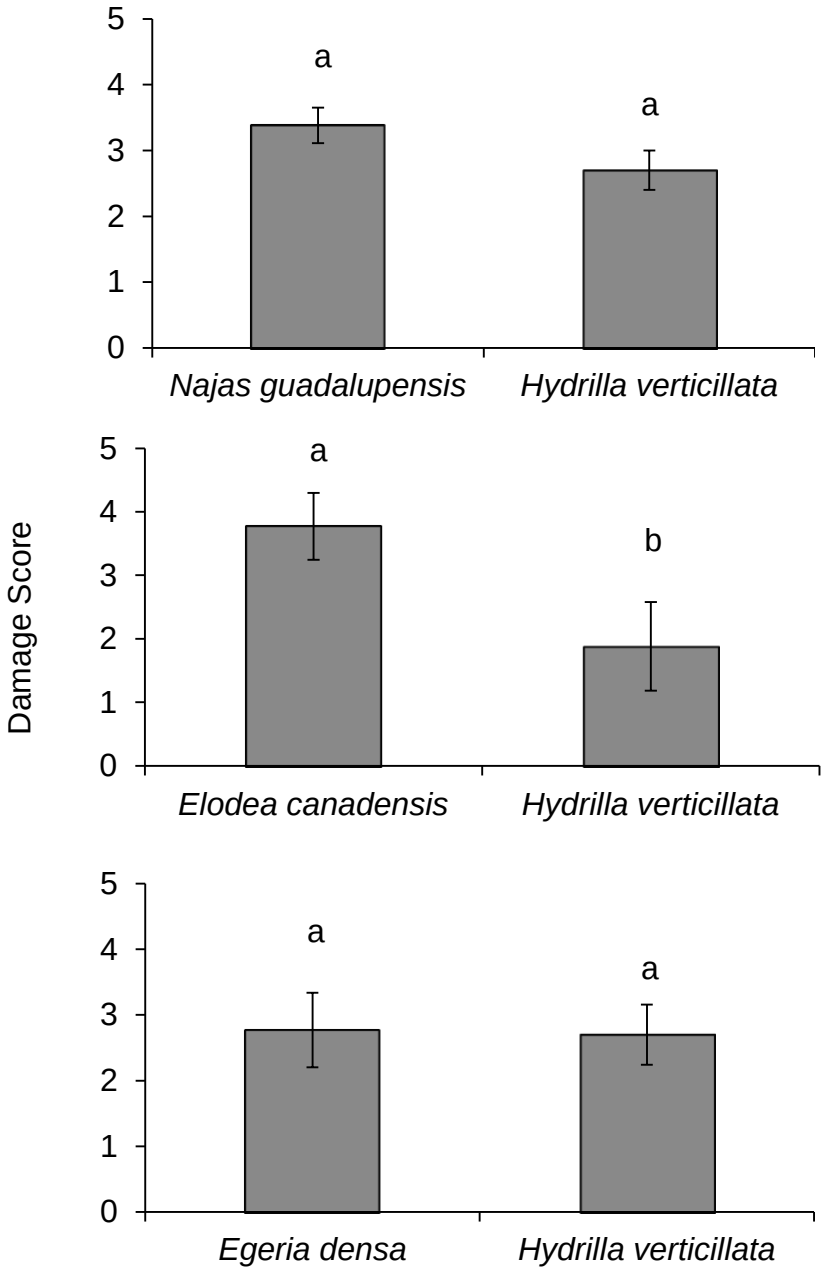


Figure 3-7. Damage scores to plants by *C. lebetis* larvae under paired-choice conditions. 0 = no damage;1 = minimal damage not visible to naked eye; 2 = light damage 10-20%; 3 = moderate damage 20-50% ;4 = significant damage >50%; 5 = tip abscission.

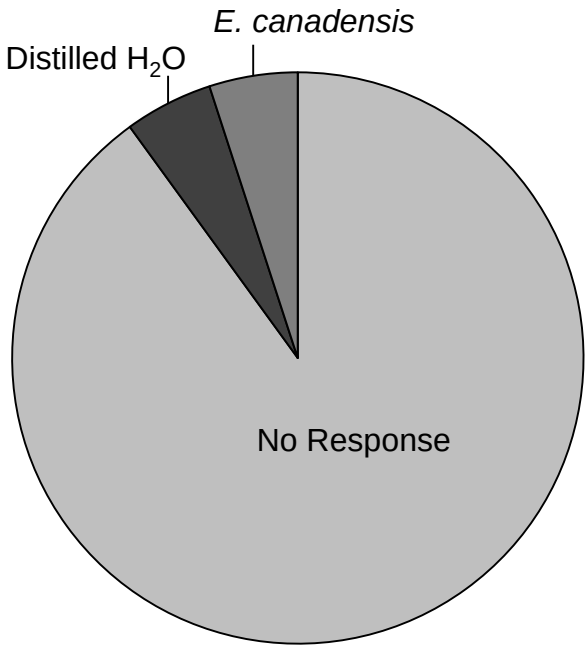


Figure 3-8. Results from the paired-choice olfactometer test. 5% of larvae associated with *E. canadensis* and distilled H_2O, respectively, 90% no response, and 0% associated with hydrilla.

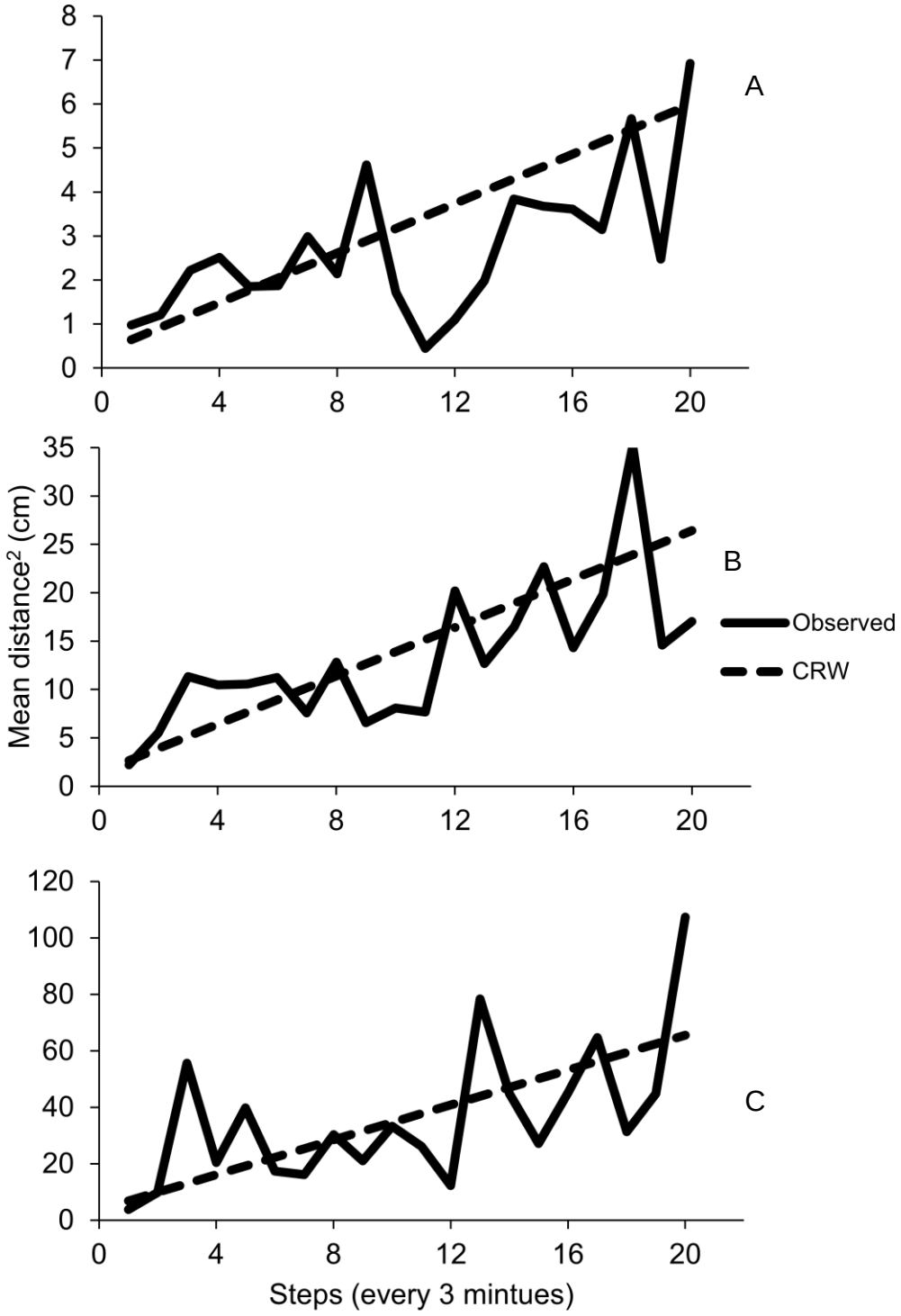


Figure 3-9. Relationship between mean squared displacement of *C. lebetis* larvae and time in Petri dish areas with A) *H. verticillata* B) *E. canadensis* C) no plant material. CRW stands for correlated random walk. Significant P-values obtained for all models show that observed values correlate to CRW, showing that movement patterns are random.

