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Mass-Rearing *Hydrellia pakistanae* Deonier, A Biological Control Agent of *Hydrilla verticillata* (L.f.) Royle, for Release and Establishment

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Preface

The work reported herein was conducted as part of the Aquatic Plant Control Research Program (APCRP), Work Unit 33028. The APCRP is sponsored by Headquarters, U.S. Army Corps of Engineers (HQUSACE), and is assigned to the U.S. Army Engineer Research and Development Center 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 Center for Aquatic Plant Research and Technology (CAPRT), Dr. John W. Barko, Director. Mr. Robert C. Gunkel, Jr., was Associate Director for the CAPRT. Program Monitor during this study was Mr. Timothy R. Toplisek, HQUSACE.

The Principal Investigator for this study was Dr. Alfred F. Cofrancesco, Aquatic Ecology and Invasive Species Branch, Ecosystem Evaluation and Engineering Division (EEED), EL. The report was prepared by Ms. Jan E. Freedman, Dr. Michael J. Grodowitz, Dr. Cofrancesco, and Ms. Robin Bare, EEED. Ms. Robin Bare is located at ERDC, Lewisville Aquatic Ecosystem Research Facility (LAERF), Lewisville, Texas.

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The investigation was performed under the general supervision of Dr. Edwin A. Theriot, former Chief, Aquatic Ecology and Invasive Species Branch; Dr. Conrad J. Kirkby, former Chief, EEED; and Dr. John Harrison, former Director, EL. Dr. Theriot is now Acting Director, EL.

At the time of publication of this report, Director of ERDC was Dr. James R. Houston. Commander and Executive Director was COL John W. Morris III, EN.

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1 Introduction

Hydrilla (*Hydrilla verticillata* (L.f.) Royle), family Hydrocharitaceae, is a submersed aquatic macrophyte that is a major problem in the United States (Sutton and Portier 1985). Problems associated with extensive growths of hydrilla include navigational interference, hindering waterflow, and detracting from recreational uses of water bodies (Yeo, Falk, and Thurston 1984). Due to its ability to multiply profusely and produce large stands under many environmental conditions, hydrilla has become a major nuisance in aquatic systems (Miller, Garrard, and Haller 1976).

Hydrilla reproduces mainly by vegetative means such as fragments (pieces of plant material that have become detached), tubers, and turions (Langeland 1990). A hydrilla fragment can easily resprout into a new plant; therefore, small amounts of plant material attached to boating equipment can cause the plant to spread readily. Specialized vegetative propagules known as tubers and turions produced by hydrilla are capable of withstanding adverse environmental and physiological conditions (Langeland 1990). Hydrilla can produce millions of propagules per hectare (Miller, Garrard, and Haller 1976), allowing it to outcompete other submersed aquatic vegetation. Tubers also provide the main method of hydrilla reinfestations (Miller, Garrard, and Haller 1976). Due to its persistent nature and efficient vegetative reproduction, hydrilla is costly to manage using herbicides and other conventional methods (Baloch and Sana-Ullah 1973). Biological control was sought as an alternative control method and resulted in major research efforts by state and federal organizations, including the U.S. Department of Agriculture and the U.S. Army Corps of Engineers (Cofrancesco 1991).

To date, four insect biocontrol agents for hydrilla have been released in the United States, including two species of flies and two species of weevils (Julien and Griffiths 1998). The Asian and Australian leaf-mining flies are ephydrids in the genus *Hydrellia*, family Ephydridae, order Diptera (Borror, DeLong, and Triplehorn 1976), and include *H. pakistanae* Deonier from southern India, Pakistan, and northern China, released in 1987, and *H. balciunasi* Bock from Australia, released in 1989. *Hydrellia pakistanae* is currently established in Florida, Alabama, Georgia, and Texas, and is spreading naturally (Julien and Griffiths 1998, Center et al. 1997, Grodowitz et al. 1999). *Hydrellia balciunasi* is only established in Texas (Grodowitz et al. 1997). The two weevil species are in the genus *Bagous*. *Bagous hydrillae,* which originates from Australia, was released in 1991 and was tentatively established in Florida and possibly Texas, but no individuals have been collected recently (Grodowitz, Center, and Snoddy

1995). The other weevil species, *B. affinis*, originates from India. It has been released at sites in Florida, California, and Texas but has never established (Grodowitz, Center, and Snoddy 1995, Julien and Griffiths 1998).

The two species of leaf-mining flies have been reared at the U.S. Army Engineer Research and Development Center, Waterways Experiment Station (WES), Aquatic and Wetlands Research and Development Support Facility, Vicksburg, MS, since 1990. *Hydrellia pakistanae* is still being reared at WES. The first colony, *H. pakistanae* – India strain, arrived in 1990 and developed through 24 generations before being disbanded in 1992. Since that time many different *H. pakistanae* colonies have been maintained at WES including several from Florida and one from China. *Hydrellia balciunasi* was brought to WES in 1991, and several different colonies were maintained before rearing was ended in 1993.

One of the most important factors to consider when mass-rearing a large number of insects is insect quality. Three major elements that need to be evaluated for quality control are production, process, and product control (Leppla and Ashley 1989). Production control manages the consistency of production output (i.e. adult emergence), process control keeps unacceptable deviations in check (i.e. eggs per female), and product control predicts the effectiveness of the product in performing its intended function (i.e. leaf damage at release sites) (Leppla and Ashley 1989). Values that can be used to monitor the quality of an insect-rearing process and to ensure continuity in production include egg viability; density and yield of immature forms; survival, size, and yield of adults; and sex ratio (Chambers 1977). Measuring certain parameters such as egg hatch, survival from egg to adult, emergence rate, and fecundity is another way to check for insect quality (Leppla and Ashley 1989). Other associated behavioral characteristics which could be studied in relation to insect quality include flight ability and affinity for small rearing containers (Grodowitz, Lloyd, and McKibben 1992).

A potential problem with maintaining insect production under laboratory conditions is that of genetic drift and the possible selection of a "laboratory strain" (Harley and Forno 1992). This is when laboratory colonies undergo rapid changes related to particular rearing procedures (Mackauer 1976). According to Leppla and Ashley (1989), insects adapted to the laboratory usually mate more frequently, and the females are more fertile with a high percentage of their eggs developing into pupae. The group Diptera, which includes *H. pakistanae* and *H. balciunasi*, is considered to be an insect group that can be mass-reared adequately (Leppla and Ashley 1989).

A successful mass-rearing program provides uniform, high-quality insects, which are available as required, and are economical to produce (Leppla and Ashley 1989). For this reason, mass production or mass-rearing of *Hydrellia* spp. has become a major objective of the WES biocontrol program.

2 Objectives

The origin, host-specificity, description, and biology of the leaf-mining flies will be discussed. Other topics will include rearing procedures used at the Aquatic and Wetlands Research and Development Support Facility, insect colony upkeep, and plant maintenance.

The rearing records of *Hydrellia* spp. produced at WES from 1990 to 1993 were examined in relationship to changes in the quantity and quality of the insects produced. Measures of success will include number of insects produced and the quality of the insects such as egg production, emergence rates, etc. Field releases, insect establishment, and future mass-rearing techniques will also be discussed.

Hydrellia pakistanae and *Hydrellia balciunasi*

Origin

Hydrellia pakistanae Deonier, a leaf-mining ephydrid fly, which has a native range including India, Pakistan, and China (McCann, Arkin, and Williams 1996), was imported to the Florida Biological Control Laboratory quarantine, Division of Plant Industry, Florida Department of Agriculture and Consumer Services, Gainesville, FL, in May 1985 for evaluation (Buckingham, Okrah, and Thomas 1989). *Hydrellia balciunasi* Bock, a native of Australia, was shipped to the Florida Biological Control Laboratory's quarantine facility in 1988 (Buckingham, Okrah, and Christian-Meier 1991). Both *Hydrellia* spp. are in the order Diptera, family Ephydridae. Their larvae are aquatic (Borror, DeLong, and Triplehorn 1976) and mine the leaves of submersed hydrilla (Buckingham, Okrah, and Thomas 1989; Buckingham, Okrah, and Christian-Meier 1991).

Host-specificity, *Hydrellia pakistanae*

Host-specificity tests were accomplished for *H. pakistanae* at the Pakistan Station, Commonwealth Institute of Biological Control, Rawalpindi, between April 1971 and March 1976. The initial tests involved releasing a single laboratory-reared male and female on each test plant. Several replications of this experiment were carried out, and the eggs deposited were left for observation on

hatching, larval feeding, and development (Baloch, Sana-Ullah, and Shah 1971). Using *H. verticillata* as the control, 19 plant species were tested. Eggs were oviposited on almost all test plants, but the larvae mined the leaves of *Potamogeton indicus*, *P. perfoliatus*, and *P. crispus* only. Only 1.6, 4.0, and 39.0 percent of these larvae developed into adults, respectively (Baloch, Sana-Ullah, and Shah 1971). Further testing indicated that *H. pakistanae* would oviposit eggs on *Potamogeton* spp., but it could not be reared through a number of successive generations (Baloch, Sana-Ullah, and Shah 1971).

