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Aquatic Plant Control Research Program

Biological and Host Range Studies with Two Species of *Hydrellia* (Diptera: Ephydridae) That Feed on Hydrilla

by *Gary R. Buckingham*
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Prepared for Headquarters, U.S. Army Corps of Engineers

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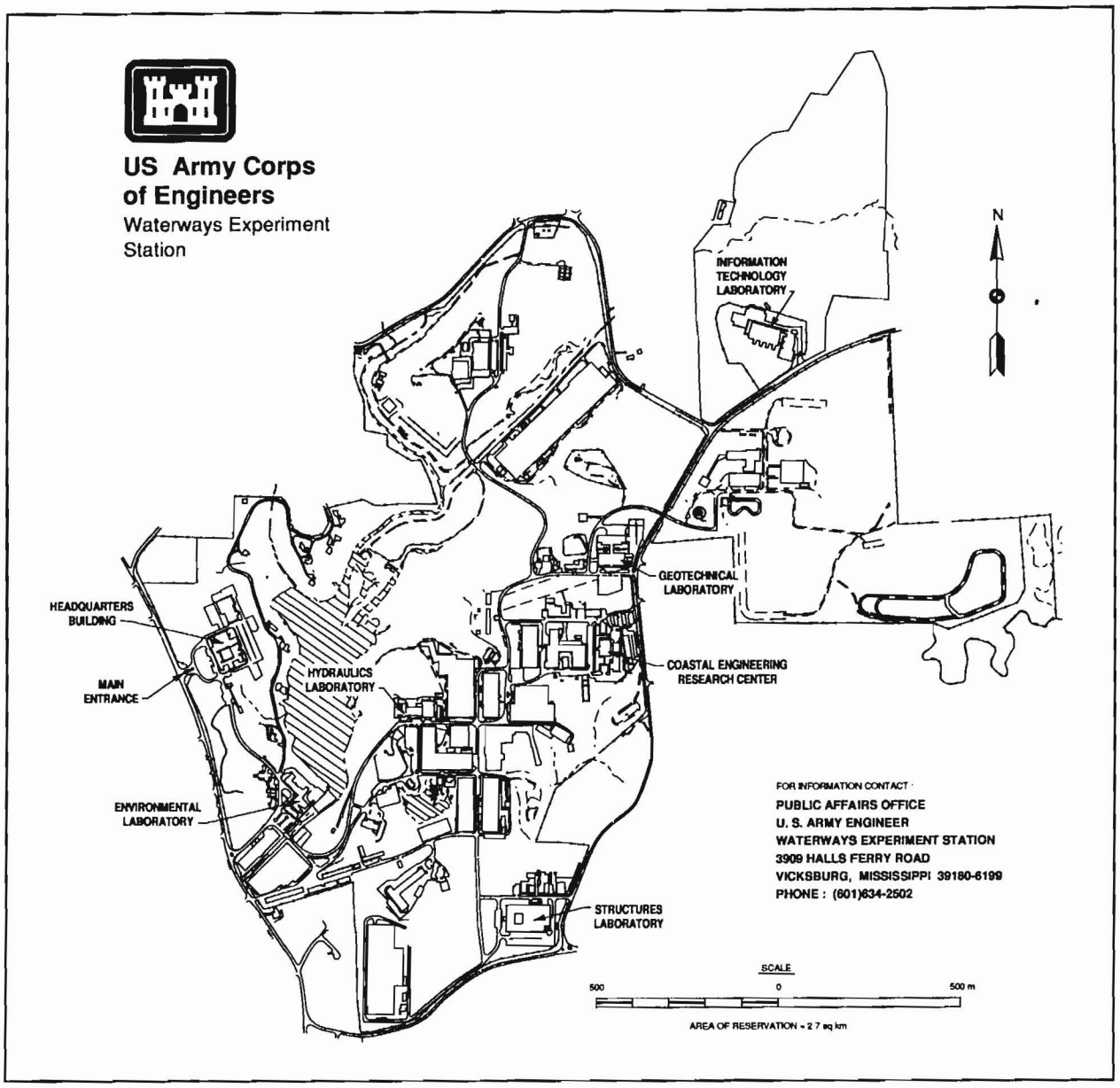

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Preface

The work reported herein was conducted as part of the Aquatic Plant Control Research Program (APCRP), Work Unit 31799. The APCRP is sponsored by the Headquarters, U.S. Army Corps of Engineers (HQUSACE), and is assigned to the U.S. Army Engineer Waterways Experiment Station (WES) under the purview of the Environmental Laboratory (EL). Funding was provided under Department of the Army Appropriation No. 96X3122, Construction General. The APCRP is managed under the Environmental Resources Research and Assistance Programs (ERRAP), Mr. J. L. Decell, Manager. Mr. Robert C. Gunkel was Assistant Manager, ERRAP, for the APCRP. Technical Monitor during this study was Ms. Denise White, HQUSACE.

The Principal Investigator for the work was Dr. Gary R. Buckingham, U.S. Department of Agriculture-Agricultural Research Service (USDA-ARS). Dr. Buckingham and Mr. Emmanuel A. Okrah, University of Florida, Gainsville, prepared this report. Special thanks goes to Mr. Don Bryne, Suwanee Labs, Lake City, FL, Mr. John Lemberger, Wildlife Nurseries, Oshkosh, WI, Dr. Don Riemer, Rutgers University, New Brunswick, NJ, and Dr. J. H. Robinson, USDA-ARS, Crowley, LA. Plant identifications were made by Mr. Kenneth Langdon and Mr. Carlos Artaud, Division of Plant Industry (DPO), Florida Department of Agriculture and Consumer Services (FDACS), and fly identifications were made by Dr. W. N. Mathis, Smithsonian Institution, Washington DC. The authors are also indebted to Ms. Christine Bennett for help with initial field collections of *H. pakistanae* in India and, along with Dr. Mike C. Thomas and Dr. Maude Christian-Meier, for help with the establishment and maintenance of the laboratory colonies; Mr. S. Krishnaswamy and Dr. M. C. Chacko, Commonwealth Institute of Biological Control, Bangalore, India, for shipments of weevils and for their invaluable assistance during our initial collecting trip and for shipment of subsequent shipments of living insects; Dr. J. K. Balciunas, University of Florida (Townesville, Australia), Mr. M. Purcell, C.S.I.R.O., Brisbane, Australia, for supplying *H. balciunasi*, and Dr. S. C. Davis for extensive help extracting and organizing information from our manuscripts and reports; and Dr. Victor Chew, USDA-ARS, Gainesville, FL, for statistical advice.

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of the USDA's Office of International Cooperation and Development. Facilities and support were provided by the DPI, FDACS, Gainesville.

The research was monitored at WES by Dr. Alfred F. Cofrancesco, Jr., Aquatic Ecology Branch (AEB) EL. The study was conducted under the general supervision of Dr. John Harrison, Director, EL; Dr. Conrad J. Kirby, Chief, Environmental Resources Division; and the direct supervision of Dr. Edwin Theriot, Chief, AEB.

At the time of publication of this report, Director of WES was Dr. Robert W. Whalin. Commander was COL Bruce K. Howard, EN.

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1 Introduction

Hydrilla (*Hydrilla verticillata* (L.f.) Royle) is a submersed weed in the southern and eastern United States (Steward and Van 1987). It is also present in California where eradication attempts are being made (Dechoretz 1989). Hydrilla, a native of the Old World, has been introduced to the United States without its natural enemies. Biological control using insects would appear to be a valid alternative or complement to other control methods. Small flies in the family Ephydridae, the shore fly family, were reported to damage hydrilla in Pakistan (Baloch, Sana-Ullah, and Ghani 1980) and in Australia (Balciunas and Center 1988).

The species reported from Pakistan, *Hydrellia pakistanae* Deonier, was collected in India and imported into the Florida Biological Control Laboratory quarantine facility at Gainesville in 1985 (Bennett 1986; Buckingham 1988). The Australian species, *H. balciunasi* Bock, was imported into quarantine in 1988. This document reports the results of studies on the biologies and laboratory host ranges of both species that were conducted to support requests to Federal and State authorities for permission to release these species from quarantine for biological control of hydrilla. *Hydrellia pakistanae* was released from quarantine directly into the field in October 1987 (Buckingham 1988). *Hydrellia balciunasi* was released form quarantine in May 1989, and field releases were made in South Florida in July 1989 (Center and Dray 1990). Much of the information in this report has been extracted from Buckingham, Okrah, and Thomas (1989) and Buckingham, Okrah, and Christian-Meier (1991).

2 Material and Methods

Rearing

The *H. pakistanae* laboratory colony was initiated with adults and immatures collected at Bangalore, India, in May 1985. Studies were conducted at the Florida Biological Control Laboratory quarantine facility, Division of Plant Industry, Florida Department of Agriculture and Consumer Services, Gainesville. Subsequent air freight shipments were received from the Indian Station, Commonwealth Institute of Biological Control (CIBC) (presently the Commonwealth Agricultural Bureau International Institute of Biological Control) at Bangalore from June to August 1985.

The *H. balciunasi* colony was initiated with adults from two airfreight shipments of infested hydrilla collected at North Pine Dam, Petrie, Queensland, Australia, by M. P. Purcell. All *H. pakistanae* were removed from quarantine prior to the arrival of *H. balciunasi* to avoid contamination of the colony.

Hydrilla was collected as needed from waterways near Gainesville and held outdoors in concrete burial vaults or in pools until used. Some hydrilla was grown in the vaults. Infested hydrilla was held in 3.8-ℓ glass jars capped with nylon organdy. Most jars were held in a temperature-controlled greenhouse that fluctuated generally between 24 and 30 °C (minimum 16 °C, maximum 34 °C), but some were held in environmental chambers at 27 ± 1 °C. All chambers had fluorescent and incandescent lighting with 16-hr photophases. The greenhouses had natural lighting with 16-hr supplemental fluorescent lighting diurnally. Average light intensity in the chamber was 125 footcandles (1,345.49 lux).

Adults generally began emerging in the jars after about 18 days and were moved to new jars or to glass-topped wooden cages for oviposition. A yeast hydrolysate-sugar solution (4 g yeast hydrolysate (Enzymatic Autolyzed Brewers Yeast), 7 g sucrose, 10 ml H_2O) was provided for food. Hydrilla in 2 to 5 cm of water was exposed in the jars or in plastic pans inside the cages to about 100 unsexed adults. The low water level ensured a maximum number of exposed leaves available for oviposition sites. Adults were moved from the ovipostion jars to new jars after 1 or 2 days but were left in the cages until they died. Three days after the adults were removed, the jars were filled

one-half to three-fourths full with water; additional hydrilla was placed in the jar below the infested hydrilla. As new adults became available, they were added to the wooden cages to replace those that died.

Because of an overabundance of eggs, removing and replacing the hydrilla in the cages after several days was necessary. The infested hydrilla was separated into several 3.8-ℓ jars with additional hydrilla below the infested hydrilla. Infested jars were closely monitored for plant damage, and plants that were heavily attacked were divided into several jars with new plants. Apical sections of stems were used most often.

The adults were removed from the jars inside a "light box." This wooden boxlike structure had translucent plastic in front of a fluorescent light for the front inside wall. The back wall was a black cloth that covered the back of the researcher reaching inside the box. If flies escaped, they could be aspirated from the lighted front wall to which they were attracted. Aspirators made from a straight glass tube capped with mesh at the back end were used. The narrow tube confined the flies in a small space and allowed them to be easily sexed and identified under a microscope.

Hydrilla infested with larvae and held in a greenhouse was air dried on nylon mesh covering a water-filled dishpan to recover larvae for experiments. Larvae dropped into the water as the plants dried and were collected with a fine paintbrush or eyedropper.

Specimens of immatures and adults of both species have been deposited in the Florida State Collection of Arthropods, Gainesville; the National Museum of Natural History, Washington, DC; and the Canadian National Collection, Ottowa, Ontario.

Biologies

All studies, including host range tests, were conducted in environmental chambers at 27 ± 1 °C and 16-hr photophase unless otherwise indicated. The studies with *H. pakistanae* were conducted from 1985 to 1987 and those with *H. balciunasi* during August and September 1988.

Measurements of immatures preserved in 75-percent isopropyl alcohol were made at 25X or 50X with an ocular micrometer in a stereomicroscope. Means are reported throughout this report with standard deviation (number, range). Larvae were killed by pouring boiling water over them.

To determine life span and fecundity, 10 pairs of newly emerged flies were held in 0.95-ℓ glass jars, 1 pair per jar, with several centimeters of water and two sprigs of hydrilla, each with five whorls of leaves. Yeast hydrolysate-sugar solution on a plastic float was provided for food. The jars were capped with nylon organdy. All eggs were counted and held for hatching; fly deaths were recorded.

Sex ratio of *H. balciunasi* was determined by sexing 663 flies that emerged from hydrilla in the larval no-choice development tests. Sex ratio of *H. pakistanae* was determined by sexing 1,030 flies that emerged throughout the study.