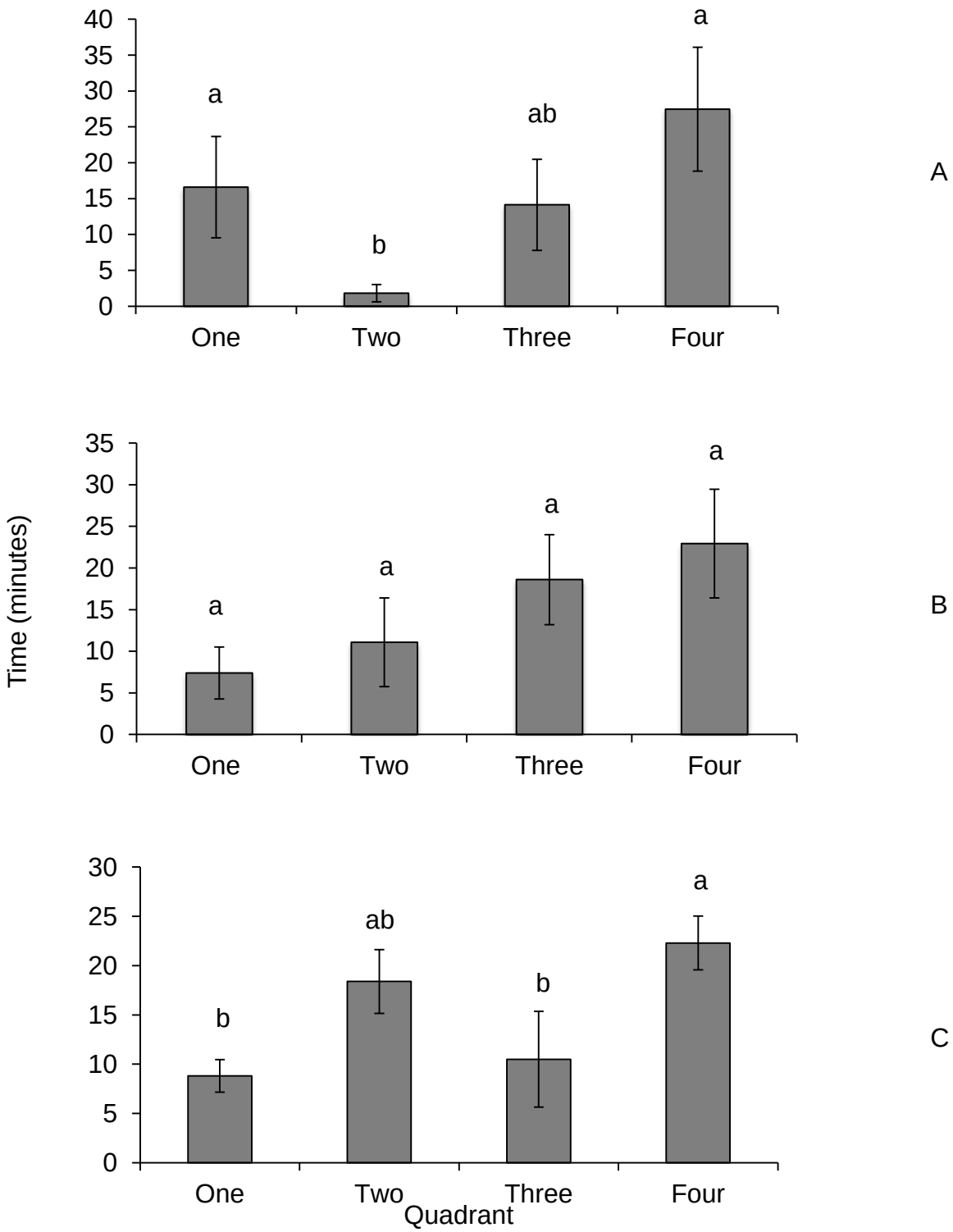
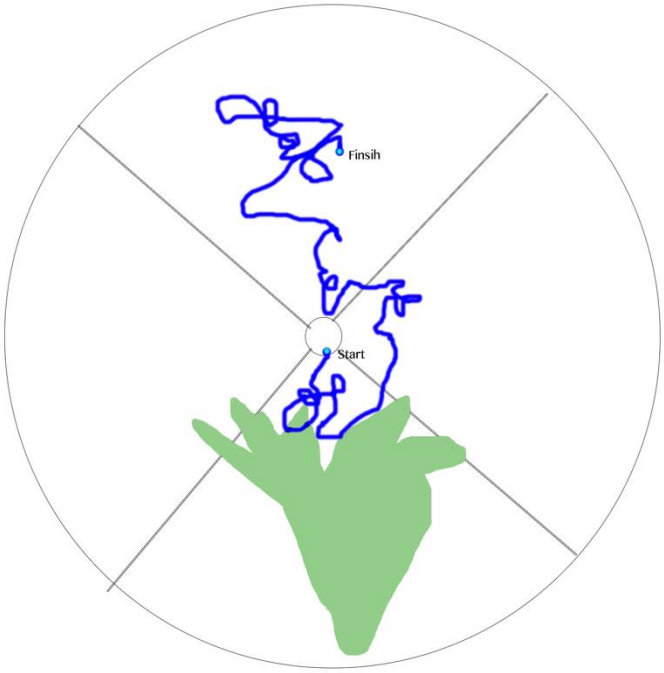


Figure 3-10. Time spent in each quadrant of Petri dish in response to pieces of A) *H. verticillata* B) *E. canadensis* or C) no plant material in quadrant four



A

B

Figure 3-11. Trace patterns of larval movement in a Petri dish from host finding behavior test with A) *H. verticillata* B) *E. canadensis.* Neonate was given 60 minutes to locate host plant in quadrant 4.

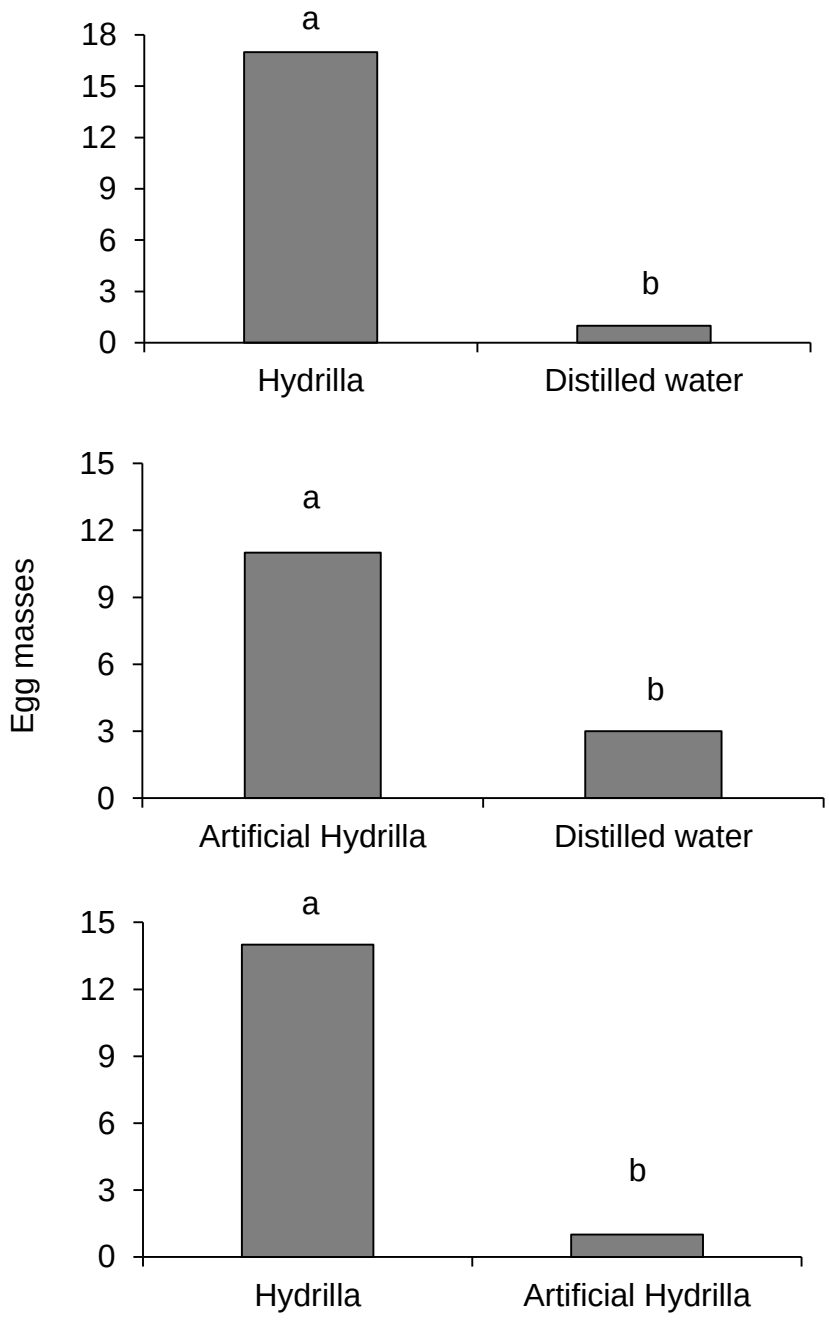


Figure 3-12. Total number of egg masses laid in each paired-choice adult oviposition trial.