In 1985, *H. pakistanae* was imported from India to the United States and tested further as a potential biocontrol agent for hydrilla. Researchers at the quarantine facility of the Florida Biological Control Laboratory, Division of Plant Industry, Florida Department of Agriculture and Consumer Services, Gainesville, FL, performed the initial tests (Buckingham, Okrah, and Thomas 1989). Fifty-one test plants were used to determine the preferred oviposition substrate of *H. pakistane*. While females tended to oviposit more eggs on hydrilla, the oviposition substrate did not appear to be an important determinant for host specificity due to the fact that the females deposited eggs readily on all plant species (Buckingham, Okrah, and Thomas 1989). Further testing indicated that *P. crispus* could serve as a temporary host for *H. pakistanae*, but it could not become successfully established (Buckingham, Okrah, and Thomas 1989). From intensive host-range tests conducted, *H. pakistanae* was determined to be safe for release as an insect biocontrol agent for hydrilla (Buckingham, Okrah, and Thomas 1989) with the first U.S. release made in Florida in October 1987 (Buckingham 1988).

Host-specificity, *Hydrellia balciunasi*

Similar host-specificity testing was performed on *H. balciunasi* at the quarantine facility in Gainesville, FL. In no-choice larval tests, 14 plant species in four families related to hydrilla plus rice were tested (Buckingham et al. 1991). Of the 15 species tested, only *P. crispus* had larvae developing into adults (i.e., 1.0 percent). There was some insect mining observed, but no development on *P. pusillus* L. (Buckingham, Okrah, and Christian-Meier 1991). An additional group of plants not closely related to hydrilla was tested in multichoice larval developmental tests. From these additional 27 plant species, no adults emerged, and no plants where damaged. Once host-range tests proved that *H. balciunasi* was safe, it was released from quarantine in May 1989 (Buckingham, Okrah, and Christian-Meier 1991).

Description

Both insects are small flies in the order Diptera, family Ephydridae (Figure 1), measuring 1.5 to 3.0 mm in length. *Hydrellia balciunasi* are generally smaller than *H. pakistanae*, but are otherwise very similar (Buckingham, Okrah, and Christian-Meier 1991). The two species can be distinguished from each other by their external male genitalia, which includes the size and shape of the macrochaetae (Buckingham, Okrah, and

Christian-Meier 1991, U.S. Army Engineer Research and Development Center (ERDC) 1998). The macrochaetae in *H. balciunasi* are large and flattened at the distal end (the end farthest from where the macrochaetea attach to the body), and the macrochaetae in *H. pakistanae* are smaller and needle-shaped (Grodowitz et al. 1996). Adult females can be identified from each other by their cerci, which are the small paired hardened structures at the tip of the abdomen (Buckingham and Okrah 1993). *Hydrellia pakistanae* have cerci that are light brown and L-shaped while those of *H. balciunasi* are dark brown and triangular (Buckingham and Okrah 1993).

Figure 1. *Hydrellia* spp. Adults are small, measuring 1.5 to 3.0 mm in length

Biology

Female *Hydrellia* spp. oviposit football-shaped eggs on sprigs of emergent vegetation (Figure 2), which, after several days, hatch into larvae (Figure 3). On the average, *H. pakistanae* eggs are 0.54 mm long and 0.16 mm wide, while the eggs of *H. balciunasi* are 0.45 mm by 0.14 mm (Buckingham and Okrah 1993). For both *Hydrellia* spp., the larvae damage the hydrilla by mining 10 to 17 (average 12) leaves (Baloch, Sana-Ullah, and Shah 1971) during three larval instars. The larval stage lasts 9 to 16 days, depending on temperature (Baloch and Sana-Ullah 1973). Once the third instar stage is completed, larvae insert their respiratory spines into the hydrilla stem and pupate (Figure 4). Pupation lasts 6 to 11 days, again depending on temperature (Baloch and Sana-Ullah 1973), after which the adult fly emerges to begin the cycle again. The entire life cycle from egg to adult takes 17 to 31 days (Baloch, Sana-Ullah, and Shah 1971), depending on temperature and light.

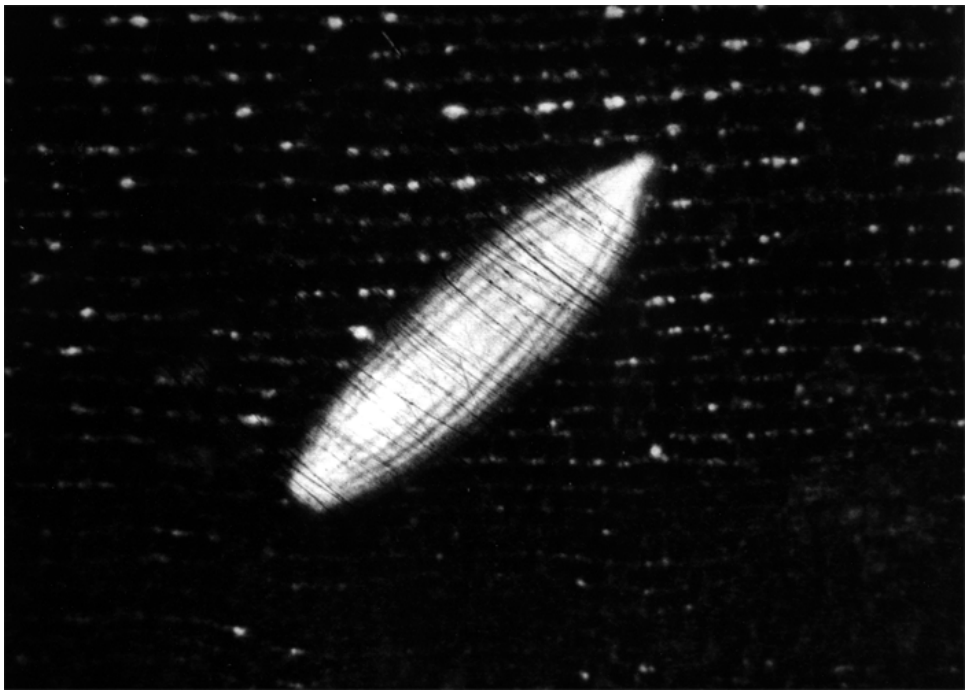

Figure 2. Egg of *Hydrellia* spp. The *H. pakistanae* eggs are 0.54 mm long and 0.16 mm wide, while the eggs of *H. balciunasi* are 0.45 mm by 0.14 mm

Figure 3. Larva of *Hydrellia* spp. Larvae damage hydrilla by mining 10 to 17 (average 12) leaves during three larval instars

Figure 4. Pupa of *Hydrellia* spp.

Greenhouse-rearing techniques

Rearing procedures used at the WES Aquatic and Wetlands Research and Development Support Facility were developed by personnel at the U.S. Department of Agriculture – Agriculture Research Service (USDA-ARS) Quarantine Facility, Gainesville, FL, and the USDA-ARS Aquatic Plant Management Facility, Ft. Lauderdale, FL. These procedures were modified for facilities available at WES, and are the same for both *Hydrellia* spp.

Insect maintenance

To maintain a *Hydrellia* spp. colony, 3-ℓ larval containers are filled with ca. 75 g of hydrilla and deionized water. Hydrilla is placed loosely into the containers leaving ample space between the plants. A 5-cm air space is left at the top of the container to give the adults a place to inhabit before being transferred to the oviposition chambers. Hydrilla sprigs inoculated with *Hydrellia* spp. eggs are placed just beneath the water surface on top of the plants in the container. The containers are placed in temperature-controlled water baths (Figure 5), maintained at ca. 23° C. The water baths are located in the greenhouse to provide maximum light levels which are important for pupal emergence and hydrilla photosynthesis.[1]

[1] Personal Communication and Unpublished Data, May 1999, Mike Grodowitz, Research Entomologist, ERDC, Vicksburg, MS.

Figure 5. Three-liter larval containers in temperature-controlled water bath

Upon completion of the pupal stage, adults emerge and are removed from the larval containers using an aspirator attached to a vacuum pump operating at 15 psig (Figure 6). Once collected, the adults are released into an oviposition chamber (Figure 7). Separate chambers are maintained for each generation of insect. Within the oviposition chamber, hydrilla sprigs are placed in petri dishes 150 by 15 mm in size filled with distilled water to provide an oviposition substrate for the females. Two types of food are provided for the adults and are

placed in smaller, 100- by 15-mm petri dishes, a yeast hydrolysate/sugar mixture (4 g yeast hydrolysate plus 7 g of sugar mixed in 10 ml of distilled water) (Buckingham et al. 1989), which provides a protein source, and a sugar/water mixture (7 g of sugar mixed in 10 ml of distilled water), which supplies carbohydrates (Figure 8).

Figure 6. When adults emerge they are removed from the larval containers using an aspirator attached to a vacuum pump

Once per week, the hydrilla sprigs are removed from the oviposition chamber and the eggs enumerated. During the field season, a portion of the eggs are placed in 150- by 15-mm petri dishes with additional hydrilla (Figure 9) and allowed to develop into second and third instar larvae. These are then used for field releases. The remaining eggs are placed in additional 3-ℓ containers filled with hydrilla and distilled water and are used for fly colony maintenance.

Rearing *Hydrellia* spp. is time-consuming, and each stage of the operation is labor-intensive. Egg enumeration should be accomplished at least once per week, and the eggs must be either placed into larval containers for colony maintenance or used for subsequent field releases. Adults should be removed from the larval containers daily for maximum insect production. Detailed records should be maintained of the number of eggs placed in each container and the number of adults removed for determination of survival. Also, records should be maintained on the total eggs and adults obtained from each generation of insect.