Hydrellia balciunasi eggs laid during 3-hr periods were used to determine immature development times. Sixteen eggs were observed every 12 hr until one or more hatched eggs were found. They were then observed three times during the following 12-hr period, and numbers of hatched eggs were recorded each time. Larval and pupal development times were determined by placing 80 eggs singly into 35-ml culture tubes containing deionized water and a sprig of hydrilla with about 15 whorls of leaves. These 35-ml culture tubes were used in all experiments unless otherwise indicated. Two groups of tubes were set up 12 days apart. The dates of pupariation and adult emergence were recorded along with sex and number of mined leaves.

Developmental threshold and degree-days for *H. pakistanae*

Developmental times were determined at 21 ± 1, 27 ± 1, 32 ± 1, and 36 ± 1 °C in environmental chambers with 16-hr photophases. Flies were exposed to hydrilla in the above chambers for 24 hr to obtain eggs. Fifty eggs from each chamber were put singly on hydrilla sprigs in culture tubes and held in their respective chambers until all adults emerged. Emergence date and sex were recorded daily. The mean rates of development were plotted against temperatures. The graph was extrapolated to obtain the fly's lower critical developmental threshold temperature. Mean degree-days was then calculated.

H. pakistanae larval development at cold temperatures

Groups of 10 larvae were placed into each of nine culture tubes containing a hydrilla sprig with about 30 whorls of leaves. The tubes were held for 1 day at 27 ± 1 °C to allow larvae to enter the leaves. Three tubes were placed randomly into each of three environmental chambers: 8 ± 1, 16 ± 1, 27 ± 1 °C. The tubes at 8 °C were transferred to 16 °C after 60 days and held there for 45 more days. Leaves were examined periodically with a stereomicroscope to follow larval development until adults emerged or larvae died.

Effect of dryness on *H. pakistanae* larval development

An experiment was conducted to test if 7- to 12-day-old larvae could complete development in plant material held under different conditions of dryness as might occur during initial stages of a drought or drawdown or during transfer on boat trailers between water bodies. Larvae were obtained, separated into culture tubes, and held for 1 day, as were those for the preceding experiment. Each infested sprig was removed from the culture tube and randomly assigned to one of nine 0.95-ℓ jars. Each of six jars was

partially filled with 350 ml of autoclaved lake sediment collected at Rodman Reservoir, Putnam County, FL. Three of these were moistened with 150 ml of deionized water and three were kept dry. The other three jars contained 350 ml of deionized water. The jars were capped with nylon organdy and held at 27 ± 1 °C. Numbers of adults, emergence dates, and sexes were recorded. The experiment was terminated 7 days after the last emergence. The general linear model (GLM) procedure of SAS Institute, Inc. (SAS) was used to compare the means.

Horizontal and vertical movements of *H. pakistanae* larvae

Hydrilla leaves containing 100 5-day-old larvae were put at one end of a 1-m glass tubing, 1 cm in diameter, and a hydrilla sprig was put at the other. The leaves were confined to the end of the tube by a small piece of 1-mm mesh nylon net inserted in front of them. Both ends of the tube were capped with nylon organdy. A similar experiment was set up with three tubes each containing leaves with 90 eggs instead of larvae. The tubes were immersed horizontally for 10 days in a tank containing water at 21 ± 1 °C. In a third experiment, pieces of *Najas guadalupensis* (Spreng) Magnus with *H. pakistanae* eggs on them were floated in mesh-bottomed styrofoam cups 1 m above hydrilla sprigs in Plexiglas tubes, 5 cm in diameter. Larvae could pass through the mesh. There were three replicates each with 75 eggs. The tubes were placed vertically in the tank. The number of larvae that moved to attack the target hydrilla was calculated as a percentage of the total number of larvae or eggs.

Mating and preoviposition of *H. pakistanae*

Female flies contained fully formed eggs at emergence. To determine how quickly they laid viable eggs after emergence and mating, adult emergence was synchronized within 30 min by immersing mature pupae (7 days old) in a high temperature, 30 to 35 °C, waterbath. All flies were confined together for 1 hr to increase the probability of mating. Flies were then separated into pairs and exposed for different durations to hydrilla sprigs with 25 whorls of leaves in 0.95-ℓ jars. Four pairs each were exposed for 3 and 6 hr and two pairs were exposed for 4.5 hr. Five of these females were also exposed singly without males to hydrilla sprigs for 3 hr. Eggs were checked after 7 days for hatching.

Comparison of the effect of two different diets on longevity, fecundity, and egg viability of *H. pakistanae*

Pupae in hydrilla sprigs from colony jars were placed singly into culture tubes. Adults were sexed as they emerged. One pair was put into each of 18 jars, 0.95-ℓ, containing a hydrilla sprig with about 20 leaf whorls. Flies in nine jars were fed the sugar-yeast hydrolysate solution, and flies in nine jars were fed a 50-percent honey solution. Hydrilla was replaced daily, and eggs

were counted and held for 7 days to determine viability. Plastic vial caps that served as floating food platforms were replaced when the food became moldy or dried. The experiment was terminated when all adults had died. The GLM procedure of SAS was used to compare means of male and female longevities, fecundity, and egg viability on the two diets.

Host Range Studies

Hydrellia pakistanae

Test plants. Test plants collected from waterways in north-central Florida were tested within a few days or were held for varying periods outdoors or indoors before testing. The following species were purchased from an aquatic plant dealer in Wisconsin: *Elodea canadensis* Michx., *Nymphaea tuberosa* Paine, *Potamogeton nodosus* C. and S., *P. pectinatus* L., and *P. richardsonii* (A. Bann.) Rydb. Additional *E. canadensis* and *Alisma subcordatum* Raf. were collected in Indiana. *Elodea nuttalli* (Planch.) St. John and *Potamogeton pulcher* Tuckerm. were from New Jersey. Test plants are listed in Tables 1 and 2.

Multichoice-with-host oviposition tests—jars. Oviposition data were obtained from the 3.8-ℓ jars in which adults were held to provide eggs for the larval tests. Approximately equal biomass of hydrilla and each of generally three test plant species were exposed to various numbers of flies for 24 hr at 27 ± 1 °C. These tests were conducted from May to December 1986 (Buckingham, Okrah, and Thomas 1989).

Multichoice-with-host oviposition tests—wooden cages. Additional tests were conducted in the greenhouse in the wooden cages described earlier with those plant species that had elicited heavy oviposition in the jars or had supported some larval development. Hydrilla was exposed along with two test plant species in each of three cages. Approximately equal amounts of each plant were exposed singly in petri dishes (14-cm-diam). Dishes were randomized to positions in a triangular pattern and then exposed to 10 pairs of adults for 5 days after which both hatched and unhatched eggs were counted. One test compared three test plant species without hydrilla. Means were calculated and analyzed after $\sqrt{x+0.5}$ transformation using 1-way analysis of variance (ANOVA) and were separated with Waller-Duncan Bayesian K-ratio t-test. Tests were conducted February to May and during August 1987 (Buckingham, Okrah, and Thomas 1989).

No-choice larval development tests. After a variety of preliminary tests were conducted using various techniques, tests were initiated with test plant sprigs, individual leaves, or portions of leaves, depending upon the morphology of the plant, in 35-ml culture tubes with deionized water. Plant material was limited by the tube length (12.5 cm) to about 10 cm. Hydrilla sprigs had about 10 to 15 whorls of leaves. Unless otherwise indicated, tests

were conducted in environmental chambers at 27 ± 1 °C with 16-hr photophase. Tubes were capped, usually at initiation, with nylon organdy held on by a plastic cap. All tests were initiated with eggs oviposited upon the respective test plant species. Five eggs on small pieces of leaves were placed on test plant sprigs in tubes or in petri dishes if the plant material was too large or if the sprigs were small floating plants. Ten tubes of each species were initiated at the same time. Generally, three plant species were compared along with the hydrilla control. Some plant species were tested several times, especially those in which partial or complete larval development occurred. The test tubes were checked after 7 days for the presence or absence of larval mining. An arbitrary scale of 0 to 5 was used to assess total damage at the time the first adults emerged. Zero indicated no damage, 1 indicated 25 percent or less of the leaf material damaged, 2—more than 25 to 50 percent, 3—more than 50 to 75 percent, 4—more than 75 percent, and 5—all the leaf material damaged. The estimates were highly subjective for comparison of different leaf types, but they did provide an additional measure of specificity. The duration of each test was 30 days, and they were conducted from May 1986 to August 1987 (Buckingham, Okrah, and Thomas 1989).

Paired-choice larval development tests. Few choice tests were conducted because the larvae were highly specific in the no-choice tests. These employed the same methods as in the no-choice tests except that two sprigs, one of hydrilla and one of a test species, were in each tube with either 5 or 15 eggs. Control treatments had two sprigs of one species, either hydrilla or the test plant. These were conducted during November and December 1986 (Buckingham, Okrah, and Thomas 1989).

Host suitability of potamogeton crispus. Fifty eggs on pieces of hydrilla or 200 eggs on pieces of *P. crispus* were placed into each of three 3.8-ℓ jars filled with the respective plant material. Adults emerging from each jar were recorded, and five males and five females from each were placed with hydrilla in 0.9-ℓ jars to observe longevity and fecundity. Hydrilla was provided to both groups to maximize oviposition. Yeast hydrolysate-sugar solution was provided for food. Deaths and egg numbers were recorded daily until all adults had died. Three samples of five eggs each were selected from each jar daily if there were sufficient eggs. These eggs were held for hatching in 29.6-ml plastic cups with moist cotton. All cages were held in environmental chambers at 27 ± 1 °C (Buckingham, Okrah, and Thomas 1989).

A second test was conducted with 60 eggs on hydrilla and 300 eggs on *P. crispus* per each of three replicates. They were placed on the respective plant material as in the preceding test. All adults produced in a *P. crispus* jar were placed each generation into a new jar with plant material and with yeast hydrolysate-sugar solution for food. Adults of the fifth generation were combined in one jar because of low numbers. The adults produced on hydrilla were treated the same as the *P. crispus* adults during the first generation, but the jars were discontinued during the second generation because the larvae destroyed the hydrilla. The jars were held in an environmental chamber at 27 ± 1 °C.

These tests were conducted from January to May 1987 (Buckingham, Okrah, and Thomas 1989).

Hydrellia balciunasi

Test plants. Plants were obtained from the same sources as those for *H. pakistanae* except that additional plants of *E. canadensis* were purchased from a biological supply company in South Carolina. Tests were conducted from April to November 1988.

No-choice larval development tests. Tests were conducted both with neonates (newly hatched larvae) and with older larvae of various ages that fed upon hydrilla. Test plants are listed in Tables 1 and 3.

Tests with neonates were conducted with the same techniques described for *H. pakistanae* except that occasionally eggs on small slivers of hydrilla were used instead of eggs on the test plant. This was necessary because females deposited insufficient numbers of eggs for testing on a few plant species. Preliminary tests demonstrated that the neonates crawled away without feeding on the slivers. Generally, two plant species were compared along with hydrilla instead of three species as with *H. pakistanae*.

Three additional tests were conducted with neonates under greenhouse conditions. In Test A, hydrilla was compared with *Potamogeton crispus* L. and *E. canadensis* in 0.95-ℓ glass jars. There were three replicates of each species, each with 10 eggs. The third replicate was set up 1 day after the others. The test lasted 33 days. In Test B, hydrilla was compared with *Egeria densa* Planch. and *N. guadalupensis* in 3.8-ℓ glass jars. There were three replicates of each species, each with 100 eggs. The first replicate was set up 2 days before the others. Test C was conducted to determine if the fly would damage rice. Forty rice seedlings grown in flooded soil in a 6.6-ℓ glass jar were exposed to 150 mixed sex flies in a wooden cage described earlier. Oviposition was not confirmed by searching for eggs, but earlier observations had indicated that females would readily oviposit on rice in the absence of hydrilla. A companion jar of rice was held outside the cage as a control. Yeast hydrolysate-sugar solution was painted on the glass every 3 days while living flies were present. After 30 days, the rice was examined for larvae and damage (Buckingham, Okrah, and Christian-Meier 1991).

Larvae of different ages that had fed on hydrilla were obtained by placing infested hydrilla over a plastic dishpan as described earlier. Small (1 to 4 days old), medium (5 to 8 days old), and large (10 to 13 days old) larvae were tested using the same technique used with neonates. The small larvae were probably 3 or 4 days old because 1- and 2-day-old larvae failed to exit from the drying leaves in a subsequent attempt to obtain small larvae of known age. Three-day-old larvae were obtained with this technique and were tested with the same methods (Buckingham, Okrah, and Christian-Meier 1991).