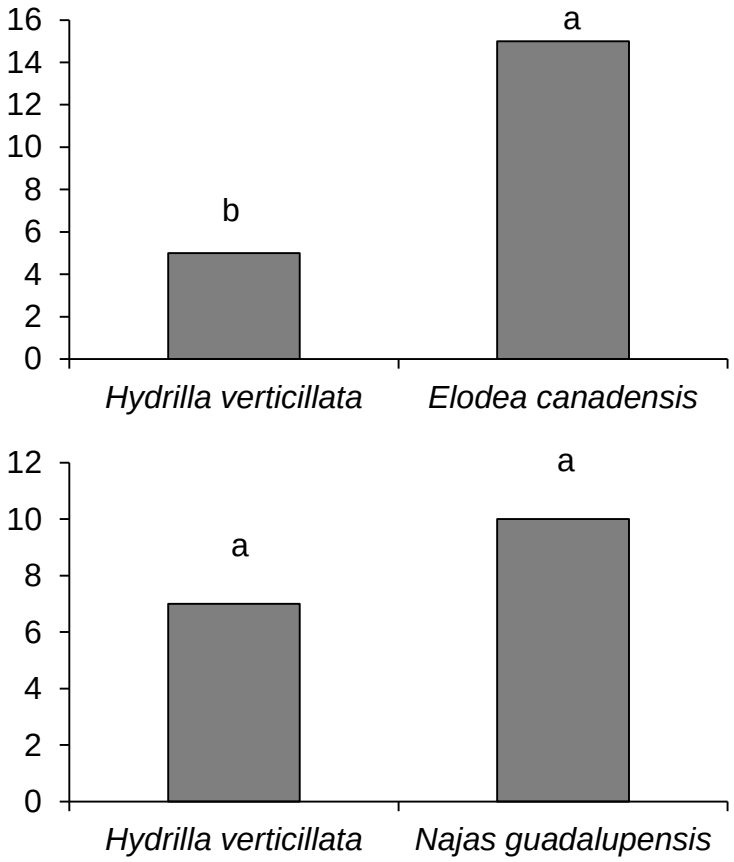


Figure 3-12. Total number of egg masses laid in each paired-choice adult oviposition trial.

CHAPTER 4
THE INFLUENCE OF WATER QUALITY ON *CRICOTOPUS LEBETIS* (DIPTERA: CHIRONOMIDAE): A HERBIVORE OF *HYDRILLA VERTICILLATA* (HYDROCHARITACEAE)

Introduction

Hydrilla verticillata (L.f. Royle) is a submersed aquatic weed that exhibits fast growth rate and is capable of forming dense monocultures. Hydrilla causes negative impacts including displacement of native vegetation (Haller and Sutton, 1975), impediment of boat traffic, recreational and commercial losses, clogging intake pipes and canals, and reductions in tourism and real estate values (Schmitz et al., 1991; Langeland, 1996). Hydrilla is difficult to manage, because few sound options are available (Hoyer et al., 2005). Biological control has been investigated, but has had little success (Buckingham and Balciunas, 1994; Forno and Julien 2000; Gurr and Wratten, 2000; Wheeler and Center, 2001). Due to the recent development of resistance to the herbicide fluridone in some Florida hydrilla populations (Michel et al., 2004), biological control is once again being explored as a possible management tool.

The family Chironomidae is comprised of non-biting midges. They have a cosmopolitan distribution, and occupy almost any habitat that is aquatic or wet (Wirth, 1949).

Water quality is an important variable that determines midge densities and distributions. Due to their sensitivity to water quality, chironomid communities have been used as indicators of water quality and pollution (Saether, 1979). Moreover, they are widely distributed in water bodies of varying quality (Paine and Gaufin, 1956). Although, some *Cricotopus* spp. have been shown to be highly tolerant to pollutants (Boesel,

1983). Given that certain midge species can be sensitive to water quality, water quality should be taken into consideration when developing biological control programs.

In 1992, the chironomid midge *Cricotopus lebetis* Sublette (Diptera: Chironomidae) was discovered in Crystal River Florida attacking the apical meristems of the invasive weed *Hydrilla verticillata* (L.f. Royle) (Cuda et al., 2002). *Cricotopus lebetis* may have potential as an augmentative biological control agent of hydrilla. This insect damages the plant by mining into the apical meristem while preparing for pupation, which causes tip abscission (Cuda et al., 2002). Studies have shown that larval feeding of *C. lebetis* is capable of causing a significant reduction in biomass of hydrilla (Cuda et al., 2011). Although hydrilla is present in many Florida water bodies, the distribution of the midge is largely unknown. During a recent survey in Florida waterways (Fall 2010), *C. lebetis* was only found in Lake Rowell, Bradford, Co. (J. P. Cuda, unpublished data).

Many different land uses can affect Florida's water bodies, including agricultural, urban, residential, and natural areas. Land use activities can influence water quality, and affect chironomid distribution and abundance. Water quality variables such as temperature, pH, dissolved oxygen, hardness, alkalinity, and presence of pesticides may be key factors that determine the distribution of this midge.

Pyrethroid pesticides are used extensively in agriculture, and chironomids have been shown to be sensitive to their presence in water bodies (Anderson, 1989). Fipronil, phenylpyraloze class insecticide, is used extensively to control terrestrial insect populations, and due to runoff, has been documented in Florida water bodies (Harmon-Fetcha et al., 2005). This chemical is highly toxic to fish and aquatic invertebrates (Schlenk et al., 2001; Key et al., 2003; Stehr et al., 2006), and could influence

distributions of *C. lebetis* and other chironomids. The presence of fipronil, even in extremely low concentrations, could be lethal to chironomid populations. Therefore, studies were conducted to monitor pyrethroid and fipronil concentrations in select locations in Florida to examine possible correlations with the distribution of *C. lebetis*. The goals of this research were to assess water quality parameters several water bodies containing hydrilla, and correlate water quality parameters to the presence or absence of *C. lebetis* and other chironomid species.

Materials and Methods

Chironomid Diversity

Six Florida water bodies were sampled quarterly from January 2011 to May 2012 to determine chironomid diversity in hydrilla tips. Locations were in the northern (Lake Rowell, Wacissa Springs), central (Lake Tohopekaliga (Toho), Bulldozer Canal), and southern (Lake Istokpoga, Lake Okeechobee) portions of the state (Fig. 4-1). A four-pronged steel hook attached to a rope was thrown into the water and dragged along the hydrosoil to collect hydrilla. Hydrilla was placed in large re-closable bags partially filled with water from the corresponding sample site to prevent desiccation of hydrilla and associated midges. All bags with hydrilla were placed in a cooler for transport to the laboratory. Several liters of water were collected from each sampling site so that the hydrilla could be maintained in the same water quality for midge rearing. In total, 300 hydrilla tips approximately 5-8 cm in length were randomly selected from each sample and placed in containers with water collected from the corresponding water body. The containers were placed in emergence cages and aerated. Emergence cages were monitored daily for midge emergence for 14 days after the sample date. Emerged adults were placed in vials containing 95% ethanol and separated by species. Individuals that

could not be identified to species were sent to John H. Epler for authoritative identification. Species richness of each sample was determined as the number of reared midge species. Diversity was calculated using the Shannon-Wiener Index (Shannon and Weaver, 1949)

Water Quality

Water quality variables, including temperature, dissolved oxygen, conductivity, and pH, were taken in the field from each location during every sampling occasion using an YSI 556 data logger (YSI, Inc., Yellow Springs, OH). Water samples were collected from the field, placed in 250 ml sterile plastic Nalgene containers, and then placed on ice. Alkalinity and water hardness were determined in the laboratory following EPA method 310.1 (alkalinity) and EPA method 130.2 (hardness) (EPA, 1971; EPA 1978).