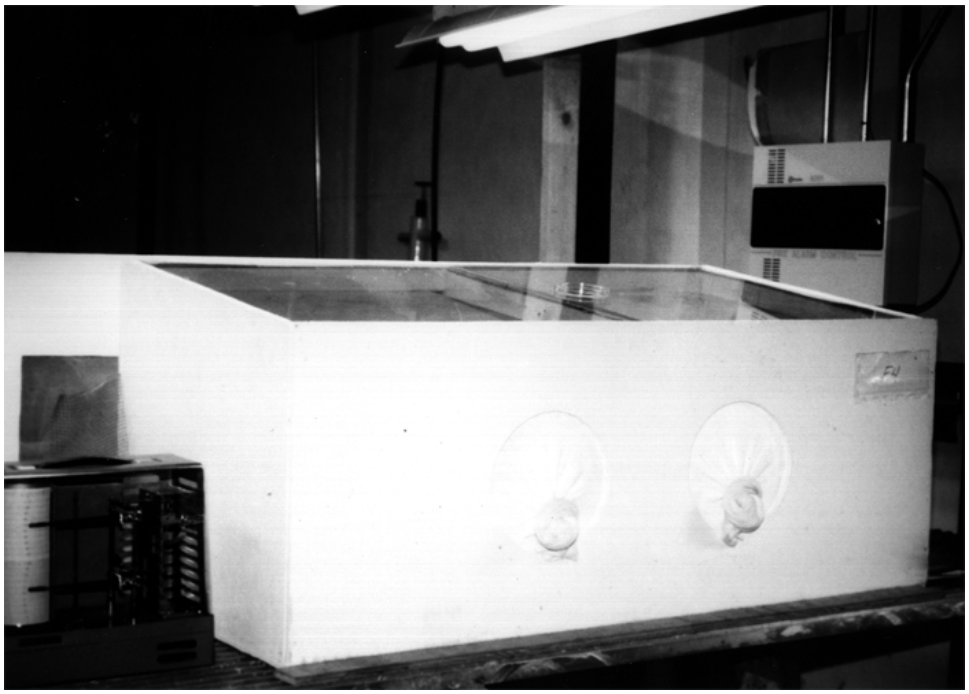

Figure 7. Once collected, *Hydrellia* spp. adults are released into an oviposition chamber for egg-laying

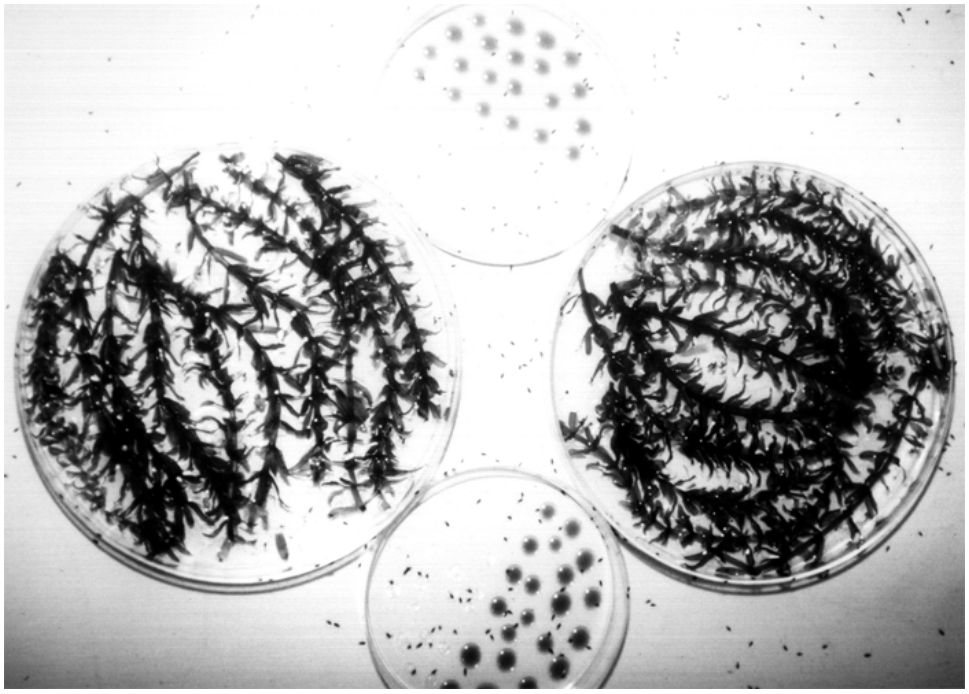

Figure 8. Within the oviposition chamber, hydrilla springs are placed in large petri dishes for egg-laying, and two types of food are provided in smaller petri dishes: a yeast hydrolysate/sugar mixture, and a sugar/water mixture

Figure 9. During the field season, a portion of the eggs are placed in petri dishes with additional hydrilla and allowed to develop into second and third instar larvae for field releases

Several problems may arise when rearing *Hydrellia* spp. The hydrilla herbivore *Paraponyx diminutalis*, order Lepidoptera, can devastate hydrilla, especially in an enclosed structure such as a larval-rearing container. The moth's feeding damage reduces the food source for the flies and makes it difficult for life cycle completion. An insect parasite, the wasp, *Trichopria columbiana*, order Hymenoptera, family Diapriidae, parasitizes *Hydrellia* spp. pupae and can greatly reduce the productivity of the colony. The wasp crawls in the water down the hydrilla stem to where the pupae are located, lays an egg inside the pupa and killing the pupa; instead of a fly emerging, a wasp emerges from the pupa. Another limiting factor associated with rearing *Hydrellia* spp. is the constant need for large amounts of high nutritive value hydrilla.

Plant maintenance

Large quantities of *H. verticillata* are necessary to sustain the *Hydrellia* spp. colonies. Since the WES rearing facilities are not located near field hydrilla populations, plant cultures must be maintained under greenhouse conditions year-round. To ensure healthy cultures, the hydrilla must be replanted periodically. Hydrilla reculturing is a labor-intensive operation involving several important steps.

Pond sediment is used as the growing medium for hydrilla. After collection, the sediment is transported to the greenhouse where it is homogenized in a mixer (Stone Construction Equipment, Inc., Honeoye, NY) to ensure uniformity. The

mixer can accommodate ca. 132 ℓ of sediment. Amendments added while the sediment is mixing include 0.5 g ammonium chloride and 1.75 g of Scotts Esmigran® (Marysville, OH) per liter of sediment. Ammonium chloride provides nitrogen for the plants, and Esmigran is a micronutrient source. After the mixing process, the nutrients need a few days to blend before the sediment is ready for use. Rubbermaid® dishpans (Wooster, OH) 29.2 cm by 34.3 cm by 13.3 cm are filled with ca. 6.5 ℓ of amended sediment and allowed to sit for 24 hr. This procedure allows water to accumulate on the sediment surface for removal. A layer of washed 8-16 silica sand (MMR Enterprises, Seagoville, TX) ca. 2 cm high is then placed on top of the sediment to minimize contact between the sediment and the culture solution (Smart and Barko 1985). Thirty apical sprigs of hydrilla (ca. 15 cm in length) are planted in each container. The containers are then submersed into 1,100-ℓ tanks filled with distilled water and a general-purpose nutrient solution (Smart and Barko 1985). Remcor® water circulators (Glendale Heights, IL) plumbed to the reculturing tanks maintain a temperature of ca. 23^{o} C. When hydrilla reaches the surface of the tanks, it can be utilized for fly rearing.

Hydrilla tank maintenance during the growing process includes keeping algae and insect infestations to a minimum. Algae can become significant in the tanks, especially during the early growth period, and can be minimized by using diatom filters (Marineland Aquarium Products, Moorpark, CA). For occasional infestations of *Paraponyx diminutalis*, the plants can be treated with DiPel® 2X Wettable Powder (North Chicago, IL) at a rate of 0.25 lb/100 gal of water (113.5 g/378.4 ℓ). DiPel® is a biological insecticide with the active ingredient *Bacillus thuringiensis*, subsp. *kurstaki,* and is highly selective for use against the larvae of lepidopterous insects. The use of DiPel can make the culture water cloudy, in which case it can be filtered with a diatom filter. In some situations, the tank may need to be drained and refilled with fresh distilled water and culture solution. Green Light 50-percent Malathion® (San Antonio, TX) is another insecticide that can also be used at a rate of 15 ml/1,100 ℓ culture tank. Plants treated with Malathion must be rinsed thoroughly before use as any residue would be detrimental to the insects.

Hydrellia spp. Generations

At WES, rearing of the Indian strain of *H. pakistanae* began in June 1990 with insects received from the USDA-ARS Aquatic Plant Management Facility, Ft. Lauderdale, FL. This colony was reared in the WES greenhouse facility until May 1992 and was allowed to develop through 24 generations. Detailed rearing data were collected and analyzed from this colony and will be discussed below.

Four different colonies of *H. balciunasi* were reared at the WES facility. The first colony originated from insects received from the USDA-ARS Aquatic Plant Management Facility, Ft. Lauderdale, FL. These insects had been in cultivation for several generations before WES received them; therefore, they were referred to as the Greenhouse (GH) *H. balciunasi* colony. They were received in April 1991 and reared through nine generations. The second colony originated from the Quarantine Laboratory Facility in Gainesville, FL. These insects had been

collected in Australia, shipped to the quarantine facility, and allowed to complete one life cycle before being sent to WES. Because this colony had only been in cultivation for one generation, they were referred to as the Field-1 (Fld-1) *H. balciunasi* colony. These insects were received in August 1991 and developed through eight generations. In March 1992 WES received a second "field" shipment from Gainesville, FL. This colony was called the Field-2 (Fld-2) *H. balciunasi* colony, and it was allowed to develop through 18 generations. A fourth colony was started from insects collected at Sheldon Reservoir, TX (*H. balciunasi* – Sheldon). It was started in August 1993 and developed through 10 generations until December 1994.