Multichoice larval development tests. Sprigs of three test plant species were placed together in 0.95-ℓ or 3.8-ℓ glass jars, depending upon the sizes of the plants, to which were added 30 eggs. Eggs were added as in the no-choice tests. The 27 plant species in 23 genera and 17 families that were tested are listed in Table 4. A control jar with hydrilla was set up each time test jars were set up. The plant species were not closely related to hydrilla and were considered to be at lower risk for attack than those in the no-choice tests. By testing three species together, testing a greater range of species with a larger number of larvae was more possible than in our no-choice tests. Three jars of each plant trio were tested (90 larvae) except only two jars were tested with *Myriophyllum spicatum* L., *Ruppia maritima* L., and *Sagittaria graminea* Michx. *Nuphar luteum* (L.) Sibth. & Small (submersed leaves) was a member of two trios (six jars). A companion jar of hydrilla with 30 eggs was set up as a control with each trio. Jars were capped with nylon organdy and held in the greenhouse with the rearing jars. Plants were inspected for damage after 10 to 12 days. Adult emergence was recorded. Each test was terminated about 7 days after the final emergence on the hydrilla control (Buckingham, Okrah, and Christian-Meier 1991).

Multichoice oviposition tests. Oviposition tests were conducted in small plastic cages (29.8 by 14 by 12 cm), in the medium-sized wooden cage described earlier, and in a similar but larger wooden cage (89 by 44.5 by ca. 44.5 cm). Hydrilla, six test plant species (Table 5), and small wooden sticks were exposed together in the small and medium-sized cages. Hydrilla and the two most accepted test plant species in the medium-sized cages were also tested in large cages. In the small and medium-sized cages, 10 pairs of flies were tested in each of three replicates. Eggs and empty chorions were counted on each plant after 5 days. Thirty pairs of flies in each of three replicates were tested for 3 days in the large cages. The small cage held 1 to 2 cm of water in the bottom so that a portion of each plant was exposed. Each plant species in the wooden cages was exposed in a petri dish, 14-cm-diam, with 1 to 2 cm of water, except in the second large-cage experiment in which small petri dishes, 8.5-cm-diam, were used (Buckingham, Okrah, and Christian-Meier 1991).

Petri dishes were randomized to positions on the floors of the wooden cages. In the first large-cage experiment, each species occupied one of three positions. In the second large-cage experiment, each position was subdivided to include three small petri dishes each with a test plant. Both large-cage experiments had the same total amount of plant material of each species (stem sections totaling 72 cm), but the distance between each species differed. In the first experiment, the species were 20 cm apart; but in the second with the small petri dishes, they were 4 cm apart. The yeast hydrolysate-sugar solution was provided in all cages for food. Comparisons of eggs per plant species were made with PROC GLM, and separation of means was made with the Waller-Duncan k-ratio t-test option ($P = 0.05$; $k = 100$) (SAS Institute, Inc. 1985). Data were transformed before analysis with $\sqrt{x+0.5}$ because of unequal variances (Buckingham, Okrah, and Christian-Meier 1991).

3 Results and Discussion

Biologies

Females oviposited on hydrilla leaves and stems at or mostly above the water's surface. Eggs were deposited singly or in loose bunches depending upon the amount of plant material available (Figure 1). Eggs were elongate with distinctive longitudinal ridges characteristic of *Hydrellia* eggs (Deonier 1971) (Figure 2). A drawing of the egg of *H. pakistanae* was included in Krishnaswamy and Chacko (1990). The moving larva was visible shortly before emerging. Mean egg measurements and egg developmental times for both species are presented in Table 6.

The neonate (newly hatched larva) usually wandered before entering a leaf, usually on the upper surface. Larval mines extended in any direction including across the midvein, but usually they were parallel to the edge of the leaf.

Figure 1. *Hydrellia balciunasi* eggs on a hydrilla leaf

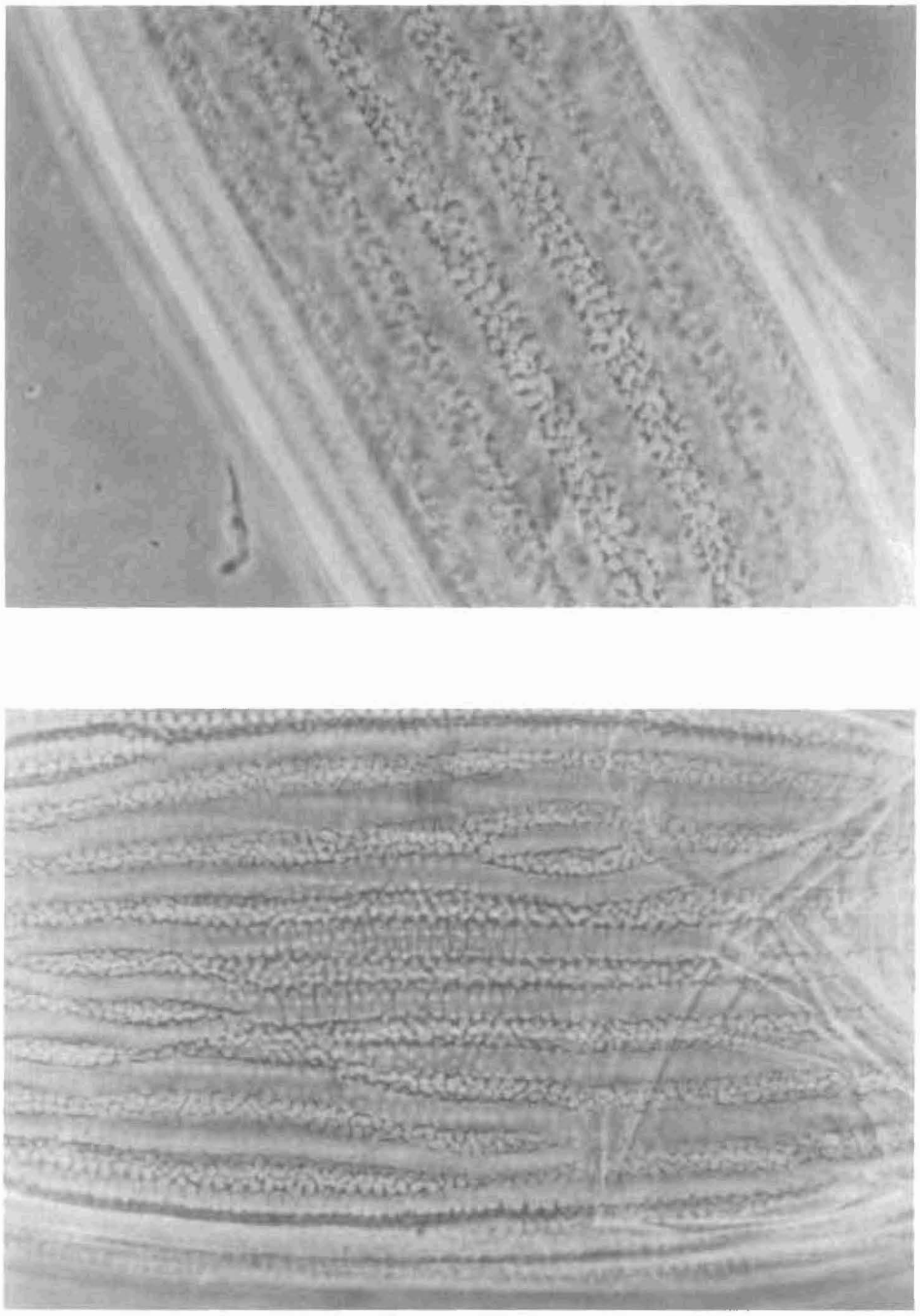


Figure 2. Closeup of longitudinal ridges on the egg chorion (eggshell), *Hydrellia pakistanae* (top) *H. balciunasi* (bottom)

Larvae mined all or almost all of the leaf before moving to an adjacent or neighboring leaf (Figures 3 and 4). They usually exited and entered a new leaf basally. They did not mine the stem. Based upon the presence of the shed-feeding apparatus in the leaves, it appeared that the first and second

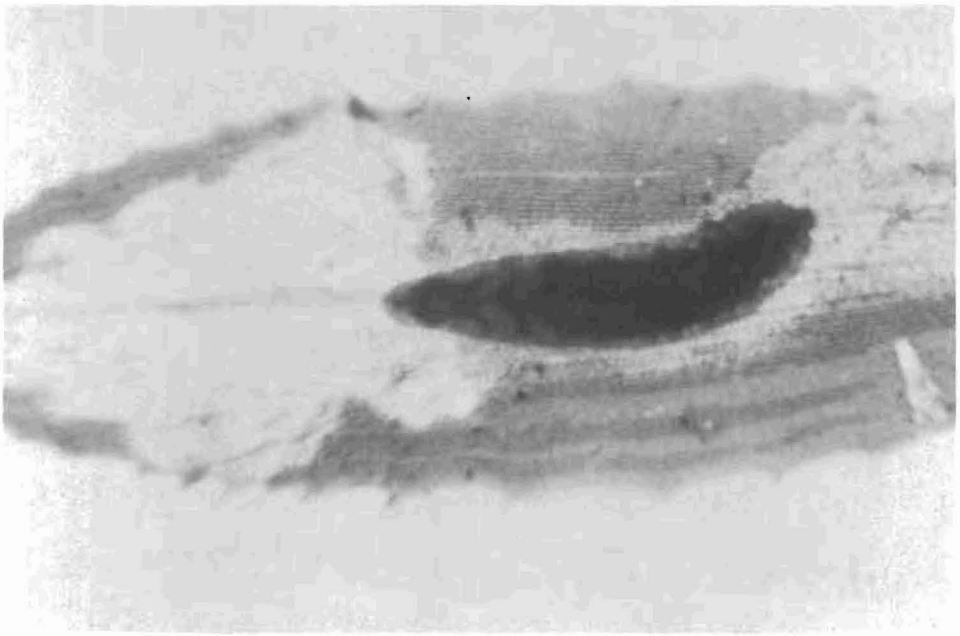


Figure 3. *Hydrellia pakistanae* larva in hydrilla leaf (Photo by M. C. Thomas)

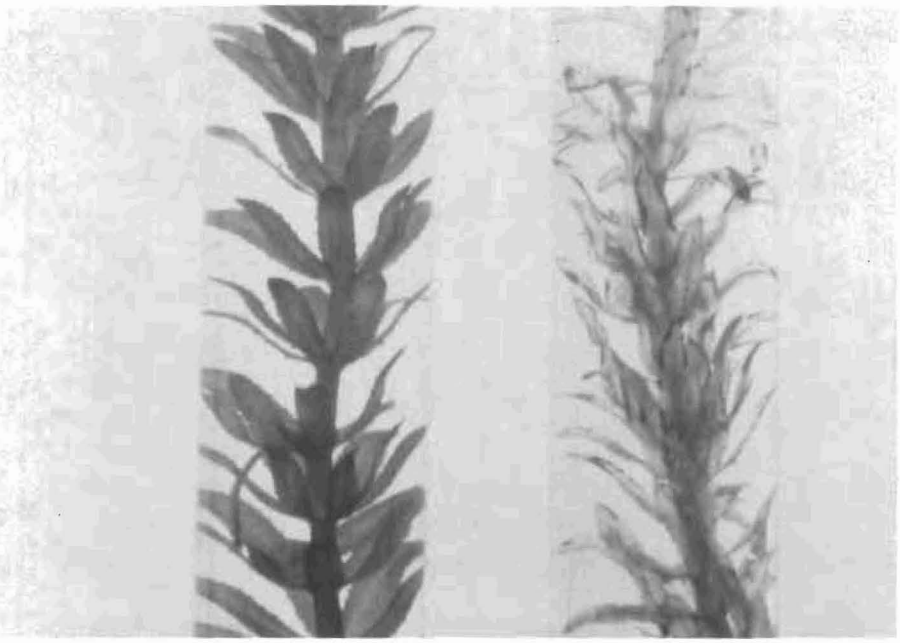


Figure 4. Hydrilla mined by *Hydrellia pakistanae* larvae (right) compared with undamaged (left)

instars were generally completed in the same leaf. Larvae continually probed the plant tissue inside the mines with their anal spines (spiracular peritremes) (Buckingham, Okrah, and Christian-Meier 1991).

Larvae have ventral transverse clusters of small spinules called creeping welts on each segment with which they cling to objects (Deonier 1971) (Buckingham, Okrah, and Christian-Meier 1991) (Figure 5).