Pesticide Analysis

Presence of pyrethroid pesticides was determined by deploying semi-permeable membrane devices (SPMD, Environmental Sampling Technologies Inc., St. Joseph, MO) at two locations (Lake Okeechobee, Lake Rowell) in the fall of 2011. These two locations were selected based on two factors; 1) *C. lebetis* was abundant in Lake Rowell in August 2010, whereas it was not found in Lake Okeechobee on any sampling date and 2) Lake Rowell is primarily surrounded by natural areas whereas Lake Okeechobee is surrounded mostly by agricultural and urban areas. Three SPMDs spiked with Permeability Reference Compound (PRC) were deployed at both locations. The canisters containing the SPMDs were not opened until they were ready for deployment. During deployment, the boat motor was shut off when in close proximity to the deployment site to avoid contamination with hydrocarbons. Three SPMDs were loaded into a single canister provided by the Environmental Sampling Technologies Inc.

(EST), then deployed into the water. A cinder block was used as an anchor, and the SPMD was suspended in the water column using a 1-gallon plastic milk jug filled with Styrofoam. The SMPDs were submersed in the water column at a depth of approximately 1 m for 1 month. SPMDs were then collected from the field, placed back into the shipping canisters, held on ice, and shipped overnight to EST for extraction and dialysis. Extracts were analyzed for pyrethroid pesticides using a gas chromatograph equipped with electron capture detectors (Wu et al., 2010). The presence of fipronil in Lake Okeechobee was determined in May 2012. Four one-liter water samples were collected in glass bottles, filtered, and analyzed for the presence of fipronil using gas chromatography (Wu et al., 2010).

Fipronil Dose Response

A preliminary range finding study was conducted to obtain initial information on the sensitivity of *C. lebetis* to fipronil. Insects were exposed to six concentrations of fipronil mixed with well water (0.0, 0.02, 0.2, 2.0, 20.0, 200.0 and 2000.0 g/L) in test tubes (35 ml) with 20 ml of the mixture. A single, undamaged, healthy hydrilla tip 3-5 cm in length was placed in each tube, along with one 8 day old larva. Test tubes were placed in a rack in an environmental growth chamber at 25°C and 14:10 (L:D) photoperiod (Model No. E36L, Percival Scientific, Inc., Perry, IA). Larval survival was assessed every 24 hours for 96 hours. A second definitive was conducted using a narrower range of fipronil concentrations (0.0, 0.5, 2.0, 5.0, 10.0, 15.0, and 20.0 µg/L). Concentrations during the definitive test were confirmed by GC-ECD using the method of Wu et al. (2010).

Effect of Alkalinity and Hardness on *C. lebetis* Larval Development

Nanopure® water was used to make three different solutions of water hardness and alkalinity; soft (alkalinity: 12, hardness: 12), moderately hard (alkalinity: 65,

hardness: 90) and very hard (alkalinity: 235, hardness: 300). Each water solution was placed in a 38 liter aquarium and aerated. Hydrilla was propagated from stems collected in Lake Toho and placed into 10.16 cm pots containing a layer of potting soil covered by sand. Hydrilla was grown in each aquarium for a minimum of 3 weeks prior to insect exposure. Tips 4-6 cm in length from each aquarium were harvested and placed individually in 35 ml test tubes filled with water from the corresponding aquarium. Control treatments used hydrilla grown in well water and test tubes filled with well water. Two neonates of *C. lebetis* larvae were transferred to each test tube using a pipette, and then each tube was stoppered with a cap with ventilation holes. All racks were placed in an environmental growth chamber maintained at 25°C and 14:10 (L:D) photoperiod (Model No. E36L, Percival Scientific, Inc., Perry, IA). Tips were checked daily to ensure that they were fully submerged in order to prevent larval desiccation. Tubes with tips that were completely destroyed received replacement tips to allow midges to complete development to adulthood. Starting approximately one week after the larvae were introduced, the test tubes were checked daily for adult emergence. Tips that supported development to the pupal stage were considered as suitable for complete development. The number of days to develop to pupation was recorded.

Data Analysis

Survival and developmental time under different water quality conditions were analyzed with ANOVA using the general linear model procedure, and means were separated with Student-Newman Keuls procedure (PROC GLM; SAS Institute 2008). The Shannon-Wiener Index was calculated for each sampling occasion and for total diversity at each sampling location. The equation was as follows:

$$H = -\sum_{i=1}^{s} p_i \ln p_i$$

Where H = the Shannon-Wiener diversity index; p_i = abundance of species *i*.

The frequency of occurrence (incidence) and abundance were calculated for each chironomid to obtain an index of prevalence (*Ip)* (Zhou et al. 2003). Incidence was determined by dividing the number of locations where the chironomid was found on each sampling date by the total number of locations sampled. Abundance was determined for each sampling occasion by summing the total number of individuals of each species found and dividing by the number of plant tips sampled on that sampling occasion. The *Ip* was calculated for each chironomid species as the product of chironomid abundance and incidence as follows:

$$Ip = \left[\frac{Fo}{N_1}\right]\left[\frac{S}{N_2}\right]$$

Where *Fo* = the number of locations a species was found, N_1 = the number of locations sampled, *S* = the sum of the abundance values for a species and N_2 = number of tips collected (Zhou et al., 2003). Confidence limits (95%) were calculated for each *Ip* value following methods provided by Buonaccorsi and Leibhold (1988), and means compared by examining overlap in confidence limits (84%) as described by Payton et al. (2003). Data from the fipronil studies were analyzed using Proc Probit in SAS (SAS Institute, 2008) to generate a logistic regression of the data.

Results

Chironomid Diversity

Species richness ranged from 8-15 taxa per location and a total of 18 species were found associated with hydrilla (Table 4-1). Bulldozer Canal had the highest

species richness and Wacissa Springs had the lowest. Species diversity, measured by the Shannon-Wiener index ranged from 1.29 – 2.27 per location (Table 4-2), with Bulldozer Canal having the highest diversity and Lake Okeechobee the lowest. *Cricotopus lebetis* was found at four of the six sampling locations (Lake Toho, Lake Rowell, Lake Istopkoga, Bulldozer Canal), but at low densities except for Lake Rowell in the fall of 2010. Total numbers of *C. lebetis* collected at each site were Lake Rowell: 27, Lake Toho: 4, Lake Istopkoga: 12, Bulldozer Canal: 3 (Table 4-3). The prevalence index was highest for *Tanytarsini* spp*.* (0.0076) and second highest for *Cricotopus sylvestris* (0.0042), a congener of *C. lebetis,* that does not feed on living plant tissues*.* The rarest species based on the index of prevalence were *Nilobezzia schwarzii* (0.000003) and *Cricotopus politus* (0.000003) (Figure 4-1). The prevalence of *Cricotopus lebetis* was intermediate with an index of 0.0023.

Water Quality

Water quality measurements at field sites indicated that there were little differences in water quality among water bodies sampled (Table 4-4). The dissolved oxygen content in Bulldozer Canal was very low for the majority of the year (<1 mg/L), except in the winter when an unseasonal storm event occurred. Other sites had dissolved oxygen concentrations ranging from 2.60-7.57 mg/L. The temperatures at each location for each sample date were within the thermal limits of *C. lebetis.* For all sampling locations the temperature ranged from 14.00 - 28.31°C. The pH for all sampling location and dates ranged from 6.34 – 8.63. Conductivity ranged from 0.196 – 40.9 (mS/cm).

Bulldozer canal and Wacissa Springs were the only sample sites with water hardness and alkalinity levels above 100 mg/L $CaCO_3$. Lake Istopkoga had alkalinity

levels ranging from 16-38 mg/L $CaCO_3$. Lake Rowell's alkalinity ranged from 31-34 mg/L $CaCO_3$ and hardness ranged from 33.0-42.5 mg/L $CaCO_3$. Lake Okeechobee had moderate alkalinity and hardness ranging from 40-98 and 40-91 mg/L $CaCO_3$, respectively. Lake Toho and Istopkoga had lower hardness ranging from 27-37 and 22-42.5 mg/L $CaCO_3$, respectively.

Pesticide Analysis

No evidence of pyrethroid pesticides was found in Lake Okeechobee or Lake Rowell from deploying SPMD units. One water sample collected in Lake Okeechobee was confirmed to have 15 ng/L of fipronil.

Fipronil Dose Response

The results from the range finding test were used to determine the range of concentrations to use in the definitive test. For the definitive study, 100% mortality occurred at 20.0, 15.0, and 10.0 µg/L of fipronil. No mortality occurred in the control, and 90%, 60%, and 40% mortality occurred at 5.0, 2.0, and 0.5 µg/L, respectively. The estimate of the LC_{50} (lethal concentration) using this data was 0.91 µg/L, and the LC_{90} was 4.52 µg/L (Fig. 4-2).