3 Results

Table 1 contains data collected from four *Hydrellia* spp. colonies reared at WES from June 1990 to June 1993: *H. pakistanae*, India strain; *H. balciunasi*-Greenhouse (GH) colony; *H. balciunasi*-Field 1 (Fld-1) colony; and *H. balciunasi*-Field 2 (Fld-2) colony. Compilation data were not collected from the *H. balciunasi*-Sheldon colony.

Table 1
Numerical Rearing Results

Hydrellia spp.	Total Eggs per Colony	Eggs Used in Larval Containers for Colony Maintenance	Eggs Used for Field Releases	Eggs Used in Colony Maintenance %	Eggs Used for Field Releases %	Adults Collected From Larval Containers	Adult Emergences From Larval Containers %	No. Days/ Generation	Avg. No. Eggs Produced Per Day
H. pakistanae India strain P – F24 6/90 - 2/92	969,605	236,911	732,694	24	76	122,549	52	49	829
H. balciunasi GH colony P – F9 4/91 - 2/92	92,041	33,641	58,400	37	63	17,452	52	39	230
H. balciunasi Fld-1 colony P – F8 8/91 - 3/92	132,955	43,219	89,736	33	67	22,106	51	52	304
H. balciunasi Fld-2 colony F2 – F18 3/92 - 6/93	314,472	141,423	173,049	45	55	96,348	68	59	346

Note: Numerical rearing results, 1990-1993. Information derived from data collected while rearing various colonies of *Hydrellia* spp. at the WES facility.

Records were kept of the total eggs per colony. A portion of the eggs was placed in larval containers for colony maintenance, and remaining eggs were used for field releases. Percent adult emergence was calculated by dividing the adults collected from larval containers with the eggs used in the larval containers for the colony. Adults were removed from larval containers for a total of 39 days. The 39-day time period was used as a cutoff point based on the fact that the entire generation should be completed by then. During the 39-day time period, females inhabiting the 5-cm air space in the larval containers could have oviposited some eggs before their removal by the vacuum pump. If left long enough, these insects

would then mature, and the next generation would start emerging inside the larval container, which would confound the generations. Even after the larval containers were discarded for a particular generation, adults would frequently still be alive in the oviposition chamber. Hence, larval containers were taken down after 39 days, but the length of the generation was based on the number of days the adults continued to inhabit the oviposition chamber.

Colony Productivity, *Hydrellia pakistanae*

The combined data for *H. pakistanae* from 1990 to 1992 was examined for number of individuals per generation (F3 – F24); percent eggs shipped per generation; eggs per female per generation; days to first adult emergence per season, month, year, and generation; days to 50-percent adult emergence per season, month, year, and generation; percent adult emergence per season, month, year, and generation; and the number of adults that emerged between day 10 and day 39.

A general trend for the *H. pakistanae* colony was a decrease in eggs per generation with an increase in total individuals shipped; i.e., the more insects shipped for field releases, the fewer adults that were available to produce eggs for the colony (Figure 10). A high percentage of eggs (over 90 percent) were removed from several generations (e.g., F11 - F13) and shipped for field releases (Figure 11). Eggs per female demonstrated a peak during the F13 generation with minimal values occurring during the F2 – F6, F15, and F20 generations (Figure 12). Low numbers of eggs per female could have been due to a several reasons, including poor quality hydrilla, high infestations of *Paraponyx diminutalis*, or high infestations of *Trichopria columbiana.*

Hydrilla leaf quality impacts the successful development of *Hydrellia* spp. (Wheeler and Center 1996) with longer developmental times associated with lower nitrogen and harder leaf tissues. Lower number of eggs per female is related to a number of hydrilla nutritional factors, including nitrogen, phosphorus, and magnesium, with a higher number of eggs associated with higher quantities of these nutritional components (Grodowitz, Freedman, and McFarland, unpublished data). In addition, weight of the females, an indicator of overall health, was higher when elevated quantities of both nitrogen and phosphorous were present in the plant tissue (Grodowitz, Freedman, and McFarland, unpublished data).

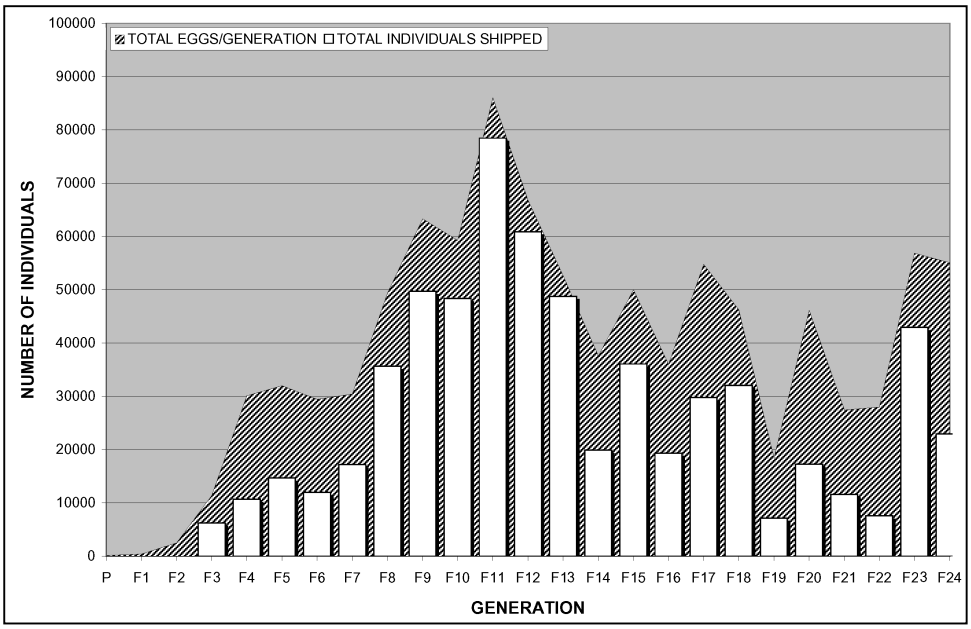

Figure 10. *Hydrellia pakistanae*, number of individuals/generation

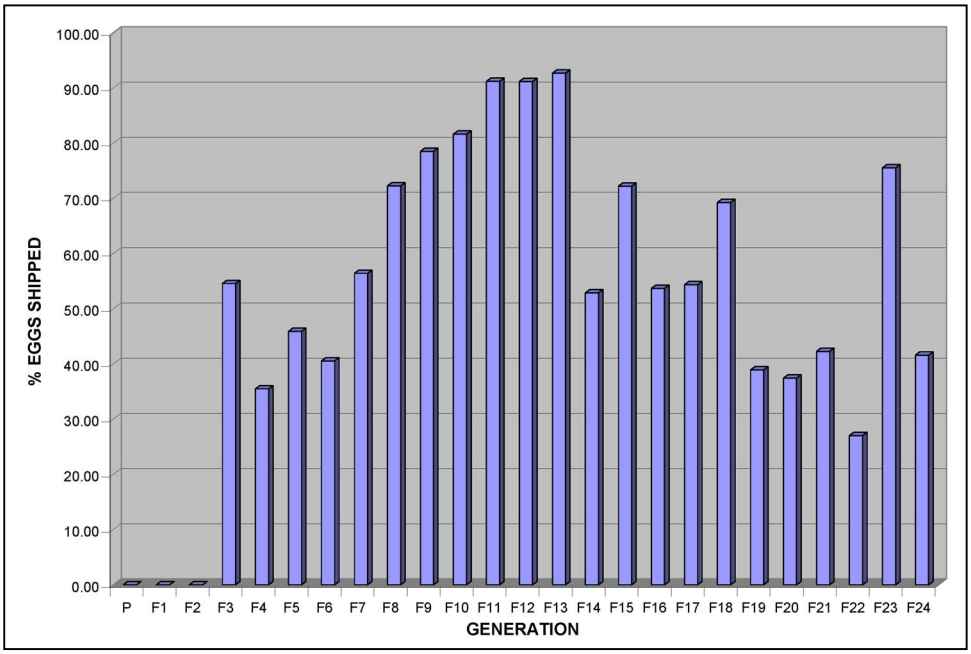

Figure 11. *Hydrellia pakistanae*, percent eggs shipped/generation

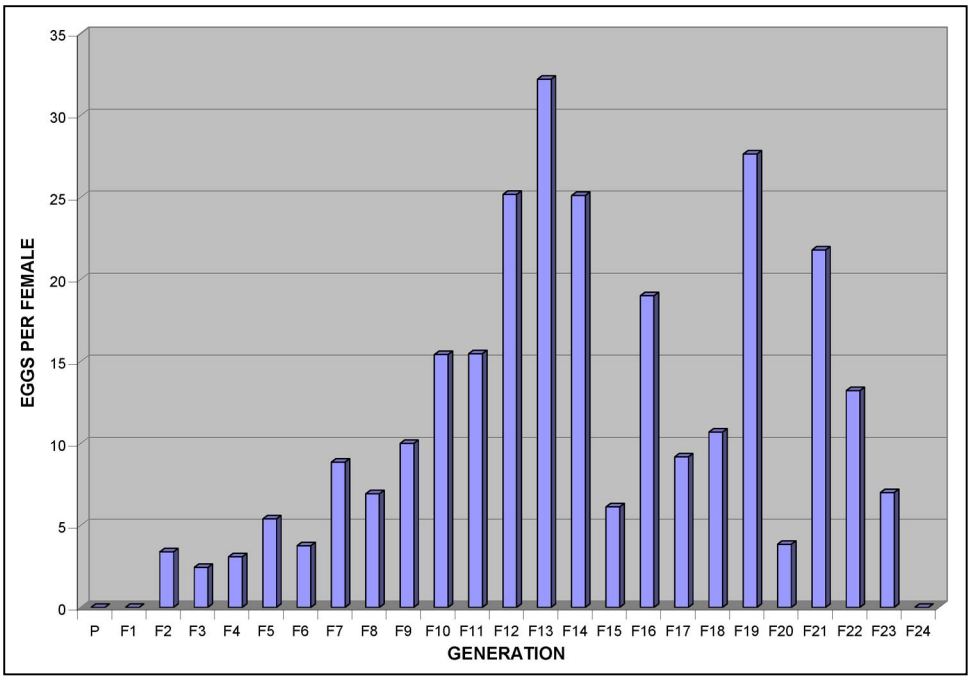

Figure 12. *Hydrellia pakistanae*, eggs per female/generation

Examining the impact of seasonality, the data was grouped into two broad categories based on growing season with the "nongrowing" season considered to be November through April, when days are shorter and there is a general assumption that hydrilla quality declines, and the "growing" season considered to be from May through October. Seasonality apparently impacted several important factors of the colony. For example, both indicators of developmental time (i.e., days to first adult emergence (Figure 13 ($p < 0.05$, df = 1, 871, F = 105.0202, p = 0.000000)) and days to 50-percent adult emergence (Figure 14 ($p < 0.05$, df = 1, 906, F = 49.67595, p = 0.000000))) were significantly lower for the growing season. In addition, while no significant differences were noted for percent adult emergence (Figure 15), there was a trend for higher survival during the growing season.