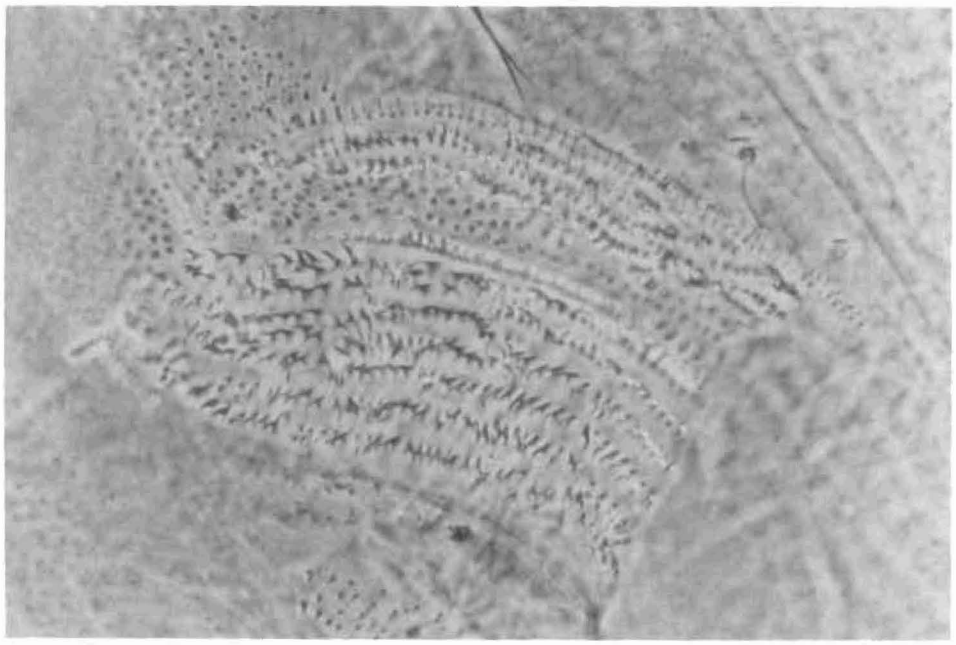
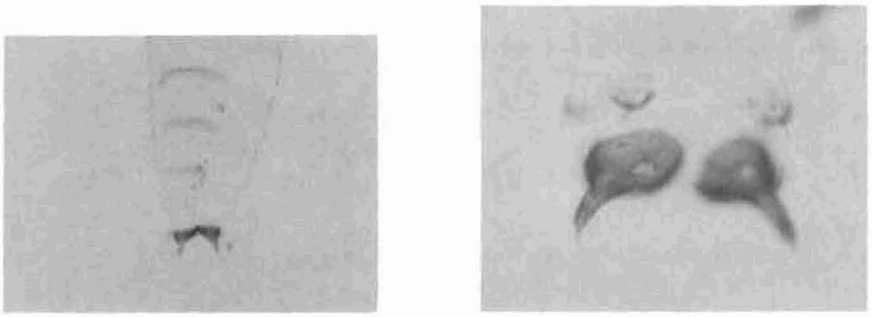
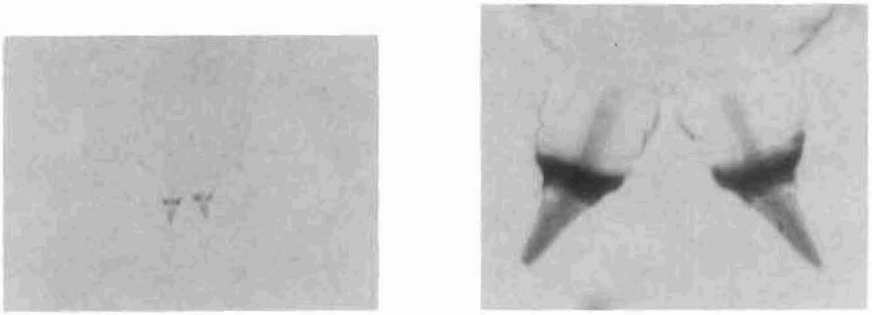


Figure 5. Creeping welts on the larva of *Hydrellia pakistanae* (3rd instar) used for locomotion

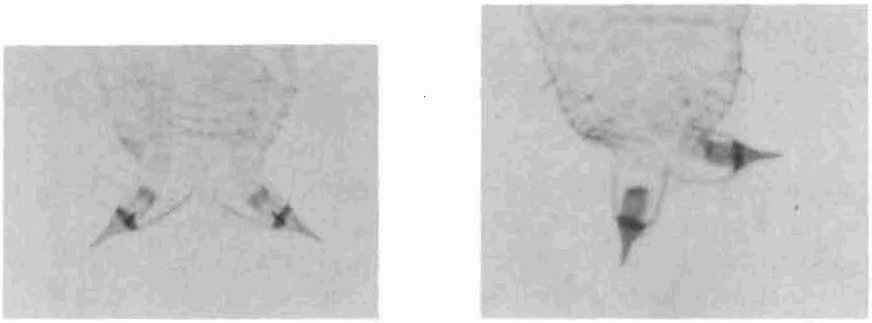
Mean larval developmental times for both species are reported in Table 6. There were three active instars. Ranges of days for each instar for both species are listed in Table 6. Instars were easily separated by the size and shape of the anal spines as illustrated for similar species by Deonier (1971) (Figure 6). These anal spines into which the spiracles open are paired spines on the tip of the posterior abdominal segment. Those of the first instar were completely heavily sclerotized (hard and dark), whereas only the tips were sclerotized in the second and third instars (Buckingham, Okrah, and Christian-Meier 1991). The tip on the third instar was more needlelike than in the second, and there was an obvious brown cylindrical extension internally. Deonier (1971) has discussed the terminology of these and other structures. Larvae appeared green when the hemolymph was green; they appeared yellow when the hemolymph was clear, but the fat bodies were yellow, probably with lipid droplets; and they appeared white when the other colors were lacking. The feeding apparatus of the living first instar was dark brown to black. Those of the other two instars were very light brown posteriorly and dark brown anteriorly. The cheliform spot to which muscles are attached was also dark brown. The lengths and shapes of the feeding apparatus also separated



a. First instar



b. Second instar



c. Third instar

Figure 6. Anal spines of *Hydrellia pakistanae* (left) and *Hydrellia balciunasi* (right) larvae (photographed at 32X except first and second instar of *H. balciunasi*, photographed at 128X)

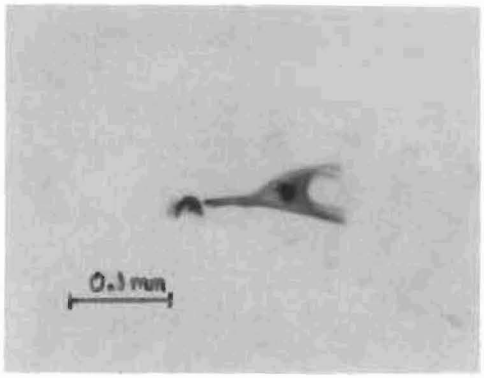
the instars easily (Figure 7). The lengths of the feeding apparatus without the mouthhooks, or mandibles, in *H. balciunasi* were as follows: first instar, 0.14 mm; second instar, 0.26 mm; and third instar, 0.35 mm (Buckingham, Okrah, and Christian-Meier 1991). They were similar in *H. pakistanae*. The mouthhook has a broad base with a narrow sickle-shaped beak. The third or last instar larvae of the two species were distinguished by the width of the mandible beak at the juncture with the enlarged base. The width measured at 400X was 0.0175 mm (10, 0.0175) in *H. pakistanae* and 0.0135 mm (8,

0.0125-0.0150) in *H. balciunasi* (Figure 8). Variability did not exist in the width for *H. pakistanae* at that magnification.

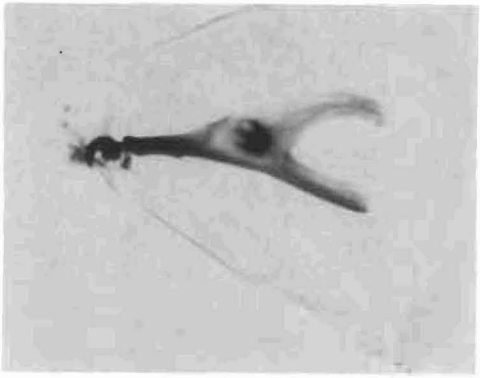
The newly formed puparium, which is the hardened larval skin containing the pupa, appeared green; but as it aged, it turned light brown. Air inside the puparium signaled that flies would soon emerge. Mean measurements and puparial developmental times are listed in Table 6. Puparia with the anal spines inserted into the stems were formed inside leaves at the axils (Figure 9). Damaged leaves often disintegrated, but the puparia remained attached to the stem. Most flies emerged around 2:00 to 4:00 p.m. in the greenhouse. When puparia scheduled for fly emergence in the afternoon were placed in warm water (32 to 35 °C) in the morning, a few flies emerged within a few minutes, indicating that they were fully formed and waiting to exit. This was a useful technique to obtain small numbers of flies of the same age (Buckingham, Okrah, and Christian-Meier 1991).

Adults are small, dark gray, and have shiny gold faces (Figures 10 and 11). A shiny face is one of the characteristics of the genus *Hydrellia*, not just of these two species. Some native species also have gold faces, although others have silver or bronze faces. For example, the native *H. bilobifera* that attacks hydrilla has a silver face. The sexes are best separated from the underside. Females have a flat, uniform stomach or abdomen (Figure 12A). Males have a distinct concavity in the middle of the abdomen in which can be seen dark brown bristles and other structures of the external genitalia (Figure 12B). *Hydrellia balciunasi* adults were generally smaller than *H. pakistanae* but were otherwise very similar. Fortunately, the external genitalia of both sexes readily distinguish the two species.

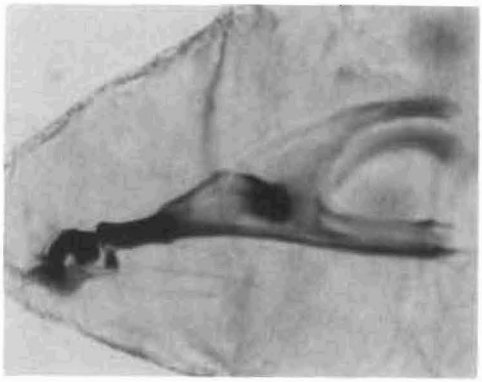
The female cerci (the small paired hardened structures at the tip of the abdomen) of *H. pakistanae* viewed from the side are light brown and L-shaped (Figure 13A) compared with those of *H. balciunasi*, which are dark brown and triangular (Figure 13B). The female cerci of the native *H. bilobifera* are dark brown and elongate (Figure 13C). The male *H. pakistanae* has a pair of large needlelike bristles on the genitalia (Figure 14A), while *H. balciunasi* has a pair of large spatulate bristles (Figure 14B). At high magnifications (>100X), the



a. First instar



b. Second instar



c. Third instar

Figure 7. Mouthhooks or feeding apparatus of *Hydrellia pakistanae* larvae (photographed at 32X)

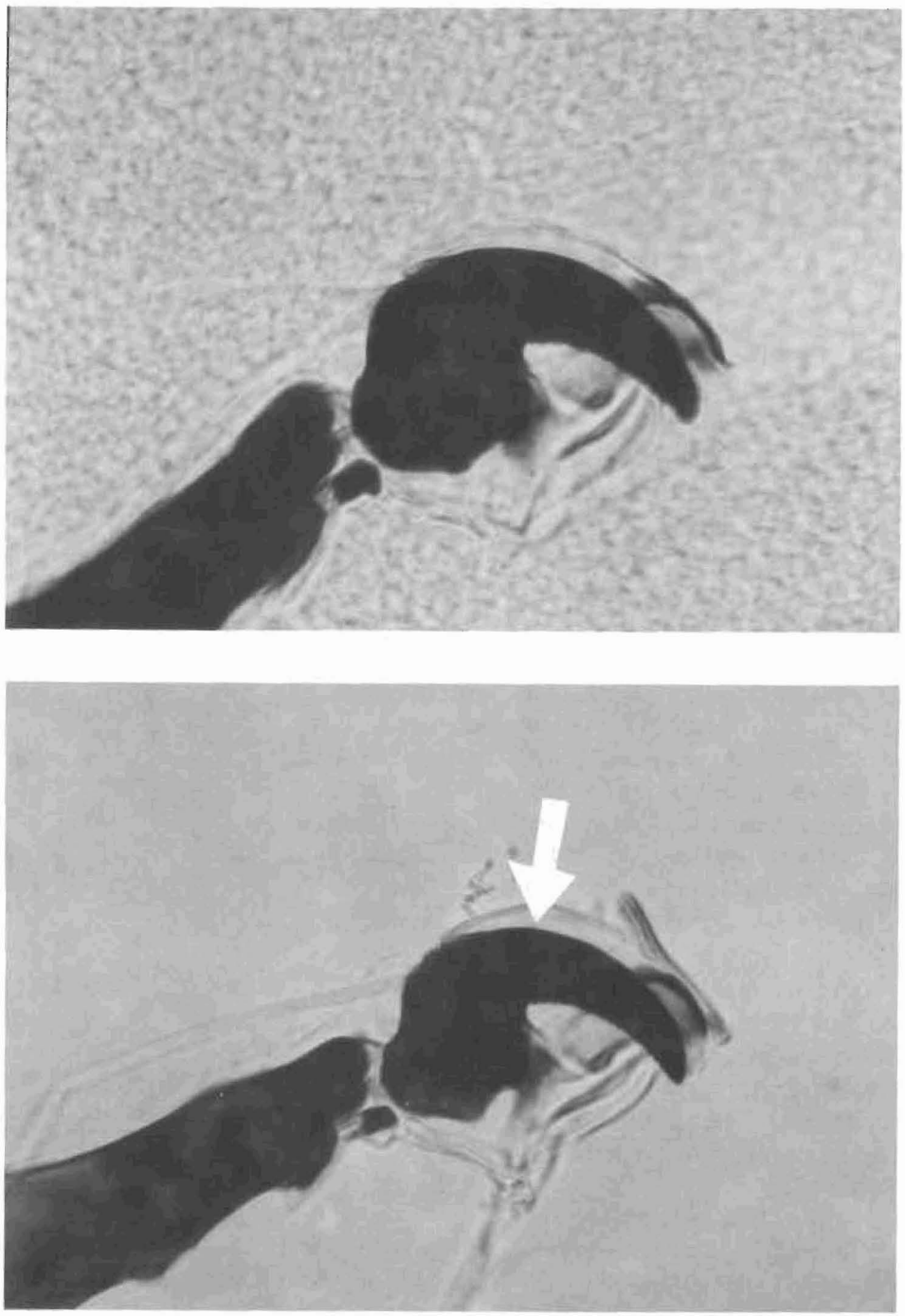


Figure 8. Larval mandibles: Third instar (photographed at 128X). *Hydrellia pakistanae* (top) *H. balciunasi* (bottom). Measurements to separate the species should be made where arrow indicates