Effect of Alkalinity and Hardness on *C. lebetis* Larval Development

There were no significant differences in development time ($F = 3.18$, $df = 3$, $P = .0848$) or survival ($F = 0.89$, $df = 3$, $P = 0.49$) of larvae reared in the different alkalinity/hardness water quality treatments (Fig. 4-3).

Discussion

Water quality is an important attribute of aquatic habitats that may influence the distribution and abundance of chironomid communities (Saether et al., 1979). In Florida, land surrounding water bodies is used in a variety of different ways that may impact

water quality. Water bodies adjacent to agricultural land may receive runoff containing pesticides and other chemicals. Chironomids are sensitive to many chemicals and may be negatively impacted by runoff (Gresens et al., 2007), but some chironmids are highly tolerant to pollutants (Boesel, 1984). SPMDs are capable of detecting pyrethroid pesticides over time, but may not detect other pesticides. Therefore, pesticide classes that were not found in the SPMD samples may have been present and influenced the distribution and abundance of *C. lebetis* and other chironomids.

The water quality data collected from the field, coupled with the laboratory study on larval development in water with different levels of alkalinity/hardness, suggest that these variables may not have a major impact on survival and distribution of *C. lebetis. Cricotopus lebetis* was found at Bulldozer Canal, which had high alkalinity and hardness and low dissolved oxygen, and also was found at Lake Istopkoga, which had low alkalinity and hardness, and average dissolved oxygen. Laboratory testing, which revealed that larval survival and development did not vary in response to differences in alkalinity and hardness, is consistent with the field data. Conductivity at Bulldozer Canal was higher than at Lake Istopkoga, and *C. lebetis* was recovered from both these locations.

Lake Okeechobee, which is surrounded largely by agriculture (Flaig and Havens, 1995), had a Shannon-Wiener Index of 1.29, which was low compared to the other sampled water bodies. Fipronil was detected in Lake Okeechobee, but at a lower concentration than what was shown to be lethal in the laboratory. Additional sampling would likely reveal temporal fluctuations, and if fipronil reaches lethal concentrations in the field. Low concentrations of fipronil have been reported to be toxic to

macroinvertebrates, and grass shrimp were reported to have a LC_{50} of 0.32 µ/L (Key et al., 2003), and thus, may affect the distribution of chironomids. The presence of fipronil could also affect colonization of *C. lebetis* in Lake Okeechobee if augmentative releases were to be made. Reducing agricultural runoff and implementing best management practices could improve water quality in Lake Okeechobee and the macroinvertebrate communities within.

The other location that *C. lebetis* was not recovered was Wacissa Springs, perhaps because of cooler water temperatures. Wacissa Springs has a constant temperature throughout the year of 21°C (Raymond Hix, personal communication). This is within the thermal limits of *C. lebetis,* but is near the lower end of the optimal temperature range for development (Chapter 2). Low water temperature would slow the midge's developmental rate compared to further south in Florida, and therefore negatively affect population growth and abundance. Moreover, Wacissa Springs was less frequently sampled than other water bodies, and therefore the probability of recovering *C. lebetis*, even if was as abundant as in the water bodies where it was found, would be lower.

The sporadic recovery of *C. lebetis* over different sampling periods suggests that this insect does not exhibit seasonality. It was found during all four sampling periods (winter, spring, summer, fall), albeit not within the same water body. In Lake Toho, its seasonal incidence was highest as it was found in the winter, spring, and fall. The seasonal abundance of the midge likely responds to changes in environmental conditions, such as temperature and food availability, which vary throughout the year. The abundance of *Cricotopus lebetis* was highest in Lake Rowell in the fall of 2010, but

surprisingly, it was not recovered from Lake Rowell on later sampling dates. The reasons for the high abundance of *C. lebetis* in the fall of 2010 in Lake Rowell are unknown, but could be a combination of biotic, abiotic and anthropogenic factors.

Overall, water quality does not seem to be as important in determining the distribution of *C. lebetis* as was hypothesized. The midge was found in four widely distributed water bodies in Florida, with different water quality characteristics. Thus, the midge does not appear to be highly sensitive to variation in the water quality parameters that were measured. Pesticides, however, may play a role, based the laboratory study on the sensitivity of the midge to fipronil. Additional sampling may reveal a correlation between the presence of pesticides and chironomid diversity.

Table 4-1.Chironomid species richness at each location from each sampling period. Means and standard deviations were calculated when there was more than one sample/location/sampling period. Plant material from Lake Rowell and Wacissa Springs was sampled separately and shipped to the facility in Fort Pierce, and these locations were not sampled during each sampling event.

	Winter	Spring	Summer	Fall	Total
Lake Toho	4 ± 2.83	5.5 ± 3.54	2	6	15
Lake Istokpoga	3 ± 1.41	3.5 ± 0.71	5	5	12
Lake Rowell	--	2	4	6	9
Lake Okeechobee	3 ± 2.83	2.5 ± 2.12	--	3	8
Bulldozer Canal	4	7	2	2	13
Wacissa Springs	--	7	7	--	8

Table 4-2. Chironomid diversity at each location for each sampling period. Means and standard deviations were calculated when there was more than one sample/water body/sampling period. Plant material from Lake Rowell and Wacissa Springs was sampled separately and shipped to the facility in Fort Pierce, and these locations were not sampled during each sampling event.

	Winter	Spring	Summer	Fall	Total
Lake Toho	1.55	1.37 ± 0.52	1.89	1.54	2.27
Lake Istokpoga	1.29	0.81 ± 0.37	1.23	1.14	1.89
Lake Rowell	--	0.67	0.64	1.43	1.42
Lake Okeechobee	1.28	1.52	--	1.05	1.29
Bulldozer Canal	1.27	1.65 ± 0.15	0.64	0.55	2.11
Wacissa Springs	--	1.79	1.48	--	1.88

Table 4-3. Chironomid species sampled from all locations during survey work.

Species	Water body					
	Lake Toho	Lake Istokpoga	Lake Okeechobee	Bulldozer Canal	Lake Rowell	Wacissa Springs
Ablabesmyia rhamphe	0	1	0	1	1	0
Apedilum elachistus	3	1	2	36	0	0
Bezzia glabra	2	0	0	0	2	0
Chironomini spp.	3	27	2	1	8	8
Cricotopus bicintus	1	0	3	5	0	0
Cricotopus lebetis	9	12	0	3	27	0
Cricotopus politus	0	0	0	0	0	1
Cricotopus sylvestris	2	3	53	25	30	5
Dicrotendipes spp.	26	11	7	20	3	12
Glyptotendipes spp.	11	0	0	6	0	0
Larsia decolorata	7	0	2	3	2	0
Nanacladius alternantherae	1	2	0	0	0	0
Nilobezzia schwarzii	0	1	0	0	0	0
Parachironomous hazelriggi	32	17	15	9	0	8
Pentaneura inconspicua	5	0	0	0	0	5
Pseudochironomous richardsoni	5	1	0	5	0	0
Tanytarsus buckleyi	10	3	0	7	9	17
Tanytarsini spp.	17	2	2	6	94	17

Table 4-4. Water quality variables at field sites from January 2011-May 2012. Means and standard deviations were calculated when there were multiple samples per location and season. Plant material from Lake Rowell and Wacissa Springs was sampled separately and shipped to the facility in Fort Pierce, and these locations were not sampled during each sampling event

Water Body	Season	Temperature (°C)	pH	Dissolved Oxygen (mg/L)	Conductivity (mS/cm)	Alkalinity (mg/L) $CaCO_3$	Hardness (mg/L) $CaCO_3$
Lake Okeechobee	Winter	18.25 ± 2.51	6.75 ± 0.46	5.62	2.18 ± 2.25	90 ± 45.26	61 ± 15.56
	Spring	24.92 ± 0.84	8.18 ± 0.04	5.55 ± 1.14	3.36 ± 4.46	86 ± 39.6	74 ± 24
	Summer	23.68	6.92	2.6	7.08	98	91
	Fall	20.45	6.52	4.42	2.73	40	40
Lake Istopkoga	Winter	18.57 ± 1.03	7.23 ± 0.42	6.41	1.03 ± 1.26	26 ± 8.49	28.5 ± 4.95
	Spring	26.34 ± 1.03	8.63	6.69 ± 0.71	1.35 ± 1.63	24 ± 2.83	42.5 ± 2.12
	Summer	27.27	8.88	6	3.07	38	37
	Fall	21.84	6.74	6.74	1.32	16	22
Lake Rowell	Winter	15.6	7.23	6.44	2.84	32	39.5 ± 0.71
	Spring	23.8	--	--	--	31 ± 4.24	42.5 ± 2.12
	Summer	29.8	--	--	--	34	33
	Fall	--	--	--	--	--	39
Lake Toho	Winter	16.9 ± 1.18	6.24 ± 1.03	4.98	1.28 ± 1.52	41 ± 1.41	20.5 ± 13.44
	Spring	26.13 ± 2.34	7.48 ± 0.08	6.74 ± 0.71	1.46 ± 1.81	42 ± 11.31	35 ± 4.24
	Summer	28.31	7.22	5.98	2.77	52	37
	Fall	22.7	7.33	5.31	1.8	34	27
Bulldozer Canal	Winter	14	7.12	4.04	40.9	156	144
	Spring	25.33 ± 1.07	7.13 ± 0.06	1.43 ± 0.78	6.62 ± 8.12	155 ± 15.56	152 ± 4.24
	Summer	27.51	7.01	0.93	11.79	124	125
	Fall	20.55	6.34	0.55	1.72	42	27
Wacissa Springs	Winter	21	--	--	--	65	128
	Spring	21	--	--	--	81	154
	Summer	21	--	--	--	111	208
	Fall	21	--	--	--	--	--