Other seasonal effects were observed in monthly and yearly emergence data. For all 12 months, the average number of days to first adult emergence was 20.83 (Figure 16), and 24 days to 50 percent of the adults had emerged (Figure 17). Percent adult emergence per month (Figure 18) produced significant differences ($p < 0.05$, df = 11,896, F = 4.316571, p = 0.000003). Yearly effects were also noted, and from 1990 to 1992, the average number of days to first adult emergence was 20.83 (Figure 19), and 24.14 to 50 percent of the adults had emerged (Figure 20). Significant differences ($p < 0.05000$, df = 2,905, F = 17.50586, p = 0.000000) were observed for percent adult emergence per year, which averaged 49.31 for all 3 years (Figure 21).

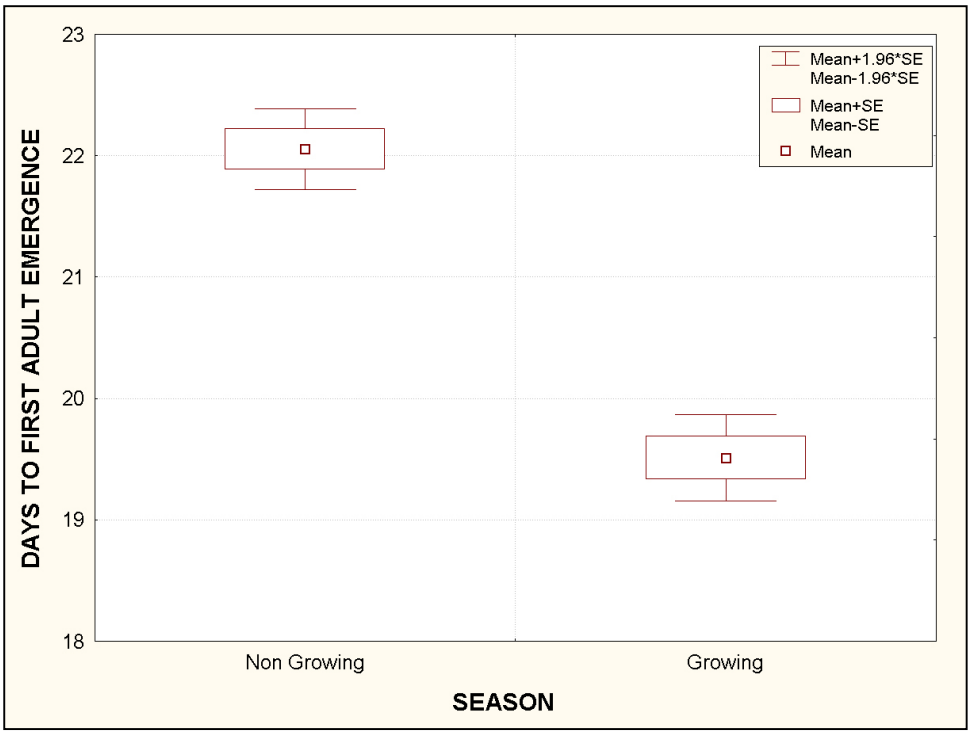

Figure 13. *Hydrellia pakistanae*, days to first adult emergence/season

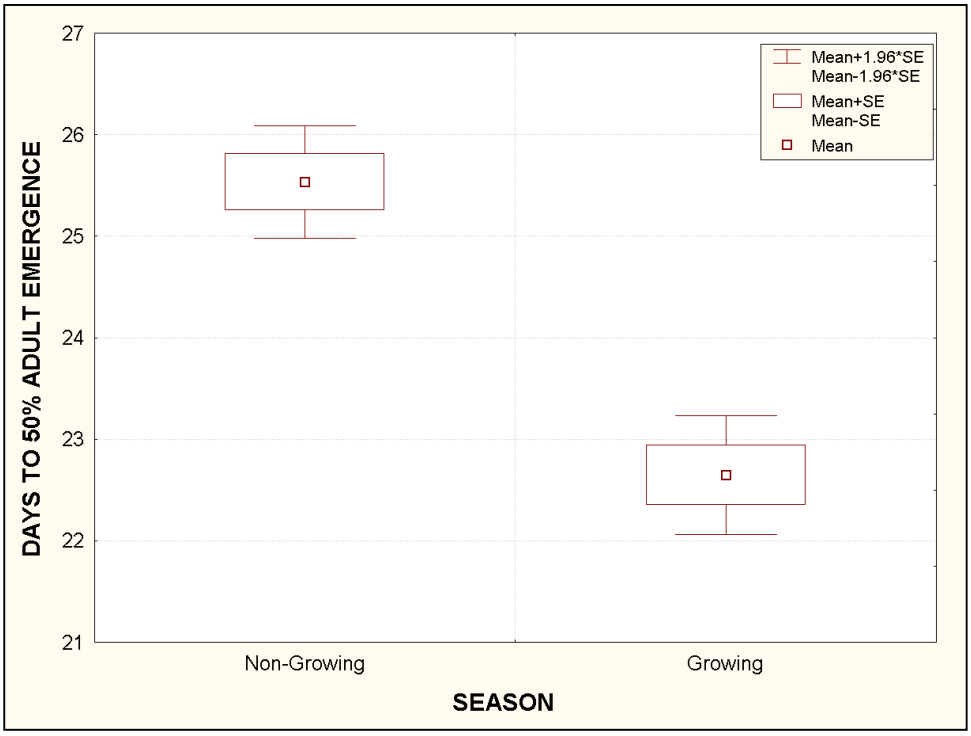