Figure 9. *Hydrellia pakistanae* puparium in hydrilla leaf (photo by Narayana Rao, CIBC Bangalor, India)

Figure 10. *Hydrellia balciunasi* adult on hydrilla leaf

Figure 11. *Hydrellia pakistanae* adult showing golden face characteristic of many species of *Hydrellia*

bristle of *H. pakistanae* has a slightly spatulate tip, but it appears pointed at low magnification (<50X). There are many other differences, but the large bristles are the most obvious (Buckingham, Okrah, and Christian-Meier 1991). The genitalia of *H. pakistanae* have been illustrated by Deonier (1978) and that of male *Hydrellia balciunasi* by Bock (1990). Deonier (1971) also illustrated the genitalia of *H. biloifera* Cresson, which occasionally attacks hydrilla (Balciunas and Minno 1985). The bristles of this species appear large and pointed, but the tips split during slide preparation revealing at least two fused bristles on each side instead of one large bristle (Figure 14C). Deonier (1971) reported five to seven bristles in a compact bundle for this species.

Males were more abundant than females in both species. The sex ratio of *H. pakistanae* was 1.5:1.0 (625 males:405 females), and that of *H. balciunasi* was 1.1:1.0 (349 males:314 females).

Mating and preoviposition of *H. pakistanae*

The four females held with males for 3 hr after the initial 1-hr mating period did not lay eggs (4 to 4.5 hr after emergence), whereas three of five females held without males for the same period did. Eggs were obtained from the two females held with males for 4.5 hr after the initial mating (5.5 to 6.0 hr after emergence) and from two of the four females held with males for 6 hr after the initial mating (7.0 to 7.5 hr after emergence). All eggs laid during this experiment hatched. This experiment demonstrated that flies could mate successfully within 1 to 1.5 hr after emergence and that the minimum

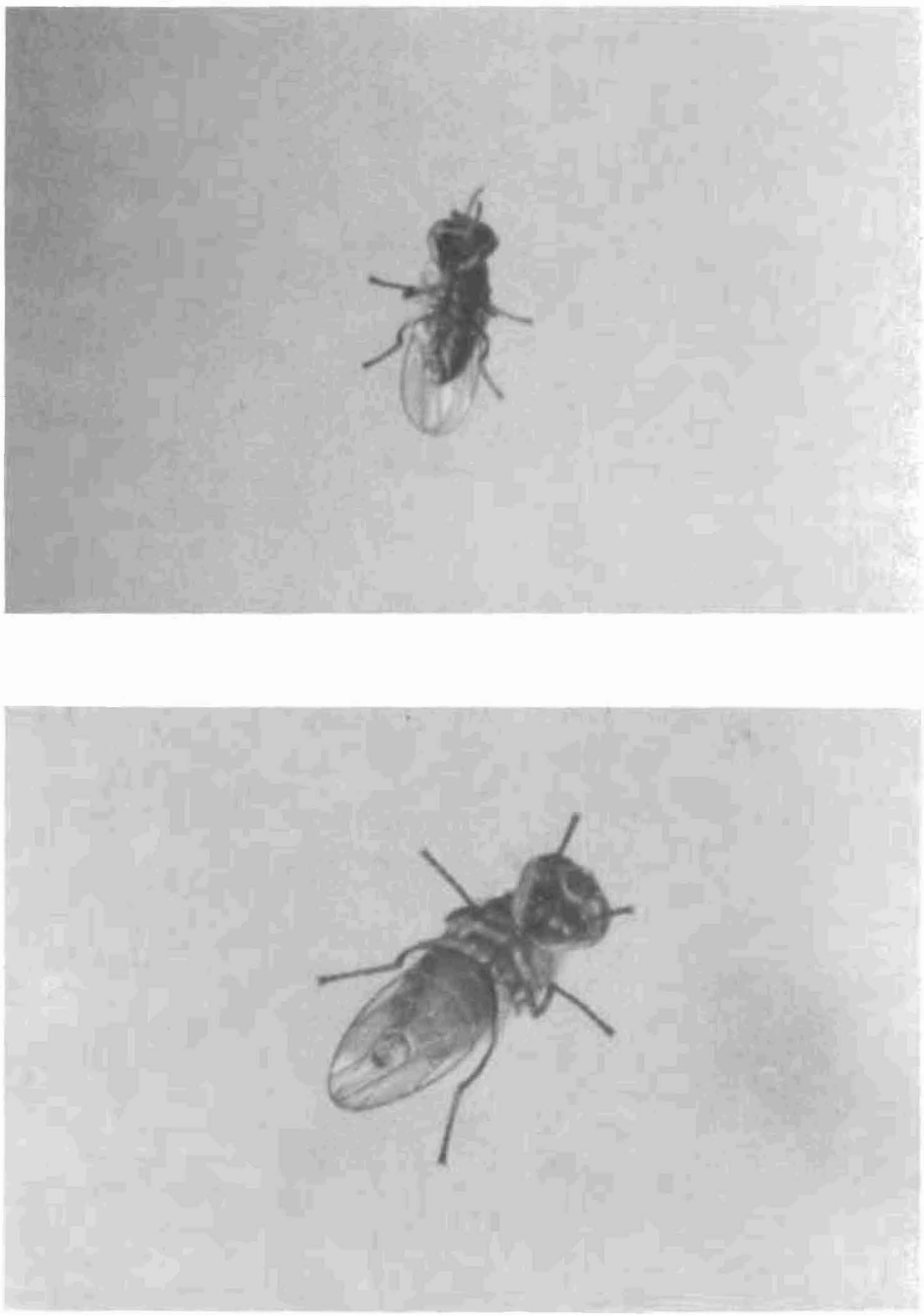


Figure 12. *Hydrellia balciunasi* adults: Ventral view (Male, top, Female, bottom)

preoviposition period was no more than 4.5 hr and possibly less. Baloch, Sana-Ullah, and Ghani (1980) reported a 1-day preoviposition period for *H. pakistanae*. *Hydrellia balciunasi* mated as quickly as 13 min after emergence, and the minimum preoviposition period was 4 hr (Buckingham, Okrah, and Christian-Meier 1991).

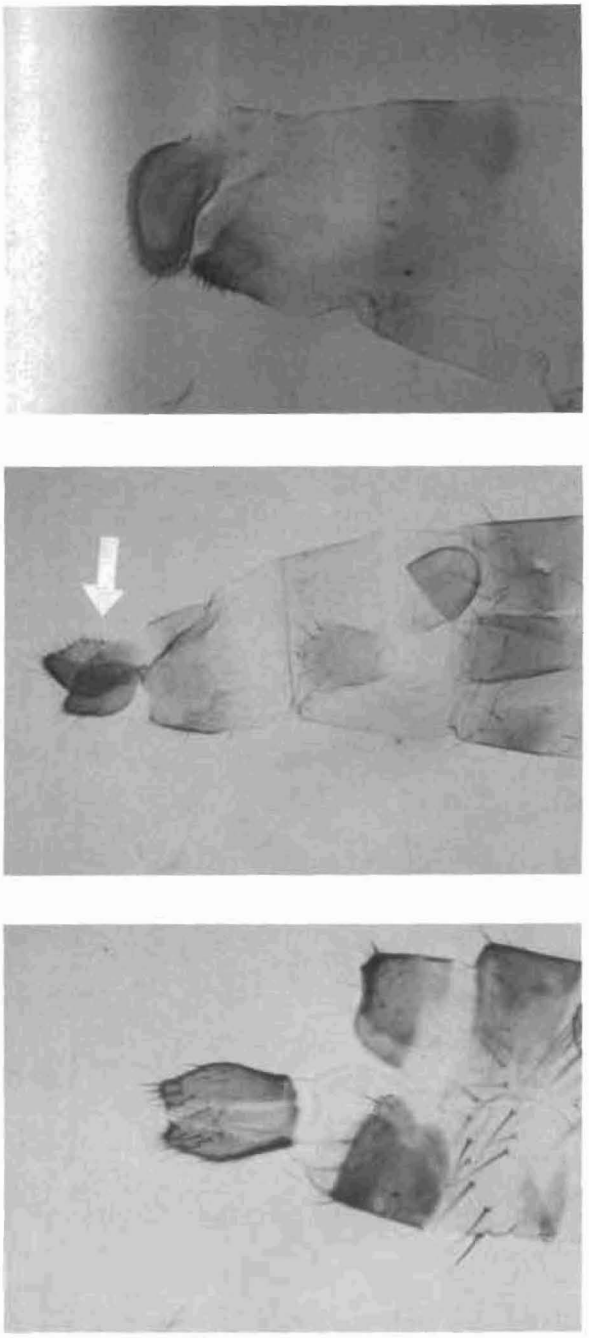


Figure 13. Female cerci or external genitalia (arrow), *Hydrellia pakistanae* (top), *H. balciunasi* (middle), and *H. bilobifera* (bottom)

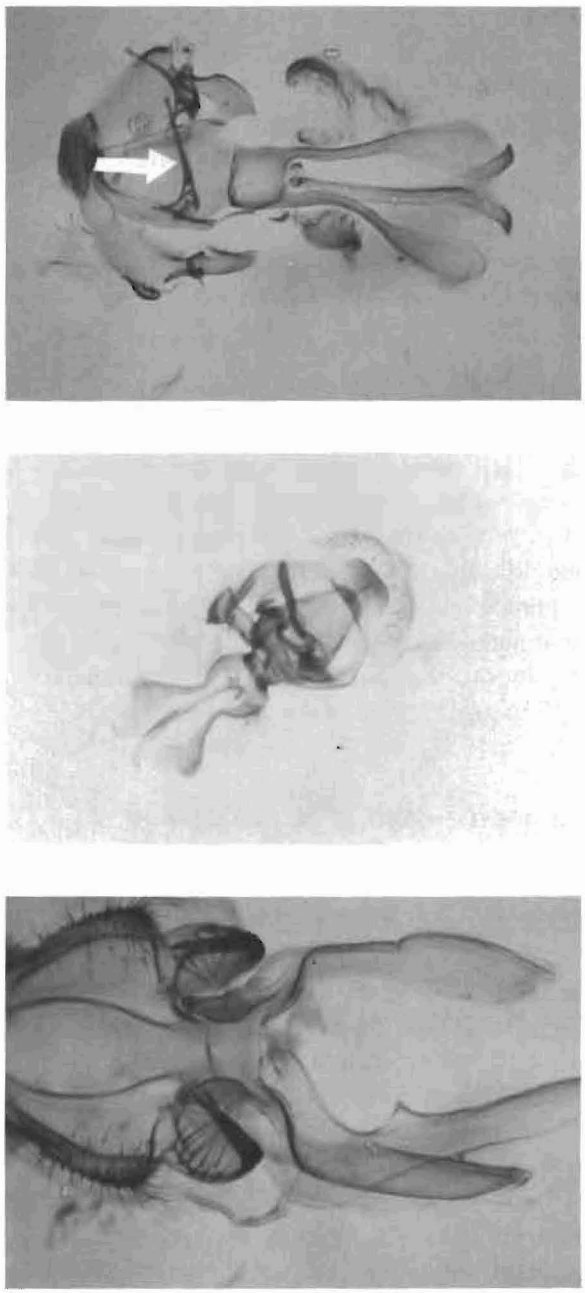


Figure 14. Male genitalia, *Hydrellia pakistanae* (top), *H. balciunasi* (middle), and *H. bilobifera* (bottom). Arrow indicates bristles that can be used to quickly separate the species

Effects of two different diets on life span, fecundity, and egg viability of *H. pakistanae*

There were no significant differences between life spans ($p = 0.33$) and egg viabilities ($p = 0.20$) of *H. pakistanae* flies fed yeast hydrolysate-sugar solution and those fed 50-percent solution. However, females fed yeast hydrolysate-sugar solution laid significantly more eggs than females fed 50-percent honey solution ($p = 0.03$). Yeast hydrolysate-sugar solution should be fed to flies to maximize fecundity, but the more easily obtained 50-percent honey solution could be used to maintain flies for short periods while awaiting release. Life span and fecundity of both fly species fed yeast hydrolysate-sugar solution are reported in Table 6.