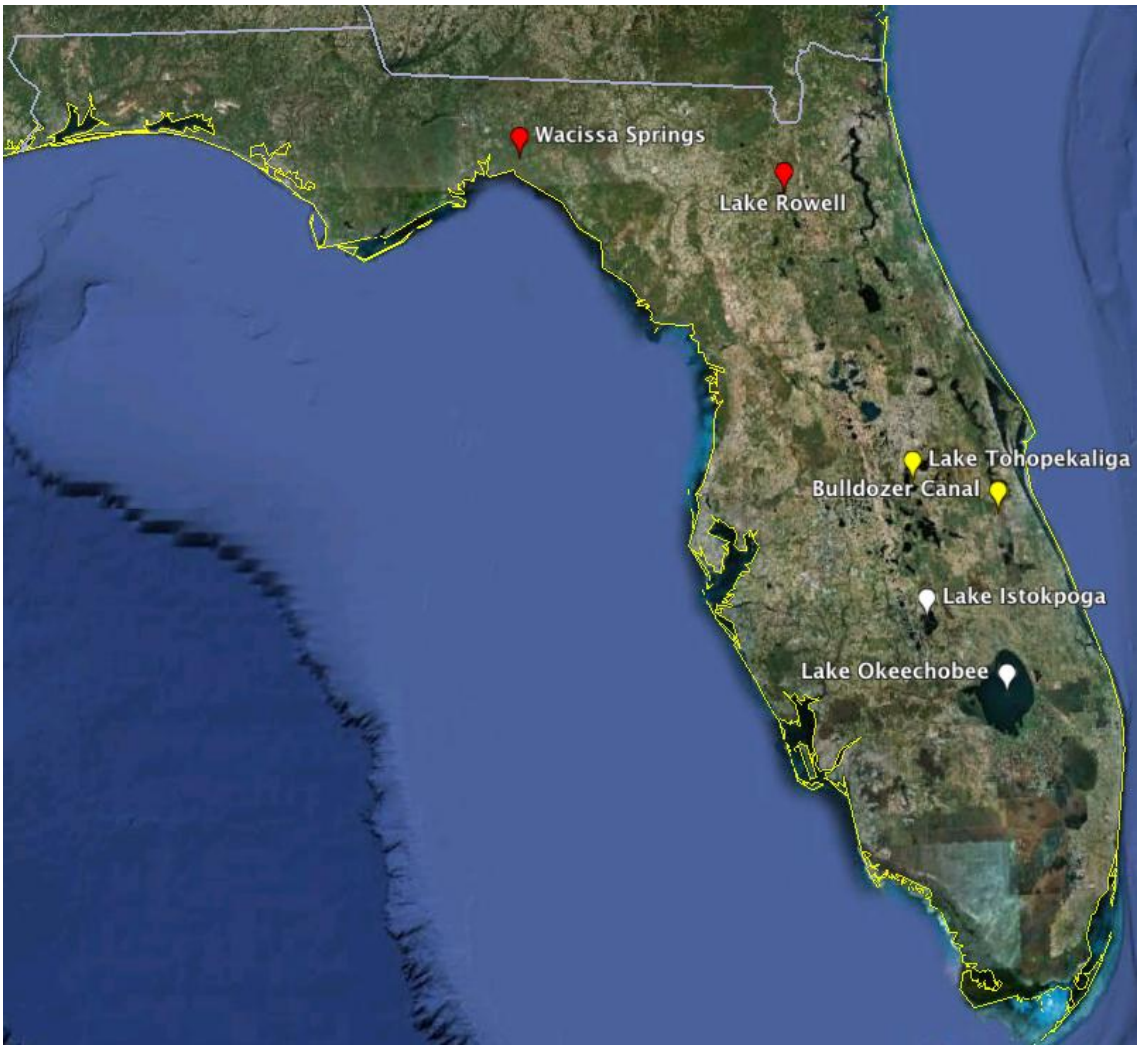


Figure 4-1. Sampling locations for survey data in Florida where hydrilla for chironomid diversity study and water quality variables were collected. Northern locations in red (Wacissa Springs, Lake Rowell), central locations in yellow (Lake Tohopekaliga, Bulldozer Canal), southern locations in white (Lake Istokpoga, Lake Okeechobee) Source: Google Earth.

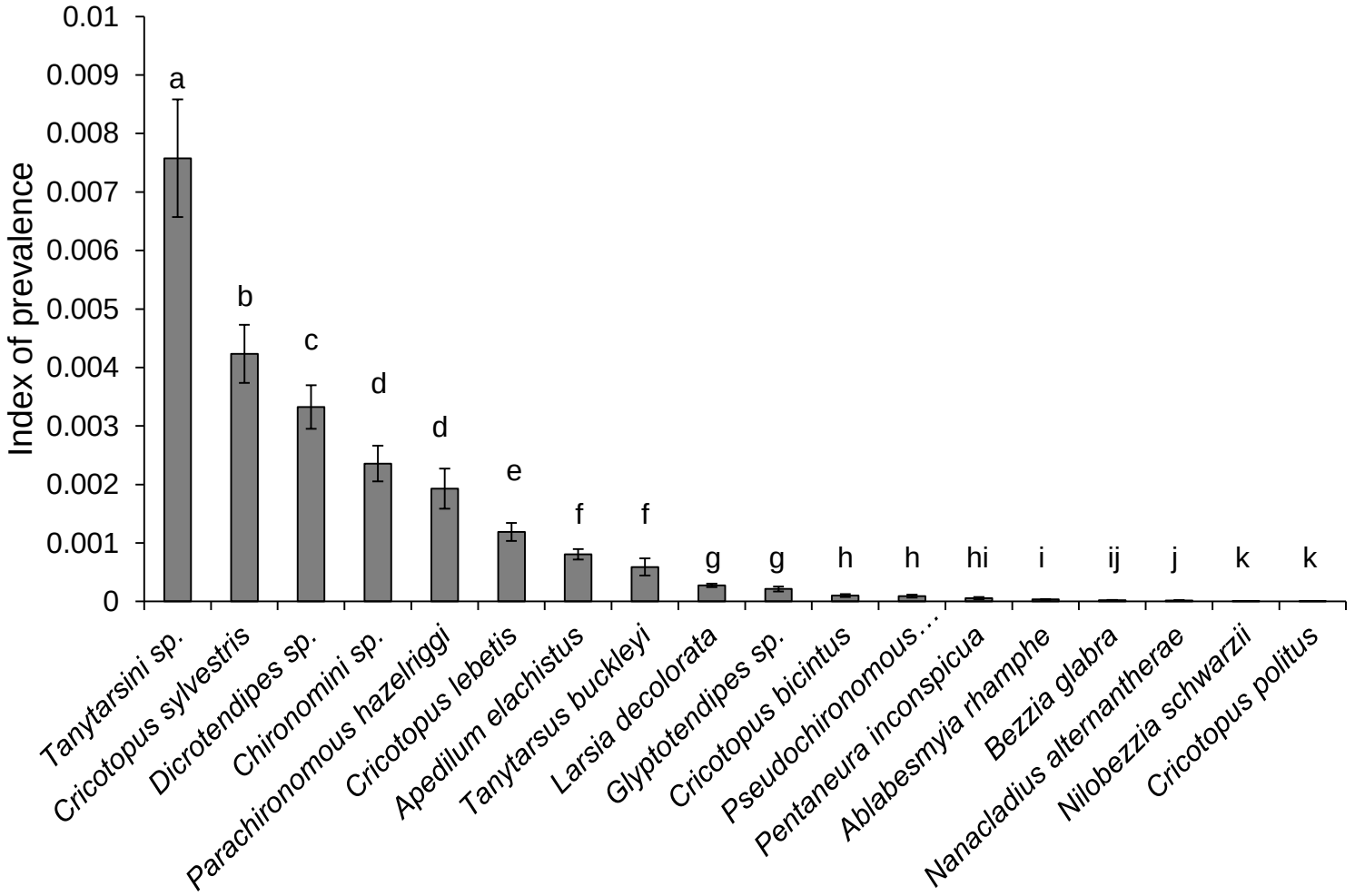
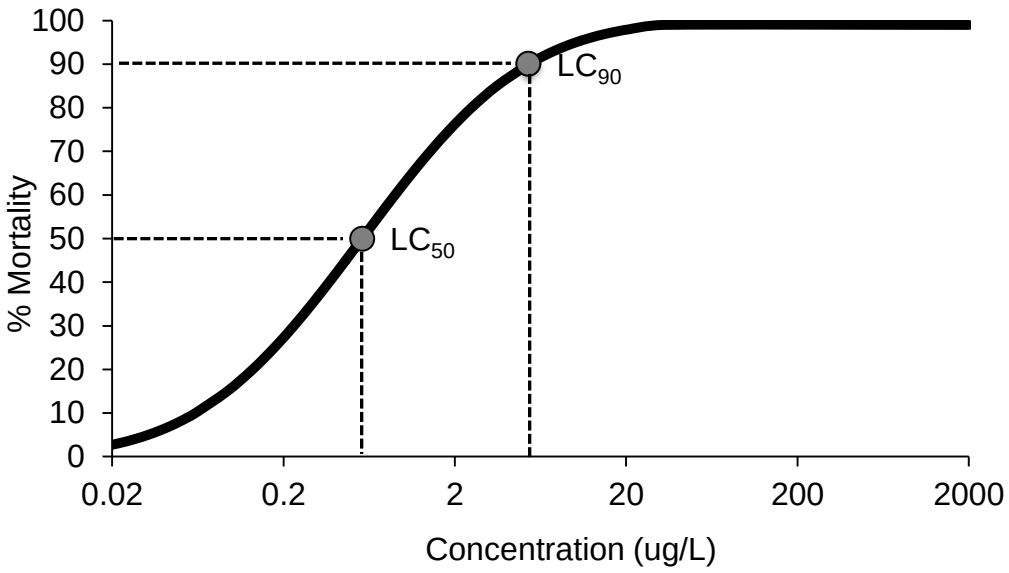