Figure 14. *Hydrellia pakistanae*, days to 50-percent adult emergence/season

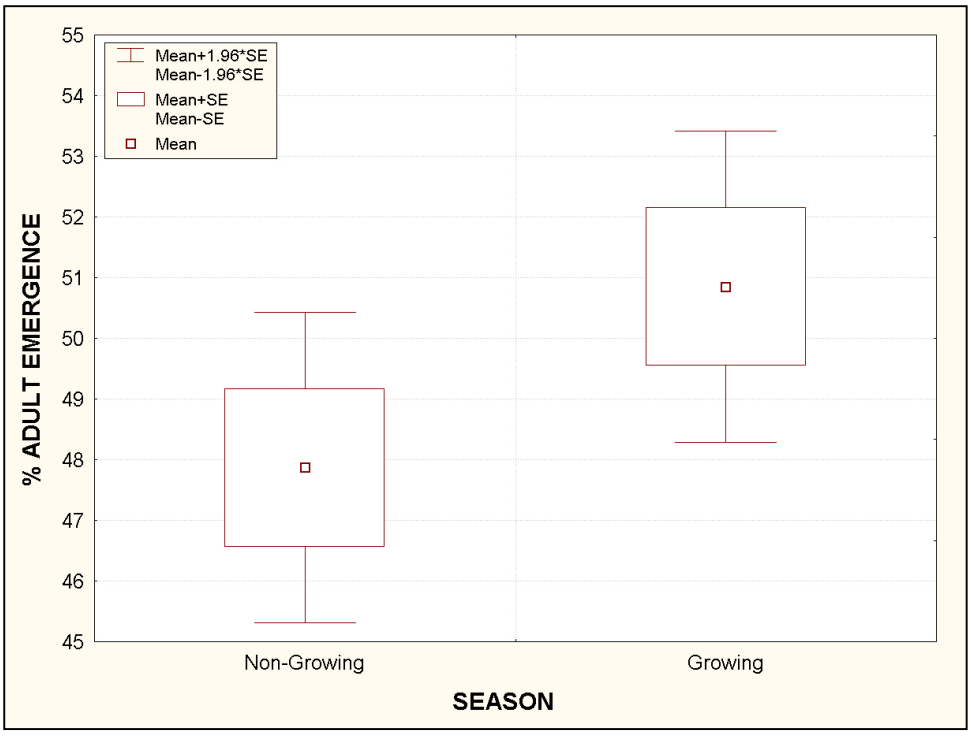

Figure 15. *Hydrellia pakistanae*, percent adult emergence/season

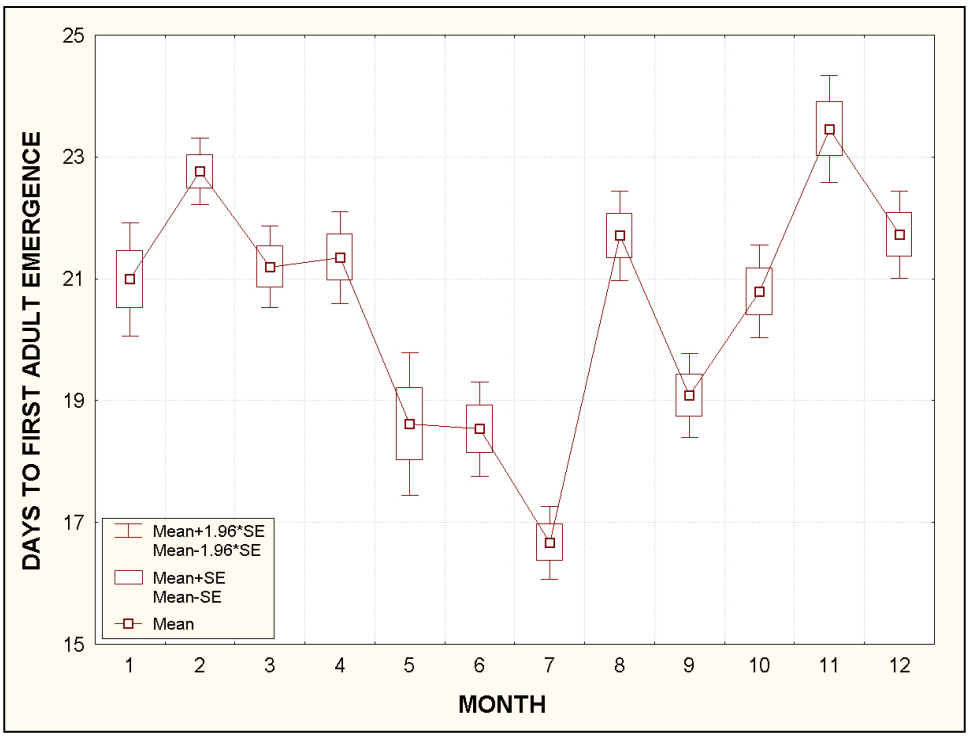

Figure 16. *Hydrellia pakistanae*, days to first adult emergence/month

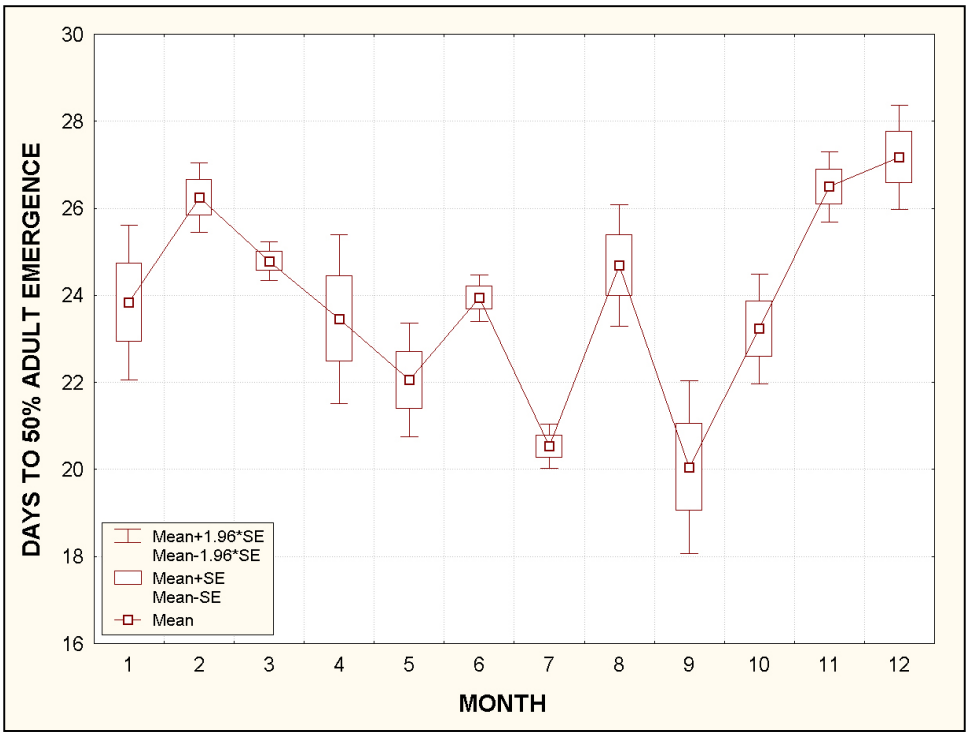

Figure 17. *Hydrellia pakistanae*, days to 50-percent adult emergence/month

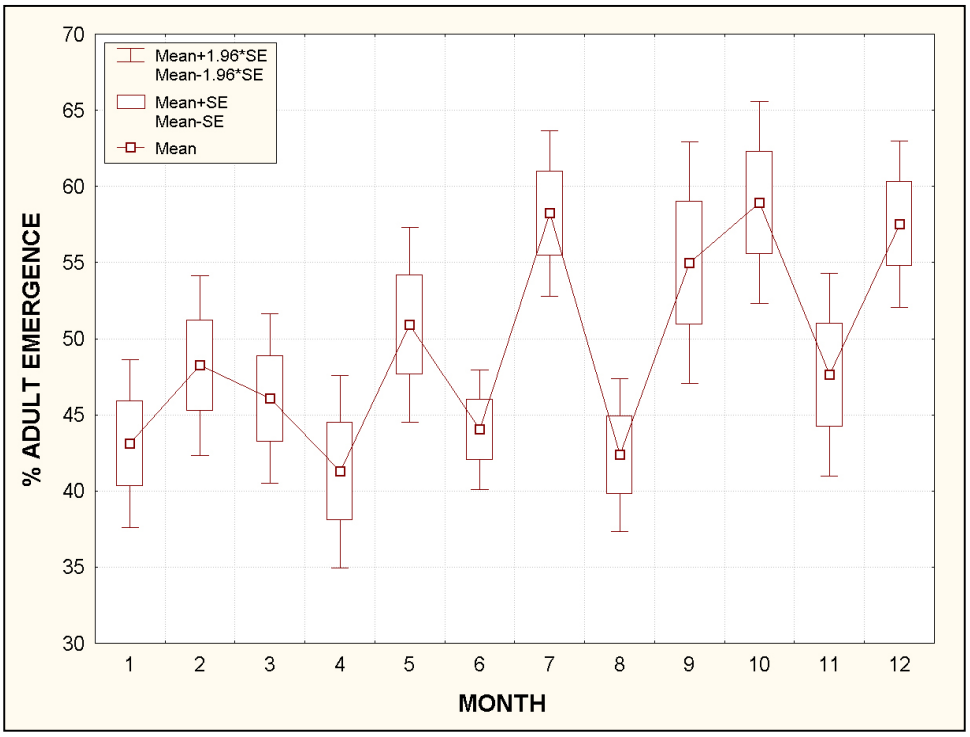

Figure 18. *Hydrellia pakistanae*, percent adult emergence/month

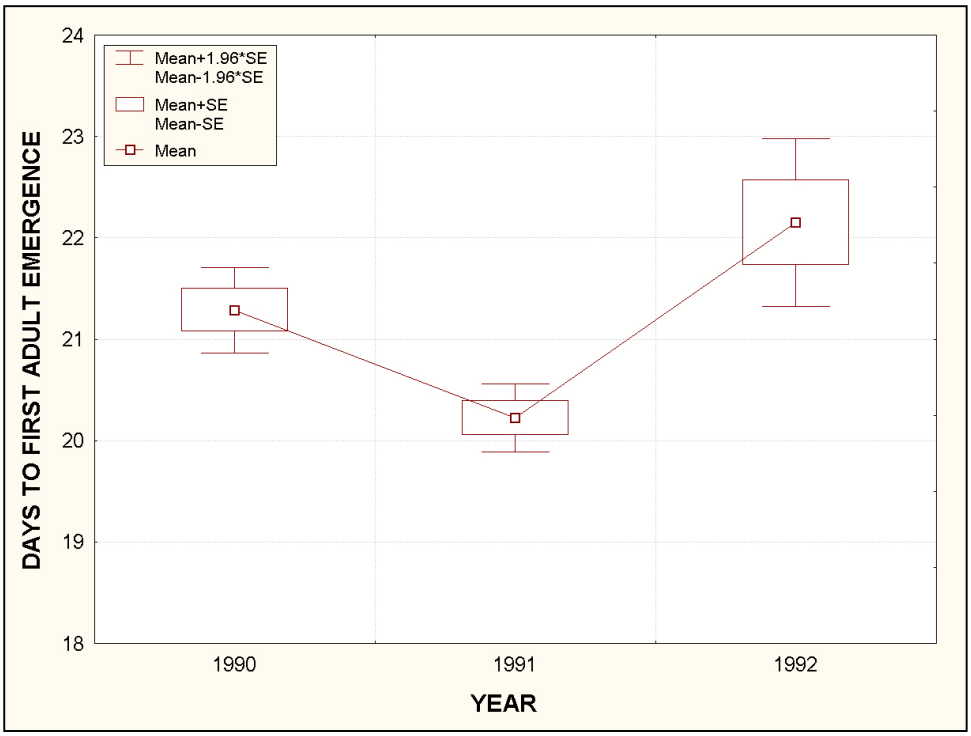

Figure 19. *Hydrellia pakistanae*, days to first adult emergence/year

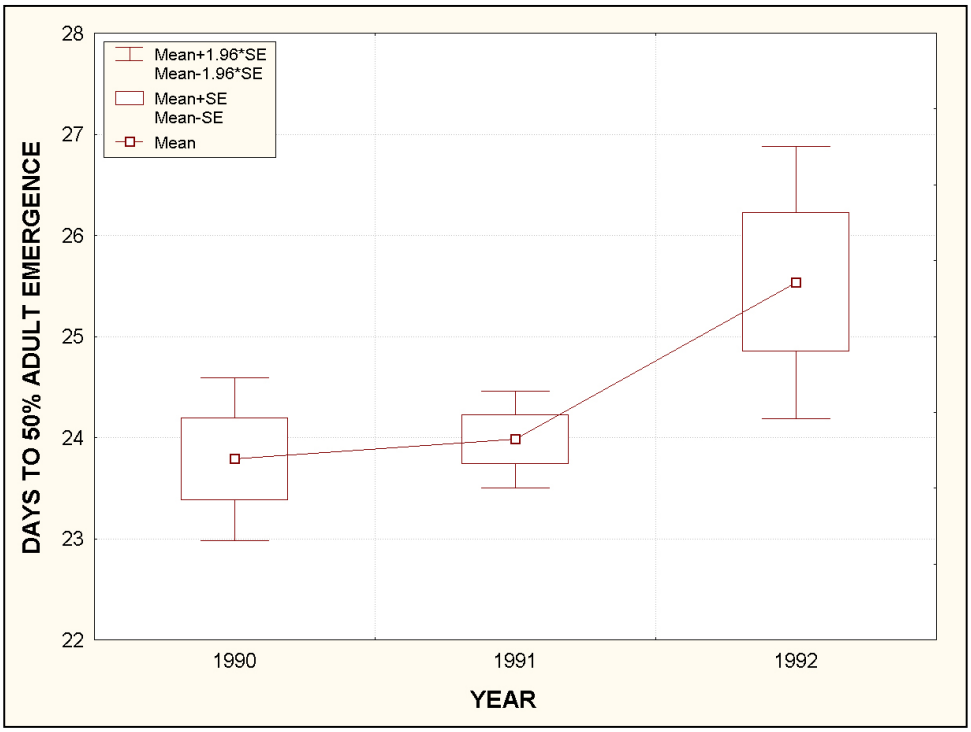

Figure 20. *Hydrellia pakistanae*, days to 50-percent adult emergence/year

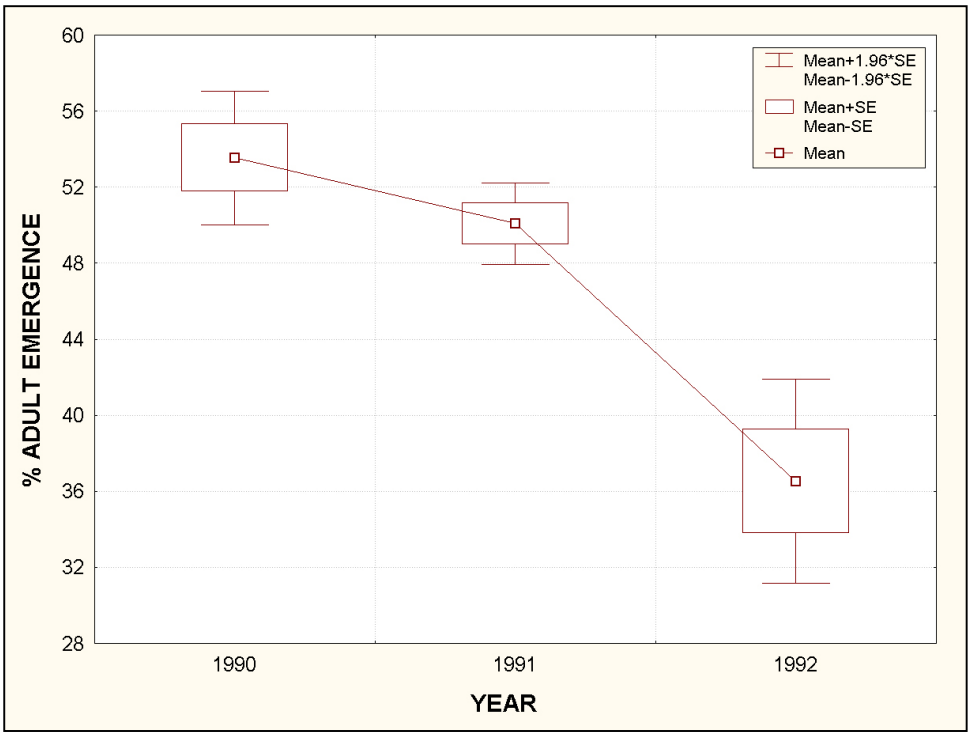

Figure 21. *Hydrellia pakistanae*, percent adult emergence/year

Combined *H. pakistanae* data from 1990 to 1992 indicated that the majority of adults emerged between day 21 and day 28 (Figure 22). Days to first emergence per generation (Figure 23) consistently fell between 17 and 25 days with an average of 21 days. Generally, 50 percent of the adults had emerged by day 24, as illustrated in Figure 24. Significant differences ($p < 0.05000$, $df = 20,887$, $F = 8.676$, and $p = 0.000000$) were observed for percent emergence of the *H. pakistanae* generations, which averaged 49.31 for all 3 years (Figure 25).

Generally, a reduction in *H. pakistane* quality was not seen based on the measured parameters, which included egg hatch, survival from egg to adult, emergence rate, and fecundity. The number of insects produced was consistent from 1990 to 1992 and seemed to be influenced more by seasonal factors, such as day length and temperature, the presence of the insect herbivore *P. diminutalis,* the pupal parasite *T. columbiana,* and the nutritive quality of the hydrilla.