The type of food eaten by the flies in nature is unknown, but they may feed on aphid honeydew. Adults were often observed in the colony congregated around and on dead flies and aphids, but whether adults were feeding on the carcasses was not determined. They touched the carcasses with their mouth-parts and even pulled and pushed them with their legs, but wounds were not observed on the carcasses after they did this. Newly emerged *H. pakistanae* females exposed to freshly killed aphids laid no more eggs and lived no longer than those without food. Both treatments were less than those with pollen, sugar, or yeast hydrolysate-sugar solution. Females fed a 10-percent honey solution were intermediate and differed from none of the other treatments.

Developmental threshold and degree-days of *H. pakistanae*

The speed of development of *H. pakistanae* increased in direct relation to temperature. Mean developmental times were 37.9 ± 2.2 days (n = 43, range 31-42) at 21.5 °C, 22.6 ± 1.5 days (n = 22, range 20-25) at 27 °C, and 16.6 ± 1.2 days (n = 39, range 15-19) at 32 °C. No adults emerged at 36 °C. The estimated lower threshold for development calculated by linear regression was 13.0 °C. Degree-days calculated for each temperature, 21.5, 27, and 32 °C, produced a mean degree-days of 311.7. These estimates should be useful for timing adult emergence in the field to confirm establishment in a water body. The number of estimated annual generations at a given location could also be estimated. Unless temperatures are measured near the water's surface within a hydrilla mat, however, many flies will emerge faster than predicted from usual water temperature measurements. On a sunny day, surface temperatures soar inside a mat where there is little water current (Carter et al. 1988). Carter et al.. (1987) reported temperatures within hydrilla mats in the Potomac River that should allow development of *H. pakistanae* from at least June through September. Degree-days were not determined for *Hydrellia balciunasi.*

Hydrellia pakistanae larval survival at cold temperatures

Large larvae of *H. pakistanae* from the greenhouse placed at 8 °C made no new mines the first week but did in the second week. None had pupated after

60 days. They were then transferred to 16 °C where 33 percent (n = 30) pupated and 10 percent emerged as adults 39.0 ± 2.6 days (range 37-42) after being transferred. Forty percent (n = 30) of the larvae placed directly at 16 °C developed into adults after 47.6 ± 14.6 days (range 29-73) compared with 73.3 percent and 14.8 ± 3.7 days (range 11-23) for larvae placed at 27 °C.

These results indicate that at least some *H. pakistanae* should be able to overwinter in north-central Florida as larvae and pupae. This conclusion was reached because winters are short and because winter water temperatures in north-central Florida lakes rarely fall below 10 °C (Okrah 1986).

Effect of dryness on *H. pakistanae* larval development

There was no emergence from hydrilla put on dry soil, but emergence from hydrilla put on damp soil did not differ statistically from emergence in water ($p = 0.07$). Flies emerged from 6 to 10 days after the hydrilla was put on the soil and from 6 to 14 days in the water. This result is interesting because it shows that mature larvae and pupae can survive and complete development outside of water provided there is sufficient moisture in the plant. Immatures might survive in hydrilla carried on boats and trailers during short journeys between water bodies, but not during long journeys that would dry the hydrilla. During drawdowns, flies might continue to emerge from hydrilla for possibly a week after it is stranded by receding waters. The top layer of hydrilla on a stranded mat dries quickly and insulates the lower layers, which remain moist and green for weeks. These emerging flies should increase the stress on the residual hydrilla population in the permanent water areas.

Horizontal and vertical movements of *H. pakistanae* larvae

Hydrilla 1 m below the water surface in vertical tubes was mined by 70.2 ± 8.9 percent of the neonates (n = 3, range = 63-80). They had dropped from eggs laid on the nonhost *N. guadalupensis* floating on the water surface. The percentage of neonates mining at 1 m in horizontal tubes was 20.7 ± 5.7 percent (n = 3, range = 14.3-25.0). The percentage of larvae mining at 1 m that had exited hydrilla in horizontal tubes was 27.6 ± 4.1 percent (n = 3, range = 24.0-32.0). This experiment demonstrated that hydrilla that has not reached the water's surface might be infested by neonates from eggs deposited on floating vegetation, and larvae might be able to move horizontally between scattered plants at low hydrilla densities.

Host Range Studies

Hydrellia pakistanae

Multichoice-with-host oviposition test—jars. A summary of oviposition on test plants confined in jars with hydrilla is presented in Table 7. Females oviposited on all species but preferred hydrilla as evidenced by the fact that oviposition on most species was less than half that on hydrilla. The total oviposition on the three test species in a jar, however, was often greater than that on hydrilla (7 out of 16 jars). Oviposition was probably strongly influenced by the number of oviposition sites available. Although the amount of plant material was approximately the same for each species in a jar, only the material exposed above the surface was a potential oviposition site. The actual amount of leaf surface of each plant species exposed above water to the flies undoubtedly varied greatly as it would in nature. Interestingly, the two species morphologically most similar to hydrilla, *E. canadensis* and *N. guadalupensis*, received the highest relative numbers of eggs. In the shallow water of the test jars, many leaves of these species protruded from the water like those of hydrilla providing an abundance of oviposition sites. Purple bladderwort, *Utricularia purpurea* Walt., also received many eggs. It presented a relatively large amount of exposed floating leaf material, but it also was paired with two poorly accepted species, *Echinodorus cordifolius* (L.) Griseb. and *Polygonum densiflorum* Meissner. Thus, instead of a choice of four plants, the females essentially had only the choice of hydrilla and bladderwort (Buckingham, Okrah, and Thomas 1989).

Because the females oviposited readily on so many plant species, a biocontrol program to conduct more intensive, replicated tests was thought to be unnecessary. The highly mobile larvae are able to leave the egg site to search for hydrilla, and thus the oviposition substrate does not appear to be an important determinant for host specificity. All eggs used in the larval tests (Tables 1, 2, 8, and 9) were deposited by females on the respective test plant, either in the presence or absence of hydrilla. Hydrilla was often added to cages to increase oviposition on marginally accepted species, since oviposition was apparently stimulated by hydrilla. Although eggs were deposited on every plant species tested, few eggs were deposited in preliminary tests on inanimate objects—for example, cork, styrofoam, and cloth (Buckingham, Okrah, and Thomas 1989). Deonier (1971) indicated that *Hydrellia* eggs were often found in the field on inanimate objects.

Multichoice-with-host oviposition test—wooden cages. Unlike the preceding test with plants mixed together in jars, the wooden cage tests compared plants exposed in separate dishes. Females thus had to respond to cues other than those of hydrilla when ovipositing on other plants. Hydrilla was strongly preferred in these tests except in comparison with *E. canadensis* (Table 10). Reduced oviposition on *P. crispus*, both in the presence and absence of hydrilla, was of special interest because that species supported most larval development among all test plants (Buckingham, Okrah, and Thomas 1989).

No-choice larval development tests. Fifty-one plant species (Table 1) were tested during these studies with emphasis on pondweeds, *Potamogeton* (eight species), Hydrocharitaceae (four species), and southern naiad, *N. guadalupensis*. Larvae mined in eight of the high risk species, but adults emerged from only five of the eight species. In the preliminary experiments, adults also emerged from *P. richardsonii* (2 from 184 eggs). Monoecious hydrilla from several northern United States locations was tested and all sprigs were mined. Unfortunately, the material that had been grown in Fort Lauderdale, FL (Steward and Van 1987), was in winter decline and disintegrated before the tests were finished. Two adults, however, did successfully develop on plants from each of two locations: Lilypons Water Gardens, Frederick County, MD, and Dyke Marsh, Alexandria, VA (Buckingham, Okrah, and Thomas 1989).

Paired-choice larval development tests. Larvae were even more specific in these tests (Table 2) than in the no-choice test. Four percent developed to adults on *P. crispus* and none from *P. nodosus* versus 8 percent and 4 percent, respectively, in the no-choice test. In addition, fewer adults emerged from hydrilla in the tubes with test plants than in the hydrilla control tubes. Apparently larvae that initially attacked the test plants either died or were unsuccessful in attempts to transfer to the sprigs of hydrilla. One adult emerged from *E. densa* paired with hydrilla, but the larva may have transferred from damaged hydrilla before pupation, since no adults were produced in the *E. densa* control tube. The relatively low percent adults from the hydrilla control with 15 eggs per tube was undoubtedly due to competition among larvae. This number of eggs was chosen to maximize the number of larvae tested against *E. densa* (Buckingham, Okrah, and Thomas 1989).

Host suitability of *Potamogeton crispus*. Results of a test comparing the survival of flies produced on hydrilla and *P. crispus* over one generation are summarized in Table 8. Survival to adulthood was four times greater on hydrilla that on *P. crispus*. Statistically significant differences were not found in the speed of development, adult longevity, and fecundity on the two plant species although all were less on *P. crispus*. Surprisingly, egg hatch was significantly greater for the females from *P. crispus* (Buckingham, Okrah, and Thomas 1989).

In a multiple generations test (Table 9), the fly population on hydrilla died out in the second generation because of total destruction of the hydrilla plants. Adults of the fifth generation on *P. crispus* were combined in one jar because of low numbers, and the population died out in the seventh generation. This test indicated that *P. crispus* could serve as a temporary but not a permanent host. Abundance of food, sexual encounters, temperature, adult exposure time, and protection from enemies were all maximized in this test, relative to the field conditions, in order to produce this small number of adults. Field populations, on the other hand, would encounter many stresses during attempts to colonize *P. crispus*. It is doubtful if they could successfully establish on this species, which is an associate of hydrilla in Pakistan and India, but not a field host of *H. pakistanae* (Baloch, Sana-Ullah, and Ghani 1980) (Buckingham, Okrah, and Thomas 1989).

Potamogeton crispus was tested more thoroughly than other species, even though it is an important introduced weed throughout much of North America and not a beneficial species, because it had supported the most larval development during no-choice tests. It was thus the best potential alternate host plant among our test plants.

Hydrellia balciunasi

Multichoice oviposition tests. Females oviposited on nonhost plants; however, their discrimination increased as the cage increased from small to medium size (Table 5). Eggs were laid only on plants, none on wooden sticks. Hydrilla was preferred in the medium-sized cage, but two species closely related to hydrilla with similar morphology were also readily accepted. There was no difference among the three in large cages possibly because relatively few eggs were deposited. Although females readily oviposited on them, neither *E. canadensis* nor *E. densa* was attacked by neonate larvae in the no-choice tests (Buckingham, Okrah, and Christian-Meier 1991).

Hydrellia balciunasi readily oviposited on many test plants in jars used to obtain eggs for no-choice tests. Field oviposition on nonhost plants would allow *H. balciunasi* larvae to attack hydrilla when it has not yet reached the water surface. Because the neonates are monophagous, they would drop or crawl from the oviposition plant to hydrilla (Buckingham, Okrah, and Christian-Meier 1991).

No-choice larval development tests. In the neonate studies, adults emerged from only 1 of 15 test plant species (Table 1). On that species, *P. crispus*, an introduced weed, only 2 of 200 larvae developed compared with 63 percent of 1,050 on hydrilla. Mining but no development was observed on *P. pusillus* (Table 1). *Hydrellia pakistanae* mined in eight of these same species and produced small numbers of adults on five of them. In the field in Pakistan, however, it was monophagous on hydrilla (Baloch, Sana-Ullah, and Ghani 1980) (Buckingham, Okrah, and Christian-Meier 1991).

Some of the experienced larvae (ones that had fed initially on hydrilla) completed development on one additional plant species, *E. canadensis*. Percent development (of these larvae) increased (compared with that of neonates) on *P. crispus*, although it was still minor (Table 3). Successful development on these nonhost plants was approximately equal for all ages of experienced larvae. Apparently, the neonate stage is the major host-isolating stage in this species (Buckingham, Okrah, and Christian-Meier 1991).

Multichoice larval development tests. Adults did not emerge from the 27 plant species representing 17 families tested in groups of three (Table 4), and no plants were damaged. Because the larvae are highly mobile, all three species would have been equally available within a jar. Therefore, it was unnecessary to retest any of these species in no-choice tests (Buckingham, Okrah, and Christian-Meier 1991).

4 Conclusions

Both *Hydrellia* species have been released in Florida where studies are underway to establish field populations and to evaluate their effects on hydrilla. Both species are from tropical to subtropical climates and might not survive outside the coastal areas of the southeastern United States. Additional species of *Hydrellia* from temperate climates in Asia should be studied for possible release in colder areas of the United States.

Although both species are specific to hydrilla, small numbers might occasionally be reared from a few additional plant species during population peaks. This should cause no undue alarm. Mature larvae often move to undamaged nonhost plants for pupariation and *H. pakistanae* might produce small numbers of adults in a few plant species related to hydrilla.