Figure 4-2. Index of prevalence of chironomids recovered from all sampling locations from January 2011-May 2012.



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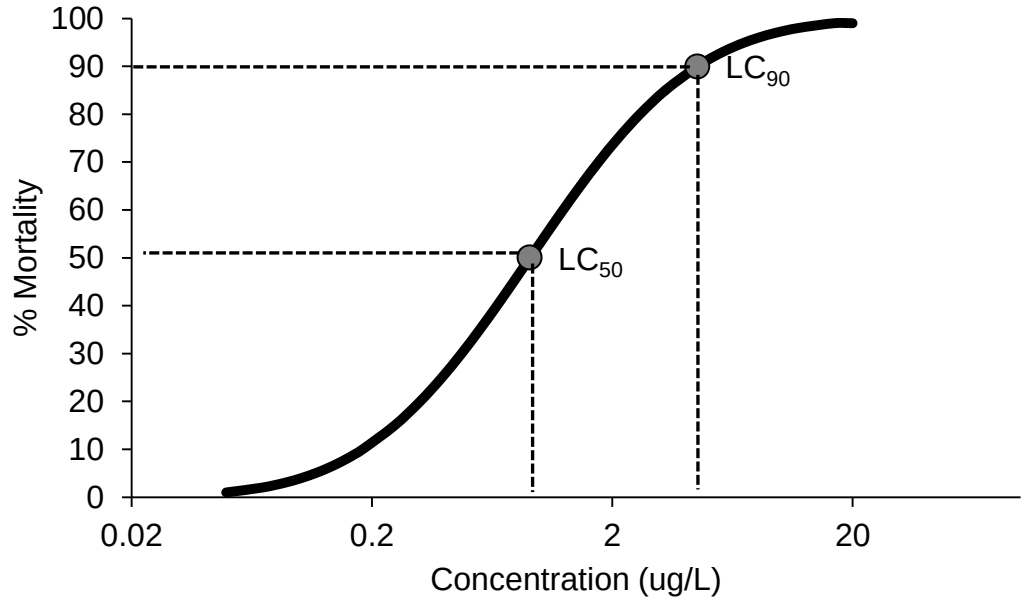

B

Figure 4-3. Fipronil laboratory bioassay with *C. lebetis* larvae A) range finding test B) dose response test. Showing lethal concentration (LC) at which 50 and 90% population mortality are predicted to occur.

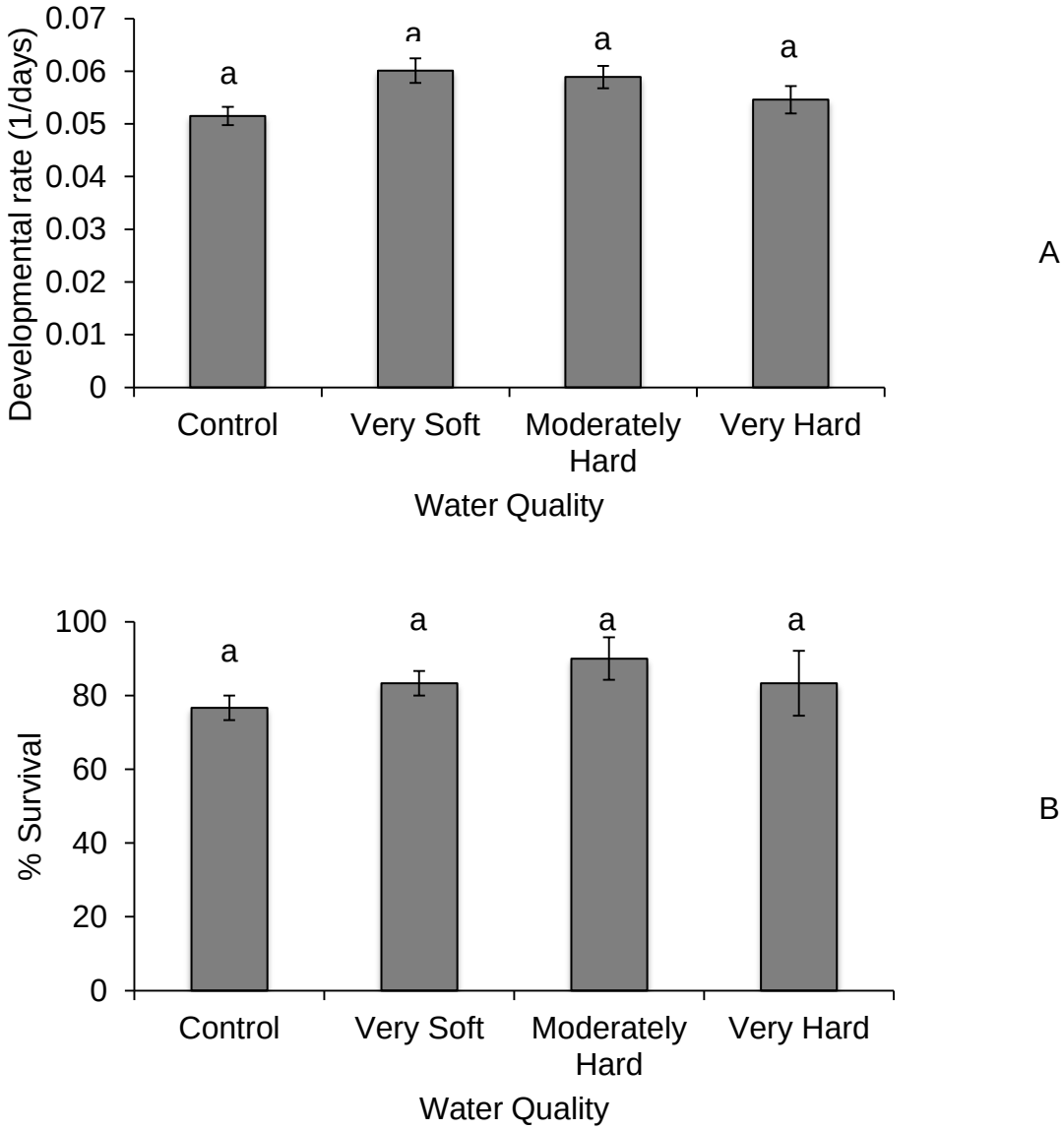

Figure 4-4. Response of *C. lebetis* to different levels of water alkalinity/hardness A) developmental rate B) percent survival.

CHAPTER 5
SUMMARY OF FINDINGS ON THERMAL REQUIREMENTS, HOST RANGE AND DISTRIBUTION OF *CRICOTOPUS LEBETIS* (DIPTERA: CHIRONOMIDAE), A NATURAL ENEMY OF *HYDRILLA VERTICILLATA* (HYDROCHARITACEAE)

Introduction

Hydrilla verticillata (L.f. Royle) is one of the most devastating aquatic invasive plants introduced into the USA. Since its introduction, costly programs have been enacted to control hydrilla, but management remains difficult (Hoyer et al., 2005). The recent advent of fluridone resistance has raised awareness of the aggressiveness of this plant if left unmanaged (Michel et al., 2004). The hydrilla miner, *Cricotopus lebetis* Sublette (Diptera: Chironomidae), was discovered in 1992 in Crystal River Florida attacking the apical meristems of hydrilla (Cuda et al., 2002). The discovery of the insect stimulated interest in assessing its potential as a biological control agent. The thermal requirements, fundamental host range, and host finding behavior were studied. Hydrilla surveys in water bodies throughout Florida were conducted to analyze water quality variables, and to search for the presence of pesticides that could impact chironomid distributions and densities. The results obtained from these studies will be used to evaluate the potential of the hydrilla miner for augmentative biological control of hydrilla in Florida, and for release as a classical biological control agent in areas where it does not occur.

Recommendations

Use of *C. lebetis* in Florida

Cricotopus lebetis may have value as an augmentative biological control agent of hydrilla. Since the midge occurs in Florida, no permits would be required to release *C. lebetis* within the state. Based on the results of field surveys, temperature-dependent

development studies, climatic mapping, host range testing and water quality studies, the midge can establish throughout Florida. However, there is some evidence that pesticide pollutants in the water may influence its occurrence, but further study and sampling is needed to confirm this statement. Following are some factors that should be considered for augmentative use of *C. lebetis*:

Water temperature

The thermal limits of *C. lebetis* are broad, as development occurred at temperatures ranging from 15-35°C. The ideal temperature range was 20-30°C, and this is within the normal limits for temperatures in Florida water bodies. Based on climatic suitability mapping and isothermal lines, *C. lebetis* is predicted to establish throughout Florida. Colder temperatures in the northern part of the state may decrease the number of generations per year. The cold tolerance studies show that *C. lebetis* does not tolerate cold temperatures for extended periods of time. Although it is unknown if this insect exhibits diapause, cold tolerance is likely to increase if it does, as has been shown for other insects (Pullin, 1996). Temperatures in vegetation mats during the summer months can be significantly higher than temperatures within the water column (Wheeler and Center, 2001; Mike Netherland, personal communication), and exceed the upper developmental threshold of *C. lebetis*. It is unknown which portion of the water column *C. lebetis* prefers, but if this insect colonizes vegetation below the surface mats, it should be able to escape temperature extremes.

Water quality

Many chironomid species are sensitive to water quality and pesticide contamination (Madden et al., 1992). In the present study, water quality parameters such as pH, conductivity, dissolved oxygen, alkalinity, and hardness did not appear to

influence the distribution of *C. lebetis*, and for hardness and alkalinity, this was supported by the laboratory study; Lake Okeechobee exhibited low species richness, and *C. lebetis* was never recovered at this location. Although *C. lebetis* was never recovered from this lake, this does not mean that is it is not present. Analysis of water samples collected from Lake Okeechobee confirmed the presence of fipronil. The results from the fipronil dose response test indicated that *C. lebetis* was sensitive to this pesticide, even in low concentrations. Fipronil contamination in Florida water bodies could play a role in the distribution and potential establishment of *C. lebetis* and other chironomid species. Other water quality variables that were not assessed in this study could also be influential in shaping chironomid communities. Further studies and sampling are needed to fully understand the distributional patterns of *C. lebetis* throughout Florida.

Potential effects on native flora

A surprising finding of this research was the discovery that *C. lebetis* is polyphagous. The host range study tested a subset of the aquatic plants that are found in Florida water bodies, and *C. lebetis* is probably able to feed and develop on additional plants that were not tested. An ideal biological control agent would attack only the target species, and this is not the case with *C. lebetis.* Monoecious hydrilla and *Elodea canadensis* were shown to be more suitable hosts than dioecious hydrilla. Most of the plants tested are native to Florida, and *C. lebetis* performed well on the native naiad, *Najas guadalupensis.* This naiad, along with other native aquatic vegetation, is commonly displaced by hydrilla (Langeland, 1996), and releasing *C. lebetis* in locations where *N. guadalupensis* is present may cause the plant additional stress. *Cricotopus lebetis* also attacked other important native aquatic flora, and although survival was not

high on these plants, *C. lebetis* could attack those plants in the field. However, the feeding habits of *C. lebetis* show that it does not kill hydrilla, but rather it changes plant architecture (Cuda et al., 2011), and the damage could be similar on other host plants. Thus, even if *C. lebetis* were to attack native plants, the damage inflicted may be insignificant. Therefore, proper risk assessment is necessary before making field releases. Given the results of the laboratory host range testing, field host range studies should be conducted to determine the difference between the ecological host range and the fundamental host range. Studies with *Parapoynx diminutalis* Snellen showed that this insect had a broad host range in the laboratory (Buckingham and Bennett, 1989), but in the field it attacked fewer plants (Buckingham, 1994). If *C. lebetis* exhibits a preference for hydrilla in the field, or has limited dispersal capabilities, it may be possible to limit non-target effects. However, based only on laboratory studies, it appears that the wide host range of *C. lebetis* may limit its value as a biological control agent.

Integrated pest management (IPM)

Cricotopus lebetis may not have significant impact on hydrilla populations when used alone, but if used in combination with the fungus *Mycoleptodiscus terrestris* (Mt) or the herbicide imazamox, there could be synergistic effects on hydrilla management (Cuda and Gillett-Kaufman, 2011). Using all three control tactics could provide a sustainable control method for hydrilla because control does not rely on one management option. Multiple management options can reduce the dependence on herbicides, and reduce the risk of herbicide resistance. When in high concentrations, Mt is lethal to hydrilla (Shearer and Nelson, 2009), but if applied at lower doses, Mt weakens the plant. Currently Mt can only be applied for experimental use, but if proven

to be effective for management of hydrilla, standards could be developed. Low doses of Mt could be used in tandem with *C. lebetis* to induce multiple stresses to hydrilla, and decrease the probability of the development of resistance to Mt. Establishing an IPM program integrating *C. lebetis,* Mt, and imazamox could be beneficial and cost effective for various reasons: 1) mass releasing insects can require costly rearing operations and continual releases, which could be reduced through integration; 2) herbicides are costly and time consuming to apply and there is a risk of hydrilla developing resistance to additional herbicides. Integration with Mt and or *C. lebetis* could lower the amount of herbicides applied, and thereby reduce the costs and the risk of herbicide resistance.

Use of *C. lebetis* Outside of Florida

Introducing an insect into areas where it is not known to occur is inherently more risky, and more complicated from a regulatory point of view, than releasing it in area where it already occurs. The broad host range of *C. lebetis* makes it very unlikely that it would be approved for release in USA states, or other areas of the world, where it is not known to occur. Furthermore, environmental conditions at northern USA locations may be unsuitable for permanent establishment of *C. lebetis*, as predicted by cold tolerance studies and species distribution modeling. If the insect is successful at reducing populations of hydrilla in Florida without evidence of non-target effects, then consideration could be given to releasing it in locations where it has not been found.

Concluding Remarks

This study provides valuable insight on the thermal requirements, host range and distribution of a recently discovered insect herbivore of hydrilla. Based on previous knowledge of the insect, the expectation was that *C. lebetis* would be host specific to hydrilla. However, laboratory-based larval host finding experiments, coupled with

observations of ovipositional behavior, suggest that the midge is an opportunistic forager, and feeds on most aquatic plants that it may encounter. Field studies should be conducted to confirm the broad the host range of *C. lebetis*. Hydrilla continues to be one of the most troublesome weeds plaguing Florida waterways. Therefore, the costs and benefits of releasing *C. lebetis* should be determined in order to assess its use as an augmentative biological control agent. Exploration for new natural enemies of hydrilla in its native range should continue.

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BIOGRAPHICAL SKETCH

Karen Stratman was born and raised in Cincinnati Ohio. Upon high school graduation in 2006, she attended Clemson University in South Carolina for her Bachelor of Science degree in environmental and natural resources. Her first experience working with invasive plants was in the summer of 2009 at Congaree National Park in Hopkins South Carolina. There, she worked to control and manage a variety of invasive plants. Since then, she has been interested in researching ways to effectively manage invasive species. In 2010, she obtained her Bachelor of Science and began a Master of Science of entomology at the University of Florida. There, she studied biological control of invasive weeds, and her research focused on researching *Cricotopus lebetis,* a potential biological control agent of hydrilla. She is a current member of the Entomological Society of America, Florida Exotic Pest Plant Council, and the Florida Aquatic Plant Management Society. Her plans for the future include continued work and research with invasive plants and developing effective management plans.