Competition between the two species in a waterway is a possibility, but the results cannot be adequately predicted. *Hydrellia pakistanae* larvae mined more leaves and females laid more eggs, but *H. balciunasi* adults lived longer and there were more females (Table 6). Immature development times were about the same. *Hydrellia pakistanae* adults contaminated the *H. balciunasi* colonies after *H. balciunasi* had been cleared for release from quarantine and after *H. pakistanae* was recolonized in quarantine for additional studies. They were very difficult to eliminate from the *H. balciunasi* rearing jars. Obviously, they were good competitors under our rearing conditions. *Hydrellia balciunasi* would appear to have a slight advantage climatically. Its range in Australia appears to include slightly greater temperature extremes than does the known range of *H. pakistanae*; however, climate data has not been carefully analyzed. Both species are expected to live together in most waterways and to complement each other. Probably more important than potential interspecific competition is the potential parasitization by native parasites moving over from native *Hydrellia*. This should be closely monitored in the field.

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Table 1
Summary of "No-choice Larval Tests" with *Hydrellia pakistanae* and *Hydrellia balciunasi* [1]

Family/Species	*Hydrellia pakistanae*				*Hydrellia balciunasi*			
	Total[2] Eggs	Tubes w/Mining, %	X̄ Damage[3] Estimate	Adults,[4] %	Total Eggs	Tubes w/Mining, %	X̄ Damage Estimate	Adults, %
Families Related To Hydrilla								
Alismataceae								
Alisma subcordatum Raf.	50	0	0	0	--	--	--	--
Echinodorus cordifolius (L.) Griseb	50	0	0	0	--	--	--	--
Sagittaria kurziana Gluck	100	0	0	0	50	0	0	0
Sagittaria latifolia Willd.	50	0	0	0	--	--	--	--
Sagittaria subulata (L.) Buchen.					50	0	0	0
Hydrocharitaceae								
Hydrilla verticillata (L.f.)Royle	2170	99	2.9	54	1050	96.7	2.1	63

[1] Adapted from Buckingham, Okrah, and Thomas (1989) and Buckingham, Okrah, and Christian-Meier (1991).
[2] Fifty eggs per test (five eggs per tube). Differences from multiples of 50 resulted from loss of sample because of plant breakdown.
[3] 0 = no mining, 1 = 1/4 or less of leaf material mined, 2 = more than 1/4 to 1/2, 3 = more than 1/2 to 3/4, 4 = more than 3/4 but not all, 5 = all leaf material mined. Estimated when the first adults emerged.
[4] $\frac{\text{Total Adults Produced}}{\text{Total Eggs}} \times 100$

Table 1 (Continued)

Family/Species	*Hydrellia pakistanae*				*Hydrellia balciunasi*			
	Total Eggs	Tubes w/Mining, %	X̄ Damage Estimate	Adults, %	Total Eggs	Tubes w/Mining, %	X̄ Damage Estimate	Adults, %
Elodea canadensis Michx.	425	29	0.3	1	300	0	0	0
Elodea nuttalli (Planch.) St. John	50	0	0	0	--	--	--	--
Egeria densa Planch.	140	18	0	0	145	0	0	0
Limnobium spongia (Bosc) Steud.	50	0	0	0	--	--	--	--
Vallisneria americana Michx.	50	0	0	0	100	0	0	0
Najadaceae								
Najas guadulupensis (Spreng.) Magnus	580	29	0.3	1	250	0	0	0
Potamogetonaceae								
Potamogeton crispus L.	245	67	1.5	7	200	20	0.2	1
Potamogeton diversifolius Raf.	150	23	0.2	0	100	0	0	0
Potamogeton illinoensis Morong	180	16	0.1	0	200	0	0	0
Potamogeton nodosus C. & S.	185	49	0.9	3	200	0	0	0
Potamogeton pectinatus L.	50	0	0	0	50	0	0	0

Table 1 (Continued)

Family/Species	*Hydrellia pakistanae*				*Hydrellia balciunasi*			
	Total Eggs	Tubes w/Mining, %	X̄ Damage Estimate	Adults, %	Total Eggs	Tubes w/Mining, %	X̄ Damage Estimate	Adults, %
Potamogeton perfoliatus L.	195	40	0.4	1	150	0	0	0
Potamogeton pulcher Tuckerm.	40	0	0	0	--	--	--	--
Potamogeton richardsonii (A.Bann.) Rydb.	45	0	0	0	100	0	0	0
Potamogeton pusillus L.	--	--	--	--	100	10	0.1	0
Miscellaneous Families								
Araceae								
Pistia stratiotes L.	50	0	0	0	--	--	--	--
Cabombaceae								
Brasenia schreberi J. F. Gmel.	50	0	0	0	--	--	--	--
Cabomba caroliniana A. Grey	100	0	0	0	--	--	--	--
Ceratophyllaceae								
Ceratophyllum demersum L.	100	0	0	0	--	--	--	--

Table 1 (Continued)

Family/Species	*Hydrellia pakistanae*				*Hydrellia balciunasi*			
	Total Eggs	Tubes w/Mining, %	X̄ Damage Estimate	Adults, %	Total Eggs	Tubes w/Mining, %	X̄ Damage Estimate	Adults, %
Characeae								
Chara sp.	50	0	0	0	--	--	--	--
Cruciferae								
Nasturtium officinale R. Br.	50	0	0	0	--	--	--	--
Cyperaceae								
Eleocharis sp. 1 (large)	100	0	0	0	--	--	--	--
Eleocharis sp. 2 (small)	50	0	0	0	--	--	--	--
Rynchospora inundata (Oakes) Fern.	50	0	0	0	--	--	--	--
Eriocaulaceae								
Eriocaulon decangulare L.	50	0	0	0	--	--	--	--
Haloragaceae								
Myriophyllum heterophyllum Michx.	100	0	0	0	--	--	--	--
Myriophyllum spicatum L.	100	0	0	0	--	--	--	--

Table 1 (Continued)

Family/Species	*Hydrellia pakistanae*				*Hydrellia balciunasi*			
	Total Eggs	Tubes w/Mining, %	X̄ Damage Estimate	Adults, %	Total Eggs	Tubes w/Mining, %	X̄ Damage Estimate	Adults, %
Proserpinaca pectinata Lam.	100	0	0	0	--	--	--	--
Juncaceae								
Juncus effusus L.	100	0	0	0	--	--	--	--
Lemnaceae								
Lemna perpusilla Torr.	50	0	0	0	--	--	--	--
Spirodela punctata (Meyer) Thomps.	50	0	0	0	--	--	--	--
Lentibulariaceae								
Utricularia purpurea Walt.	50	0	0	0	--	--	--	--
Menyanthaceae								
Nymphoides aquatica (floating leaves) (S. G. Gmel.) Kuntze.	50	0	0	0	--	--	--	--
Nymphoides aquatica (submersed) (S. G. Gmel.) Kuntze	50	0	0	0	--	--	--	--

Table 1 (Continued)

Family/Species	*Hydrellia pakistanae*				*Hydrellia balciunasi*			
	Total Eggs	Tubes w/Mining, %	X̄ Damage Estimate	Adults, %	Total Eggs	Tubes w/Mining, %	X̄ Damage Estimate	Adults, %
Nymphaeaceae								
Nuphar luteum (L.) Sibth. & Sm.	100	0	0	0	--	--	--	--
Nymphaea tuberosa Paine	100	0	0	0	--	--	--	--
Onagraceae								
Ludwigia repens Forst.	150	0	0	0	--	--	--	--
Poaceae								
Oryza sativa L.	100	0	0	0	200	0	0	0
Hydrochloa carolinierisis Beauv.	50	0	0	0				
Zizaniopsis miliacea (Michx.)	50	0	0	0				
Polygonaceae								
Polygonum densiflorum Meissner	50	0	0	0	--	--	--	--
Pontederiaceae								
Pontederia cordata L.	50	0	0	0	--	--	--	--

Table 1 (Concluded)

Family/Species	*Hydrellia pakistanae*				*Hydrellia balciunasi*			
	Total Eggs	Tubes w/Mining, %	X̄ Damage Estimate	Adults, %	Total Eggs	Tubes w/Mining, %	X̄ Damage Estimate	Adults, %
Rupplaceae								
Ruppia maritima L.	200	0	0	0	--	--	--	--
Salviniaceae								
Azolla caroliniana Willd.	50	0	0	0	--	--	--	--
Salvinia rotundifolia Willd.	50	0	0	0	--	--	--	--
Scrophulariaceae								
Bacopa monnieri (L.) Penell	50	0	0	0	--	--	--	--
Typhaceae								
Typha latifolia L.	50	0	0	0	--	--	--	--
Umbelliferae								
Hydrocotyle umbellata L.	50	0	0	0	--	--	--	--
Filamentous Algae								
Unidentified green alga	50	0	0	0	--	--	--	--

Table 2
Summary of "Pair-choice with Host" Larval Tests with *Hydrellia pakistanae*

Test Plant	Total Eggs	Tubes w/ Mining, %	Adults[a] %
Controls			
Hydrilla	50[b]	100	70
Hydrilla	150[c]	100	39
E. densa	150	20	0
Pair 1			
Hydrilla	150	100	11
E. densa		70	1
Pair 2			
Hydrilla	50	100	48
N. guadalupensis		40	0
Pair 3			
Hydrilla	50	100	48
P. crispus		60	4
Pair 4			
Hydrilla	50	100	40
P. nodosus		30	0

Note: After Buckingham, Okrah, and Thomas (1989).
a. Values derived using the following:

$$\frac{\text{Total adults from pupae in test plant}}{\text{Total eggs}} \times 100$$

b. Five eggs per 35 ml-tube.
c. Fifteen eggs per tube.

Table 3
No-choice Larval Development Test with Different Age Larvae of *Hydrellia balciunasi* Fed Initially on Hydrilla[1]

Test Plants	No. Larvae Per Replicate[2]	Mean No. Adults Emerged ± s.d. (Range)		Adults, %
		Male	Female	
1- to 4-Day-Old Larvae				
Hydrilla	10	4.3 ± 1.5 (3-6)	4.7 ± 2.1 (3-7)	90.0
Potamogeton crispus	10	1.3 ± 0.6 (1-2)	0.7 ± 0.6 (0-1)	20.0
Elodea canadensis	10	0.3 ± 0.6 (0-1)	0	3.3
3-Day-Old Larvae				
Hydrilla	10	4.0 ± 1.0 (3-5)	4.0 ± 0 (4)	80.0
Potamogeton crispus	10	1.3 ± 0.6 (1-2)	1.0 ± 0 (1)	23.3
Elodea canadensis	10	0	0	0
5- to 8-Day-Old Larvae				
Hydrilla	10	3.4 ± 0.6 (3-4)	5.7 ± 1.5 (4-7)	90.0
Potamogeton crispus	10	1.7 ± 1.2 (1-3)	0.7 ± 0.6 (0-1)	23.3
Elodea canadensis	10	0.3 ± 0.6 (0-1)	0	3.3
10- to 13-Day-Old Larvae				
Hydrilla	30	8.3 ± 3.8 (4-11)	10.0 ± 2.6 (7-12)	61.0
Potamogeton crispus	30	2.7 ± 0.6 (2-3)	3.0 ± 1.0 (2-4)	18.7
Hydrilla	30	11.7 ± 3.2 (8-14)	14.0 ± 1.0 (13-15)	85.6
Elodea canadensis	30	1.0 ± 1.0 (1-2)	1.3 ± 0.6 (1-2)	7.8

[1] After Buckingham, Okrah, and Christian-Meier (1991).
[2] Three replicates; 0.45-ℓ glass jars.

Table 4
Plant Species Tested in Multichoice Larval Development Tests with *Hydrellia balciunasi* [1]

Alismataceae
Echinodorus sp.
Sagittaria graminea Michx.
Aponogetonaceae
Aponogeton sp. (exotic aquarium species)
Azollaceae
Azolla caroliniana Willd.
Cabombaceae
Brasenia schreberi J. F. Gmel.
Cabomba caroliniana A. Grey
Ceratophyllaceae
Ceratophyllum demersum L.
Cruciferae
Nasturtium officinale R. Br.
Haloragaceae
Myriophyllum aquaticum (Velloso) Verdc.
Myriophyllum spicatum L.
Hydrocharitaceae
Hydrilla verticillata (L.f.) Royle
Limnobium spongia (Bosc) Steud.
Lemnaceae
Lemna Valdiviana Phil.
Spirodela polyrhiza (L.) Schleid.
Lentibulariaceae
Utricularia floridana Nash.
Utricularia purpurea Walt.
Menyanthaceae
Nymphoides aquatica, (S. G. Gmel.) Kuntze. (submersed leaves)

(***Continued***)

[1] No adults were produced on any of the plants.

Table 4 (Concluded)
Nymphaeaceae
Nuphar lutuem (L.) Sibth. & Sm. (submersed leaves)
Nymphaea odorata Aiton (submersed leaves)
Onoagraceae
Ludwigia repens Forst.
Poaceae
Hydrochloa caroliniensis Beauv.
Oryza sativa L.
Panicum hemitomon Schult.
Panicum dicotomiflorum Michx.
Polygonaceae
Polygonum densiflorum Meissner
Scrophulariaceae
Bacopa monnieri (L.) Penell.
Ruppiaceae
Ruppia maritima L.

Table 5
Multichoice Oviposition Tests with *Hydrellia*

Test Plant	Small Cage[1]			Medium-sized Cage[1]			Large Cage–First Test[2]			Large Cage–Second Test[2]		
	X̄ No. Eggs[3]	S.D.	Range	X̄ No. Eggs	S.D.	Range	X̄ No. Eggs	S.D.	Range	X̄ No. Eggs	S.D.	Range
Hydrilla	16.3a	6.0	10-22	44.0a	4.4	41-49	17.3a	9.0	8-26	11.8a	7.9	3-27
Oryza sativa	11.0ab	7.0	6-19	0c	0	0	--	--	--	--	--	--
Ceratophyllum demersum	8.3abc	6.8	3-16	0.7c	1.2	0-2	--	--	--	--	--	--
Elodea canadensis	3.7bcd	2.1	2-6	25.3b	5.5	20-31	32.0a	19.7	14-53	12.2a	13.6	2-43
Lemna-Azolla mix	3.3bcd	1.5	2-5	0.3c	0.6	0-1	--	--	--	--	--	--
Egeria densa	3.0bcd	3.6	0-7	22.0b	16.1	7-39	10.7a	11.5	4-24	8.9a	6.0	3-21
Potamogeton crispus	2.0cd	2.0	0-4	0c	0	0	--	--	--	--	--	--
Wooden stick	0d	0	0	0c	0	0	--	--	--	--	--	--

Note: After Buckingham, Okrah, and Christian-Meier (1991).

[1] Ten pairs per each of three replicates; 5 days.

[2] Thirty pairs per each of three replicates; 3 days.

[3] Means within a column followed by the same letter are not significantly different; GLM procedure (P = 0.05), Waller-Duncan k-ratio t-test (k = 100) option (SAS Institute 1985).

Table 6
Biological Data for *Hydrellia pakistanae* and *Hydrellia balciunasi* Reared at Constant 27 ± 1 °C[1]

Parameter	*Hydrellia pakistanae*				*Hydrellia balciunasi*			
	Mean	S.D.	N.	Range	Mean	S.D.	N.	Range
Development Time, days								
Egg	3.2	0.1	50	2.9-3.7	3.0	0.1	16	2.5-3.0
Larva								
Male		--			12.0	1.7	19	9-16
Female		--			12.2	1.8	25	10-18
Mixed	12.8	1.6	21	10-16		--		
Puparium								
Male		--			8.5	0.7	19	7-9
Female		--			8.0	0.8	25	7-10
Mixed	6.6	0.6	21	6-8		--		
Total								
Male		--			23.5	1.5	19	21-27
Female		--			23.2	1.8	25	20-29
Mixed	22.6	1.7	21	20-25		--		
Life Span, days								
Male	7.9	3.4	9	4-15	15.6	12.9	10	1-38
Female	10.2	4.5	9	6-21	19.7	8.9	10	3-31
Fecundity (Eggs per female per lifetime)	68.4	29.9	9	27-107	35.5	28.3	10	2-83
No. Leaves Mined per Larva								
Male		--			5.4	1.2	8	4.0-7.5
Female		--			7.2	1.1	15	5.0-9.0
Mixed	11.9	3.4	28	6-21		--		
Measurements, mm								
Egg								
Length	0.54	0.03	20	0.48-0.58	0.45	0.02	10	0.44-0.48
Width	0.16	0.01	20	0.14-0.18	0.14	0.01	10	0.14-0.16
Puparium								
Length	3.00	0.21	25	2.60-3.56	2.76	0.16	28	2.40-3.16
Width	0.83	0.06	25	0.72-0.96	0.76	0.04	28	0.68-0.88

[1] Partially adapted from Buckingham, Okrah, and Christian-Meier (1991).

Table 7
Summary of "Multichoice with Host—Jars" Oviposition Tests with *Hydrellia pakistanae*[1]

Family/Species	Oviposition Tests		
	Test[2] Symbol	Host Eggs,[3] %	
		Mean	Range
Families Closely Related To Hydrilla			
Alismataceae			
Echinodorus cordifolius (L.) Griseb.	A	5	--
Sagittaria latifolia Willd.	B	40	--
Hydrocharitaceae			
Elodea canadensis Michx.	C	84	--
Limnobium spongia (Bosc) Steud.	D	43	--
Najadaceae			
Najas guadulupensis (Spreng.) Magnus	C	87	--
Potamogetonaceae			
Potamogeton crispus L.	E,F	21	18-25
Potamogeton nodosus C. & S.	E,F,G,H	27	13-43
Potamogeton pectinatus L.	C	55	--
Miscellaneous Families			
Ceratophyllaceae			
Ceratophyllum demersum L.	I	41	--
Characeae			
Chara sp.	J	28	--
Cruciferae			
Nasturtium officinale R. Br.	K,L	20	14-26

Sheet 1 of 3

[1] Adapted from Buckingham, Okrah, and Thomas (1989).
[2] Hydrilla and three test plant species were exposed in one 3.8-ℓ jar. Plant species with the same letter were exposed together. These plants were chosen for the no-choice larval feeding tests in which the eggs from this test were used.
[3] $\frac{\text{Eggs on test plant}}{\text{Eggs on hydrilla}} \times 100$

Table 7 (Continued)

Family/Species	Test[2] Symbol	Oviposition Tests: Host Eggs,[3] % Mean	Host Eggs,[3] % Range
Cyperaceae			
Eleocharis sp. 2 (small)	M	50	--
Haloragaceae			
Myriophyllum heterophyllum Michx.	I,K	34	15-53
Proserpinaca pectinata Lam.	J	32	--
Juncaceae			
Juncus effusus L.	M	45	--
Lemnaceae			
Lemna perpusilla Torr.	N	47	--
Spirodela punctata (Meyer) Thomps.	N,O	21	11-30
Lentibulariaceae			
Utricularia purpurea Walt.	A	70	--
Nymphaeaceae			
Nuphar lutuem (L.) Sibth. & Sm.	D,H,P	25	9-44
Nymphaea tuberosa Paine	D,H,P	24	10-47
Onagraceae			
Ludwigia repens Forst.	K	47	--
Poaceae			
Oryza sativa L.	E,G,I	29	12-58
Hydrochloa caroliniensis Beauv.	M	42	--
Polygonaceae			
Polygonum densiflorum Meissner	A	19	--
Pontederiaceae			
Pontederia cordata L.	B	39	--
Ruppiaceae			
Ruppia maritima L.	F	23	--

Table 7 (Concluded)

Family/Species	Oviposition Tests		
	Test[2] Symbol	Host Eggs,[3] %	
		Mean	Range
Salviniaceae			
Azolla caroliniana Willd.	N	54	--
Typhaceae			
Typha latifolia L.	B	38	--
Umbelliferae			
Hydrocotyle umbellata L.	J	26	--

Table 8
Hydrilla versus *Potamogeton crispus*: Comparison of Host Suitability for *Hydrellia pakistanae*

Test Plant	Replicate	Eggs Per Adults,[1] %	Days to $\bar{X}$ Longevity in Days 50% Emergence	Males	Females	Eggs/Female[2]	$\bar{X}$ % Egg Hatch[3]
Hydrilla	50	59.3 ± 7.6a(54-68)	19.7 ± 0.6a(19-20)	11.4 ± 3.3a(9.4-15.2)	10.3 ± 2.3a(7.8-12.2)	78.0 ± 19.0a(66-100)	85.6 ± 4.3a(82.3-90.4)
P. crispus	200	14.0 ± 2.0b(12-16)	21.0 ± 2.0a(19-23)	10.5 ± 1.0a(9.8-11.6)	8.9 ± 3.2a(5.4-11.6)	63.0 ± 16.1a(48-80)	94.3 ± 2.0b(92.0-95.5)

Note: After Buckingham, Okrah, and Thomas (1989).
[1] $\bar{X}$ ± SD(range). Means within a column followed by the same letter were not significantly different ($p \leq 0.05$, t-test).
[2] Data from samples of five pairs selected from total adults emerged in each replicate.
[3] Three samples of five eggs selected from total eggs deposited each day in each replicate. Mean of all samples in the replicate.

Table 9
Hydrilla versus *Potamogeton crispus*: Multiple Generations of *Hydrellia pakistanae*

Test Plant	Replicate	Initial No. Eggs	No. Adults in Generation No.							
			1[a]	2[b]	3[c]	4	5[d]	6	7	8
P. crispus	1	300	69 (23)	19	30	5	2	6	8	0
	2	300	39 (13)	7	15	4	1	--	--	--
	3	300	64 (21)	8	22	5	3	--	--	--
	Totals	900	172 (19)	34	67	14	6	6	8	0
Hydrilla	1	60	36 (60)	--	--	--	--	--	--	--
	2	60	51 (85)	--	--	--	--	--	--	--
	3	60	44 (73)	--	--	--	--	--	--	--
	Totals	180	131 (73)							

Note: After Buckingham, Okrah, and Thomas (1989).

[a] $\frac{\text{No. adults}}{\text{Initial No. Eggs}} \times 100$. Egg nos. were unknown in subsequent generations. Adults in each generation were held until death in jars, which were then held for larval development.

[b] Larvae of the second generation on hydrilla died when they destroyed the plants.

[c] Numbers might also include fourth generation individuals produced from eggs deposited by newly emerged third generation females before they were removed to new jars.

[d] Adults of fifth generation were pooled to ensure mating success.

Table 10
Summary of "Multichoice with Host—Wooden Cage" Oviposition Tests with *Hydrellia pakistanae*

Test Plant[1]	No. Eggs ($\bar{X} \pm SE$)[2]			
	Test 1	Test 2	Test 3	Test 4
Hydrilla verticillata	107.3 ± 22.1a	92.0 ± 21.2a	--	99.7 ± 68.7a
Najas guadalupensis	--	47.7 ± 7.8b	51.3 ± 3.8a	--
Egeria densa	--	2.0 ± 0.6c	43.0 ± 9.5a	--
Potamogeton crispus	10.0 ± 4.2b	--	6.3 ± 4.8b	--
Potamogeton nodosus	1.0 ± 0.6b	--	--	--
Potamogeton pectinatus	--	--	--	0b
Elodea canadensis	--	--	--	90.3 ± 35.1a

Note: After Buckingham, Okrah, and Thomas (1989).

[1] These plants were tested because they were mined in larval tests except *Potamogeton pectinatus*, an important species for wildlife. *Potamogeton pectinatus* was included to compare results of this test with the jar test in which it received 55 percent as many eggs as hydrilla. Each test had three replicates.

[2] Means within a column followed by the same letter are not significantly different (K = 100, Waller-Duncan Bayesian K-ratio t-test).

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Hydrilla verticillata (L. fil.) Royle (common name hydrilla) is a noxious aquatic plant introduced into the United States from Africa through the aquarium industry and sold under the name "oxygen plant" or "Star Vine." Hydrilla has long branching stems that often fragment and form large floating mats and can grow in water depths up to 15 m. Two reproductive structures that enable hydrilla to withstand extremely harsh weather conditions are turions or winter buds (dense clusters of apical leaves that are produced in the leaf axils, green and ovoid-conical shaped buds), and bubil-like hibernacular commonly, but incorrectly, called tubers (these are formed at the ends of stolons buried in the substratum). Plants are found in lakes, rivers, drainage and irrigation canals, ponds, and streams. Severe infestations of hydrilla can restrict boat traffic and interfere with fisheries and waterflow.

Biological control methods using insects appear to be an excellent alternative to conventional methods (mechanical harvesting or herbicide application) or may be used in conjunction with conventional methods to enhance control or possible eradication of nuisance aquatic plants.

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This report presents results of studies on the biologies and laboratory host ranges of two species of flies that feed on hydrilla, *Hydrellia*, *Hydrellia pakistanae* Deonier and *Hydrellia balciunasi* Boch. Biological and host range studies for *H. pakistanae* were conducted from 1985-1987, while the studies for *H. pakistanae* were conducted during August and September 1988. All studies were conducted in environmental growth chambers at 27 ± 1 °C with a 16-hr photophase.

Results showed that *H. pakistanae* flies fed with yeast hydrolysate-sugar solution exhibited no significant differences in life span or egg viability from those fed with 50-percent honey solution; however, fecundity increased significantly with the yeast hydrolysate-sugar solution. These results also show a direct relationship between the rate of development for *H. pakistanae* and temperature (as temperature increased so did the development rate).

At present, both species of *Hydrellia* have been released in Florida, and studies are being conducted to establish field populations and evaluate their effectiveness as biocontrol agents on hydrilla.