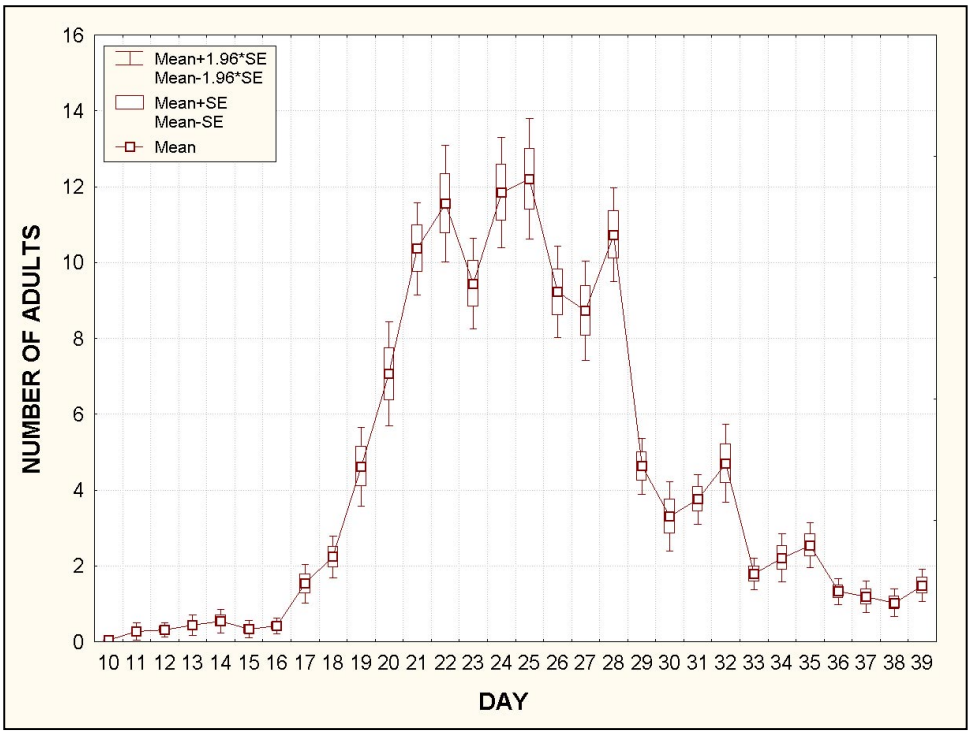

Figure 22. *Hydrellia pakistanae*, number of adults/day

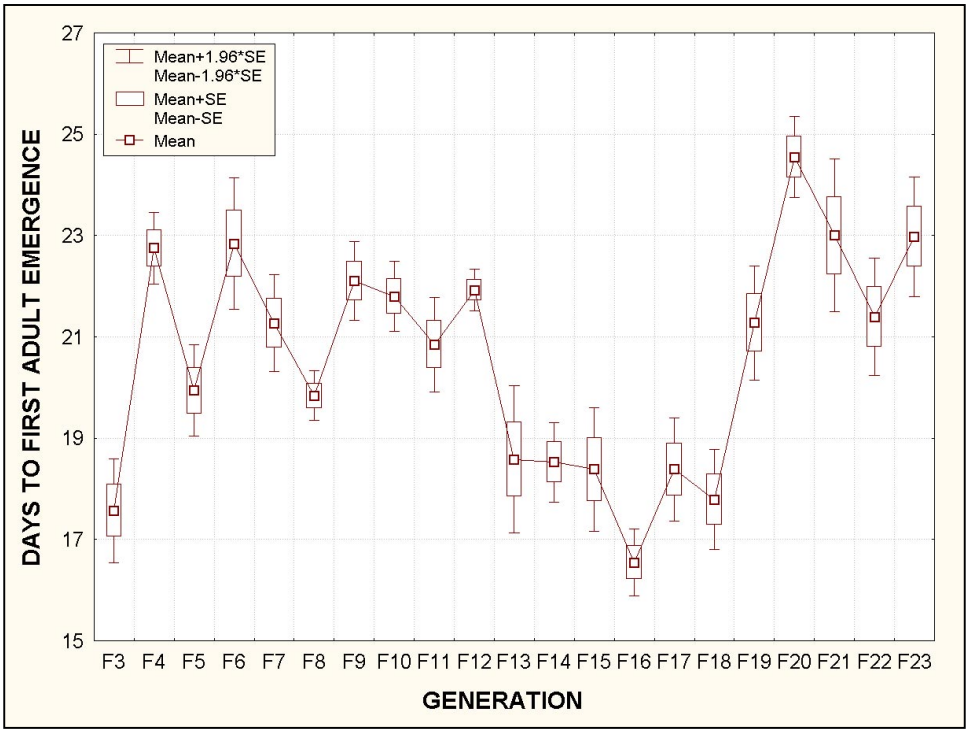

Figure 23. *Hydrellia pakistanae*, days to first adult emergence/generation

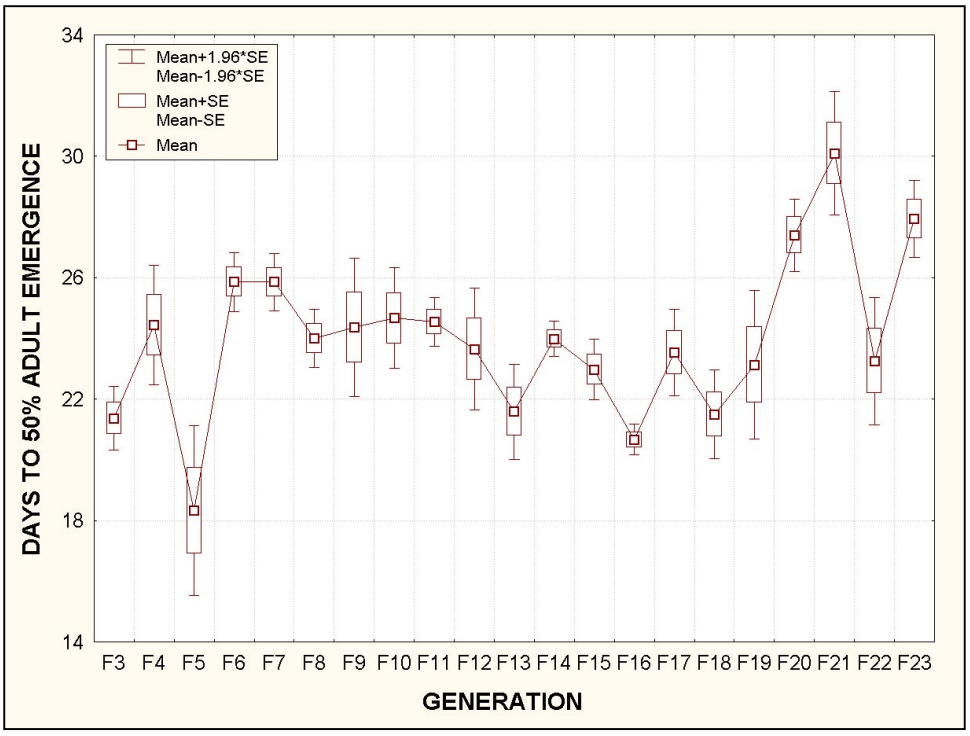

Figure 24. *Hydrellia pakistanae*, days to 50-percent adult emergence/generation

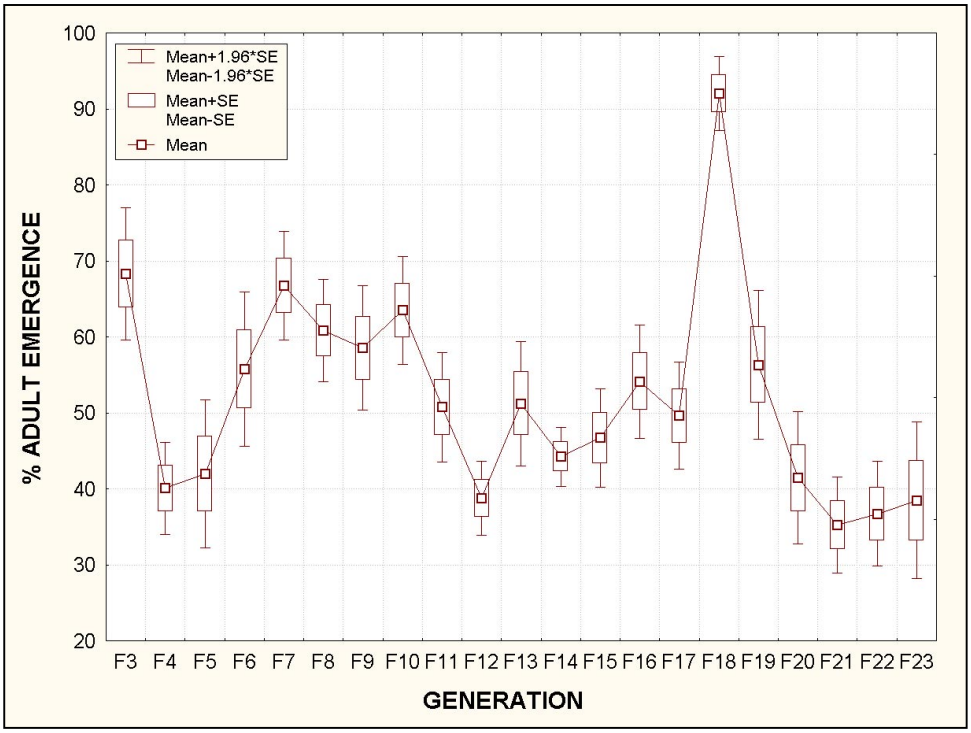

Figure 25. *Hydrellia pakistanae*, percent adult emergence/generation

Field Releases and Establishments, *H. pakistanae* and *H. balciunasi*

In the United States to make a field release in any state of an introduced biocontrol insect, several additional steps are required. First, permission must be obtained from each state's plant regulatory official. Permit release applications (PPQ Form 526) are filed with the USDA Animal and Plant Health Inspection Service (APHIS) for the introduction of biocontrol insects into states where field releases will be made. Accompanying each field shipment of insects, a USDA Biological Shipment Record (Form AD-943) must be filled out. These forms provide information and records for each party involved in the field release.

Table 2
***Hydrellia Pakistanae,* India Strain, Releases from WES Insects, 1990 to 1992**

Site	Years Released	Total
Lake Seminole, FL	1990 – 1992	>200,000 immatures
Guntersville, AL	1990 – 1991	>36,000 immatures
Ft. Lauderdale, FL	1990 – 1991	300,000 immatures
Lake Bouef, LA	1991	>85,000 immatures
Muscle Shoals, AL	1991	>4,700 immatures
Total Released	1990 – 1992	> 625,700 immatures & adults

Between 1993 and 1997 many more releases of WES-reared *H. pakistanae* were made (Table 3), and several sites are established. Sites with established populations that the WES colony contributed insects to included: Ft. Lauderdale, FL; Lake Seminole, FL/GA; Choke Canyon and Coleto Creek, TX; and Guntersville, AL.

Table 3
***Hydrellia pakistanae,* India Strain, Releases from WES Insects, 1993 to 1997**

Site	Years Released	Total
Choke Canyon, TX	1993 – 1995	>100,000 immatures & adults
Coleto Creek, TX	1994 – 1995	> 30,000 immatures & adults
Sheldon Reservoir, TX	1995	> 50,000 immatures
Lake Cypress Springs, TX	1996 – 1997	> 10,000 immatures
Total Released	1993 – 1997	> 190,000 immatures & adults

Table 4
***Hydrellia balciunasi* Releases from WES Insects, 1991 to 1994**

Site	Years Released	Total
Sheldon Reservoir, TX	1991 - 1992	> 74,000 immatures
Ft. Lauderdale, FL	1991 - 1994	> 59,000 immatures & adults
Huntsville State Park – Lake Raven, TX	1992 - 1994	> 71,000 immatures
Coleto Creek Reservoir, TX	1992 - 1993	> 33,000 immatures
Gainesville, FL	1993	2,000 immatures
Choke Canyon, TX	1993 - 1994	> 28,000 immatures
Total Released	1991 - 1994	>260,000 immatures & adults

Hydrellia balciunasi is established at Sheldon Reservoir, TX, from insects produced by the WES colony. Insects are also established at Lake Cypress Springs and Lewisville, TX, although *H. balciunasi* has never been released at either location (Grodowitz et al. 1999). These insects have probably moved to these two locales through natural dispersion.

The rearing program at WES has resulted in successful establishment of many field populations. Of the nine areas where WES-reared *H. pakistanae* were released, five are established. Flies are established at one of the six *H. balciunasi* release areas and also at two areas where the insects were never released. The quantitative data from field sites indicate that releasing flies seems to result in greater establishment success with higher populations. This points to the fact that rearing is needed to get fly numbers high at a given site.

Natural dispersion of *Hydrellia* spp. may work, but it seems to take a longer time period to occur. Also, since the flies are weak flyers, expansion of their range is slow in areas where hydrilla infestations are not contiguous. Figure 26 demonstrates combined data from both introduced *Hydrellia* spp. of immatures per kilogram of plant material, and percent leaf damage at release sites versus nonrelease sites. A large difference is observed between immatures per kilogram at release sites (650 immatures/kg) as compared to nonrelease sites (75 immatures/kg), and percent leaf damage at release sites (8.0 percent) as compared to nonrelease sites (0.5 percent). The number of flies and associated hydrilla leaf damage are higher at sites where the flies were actually released.

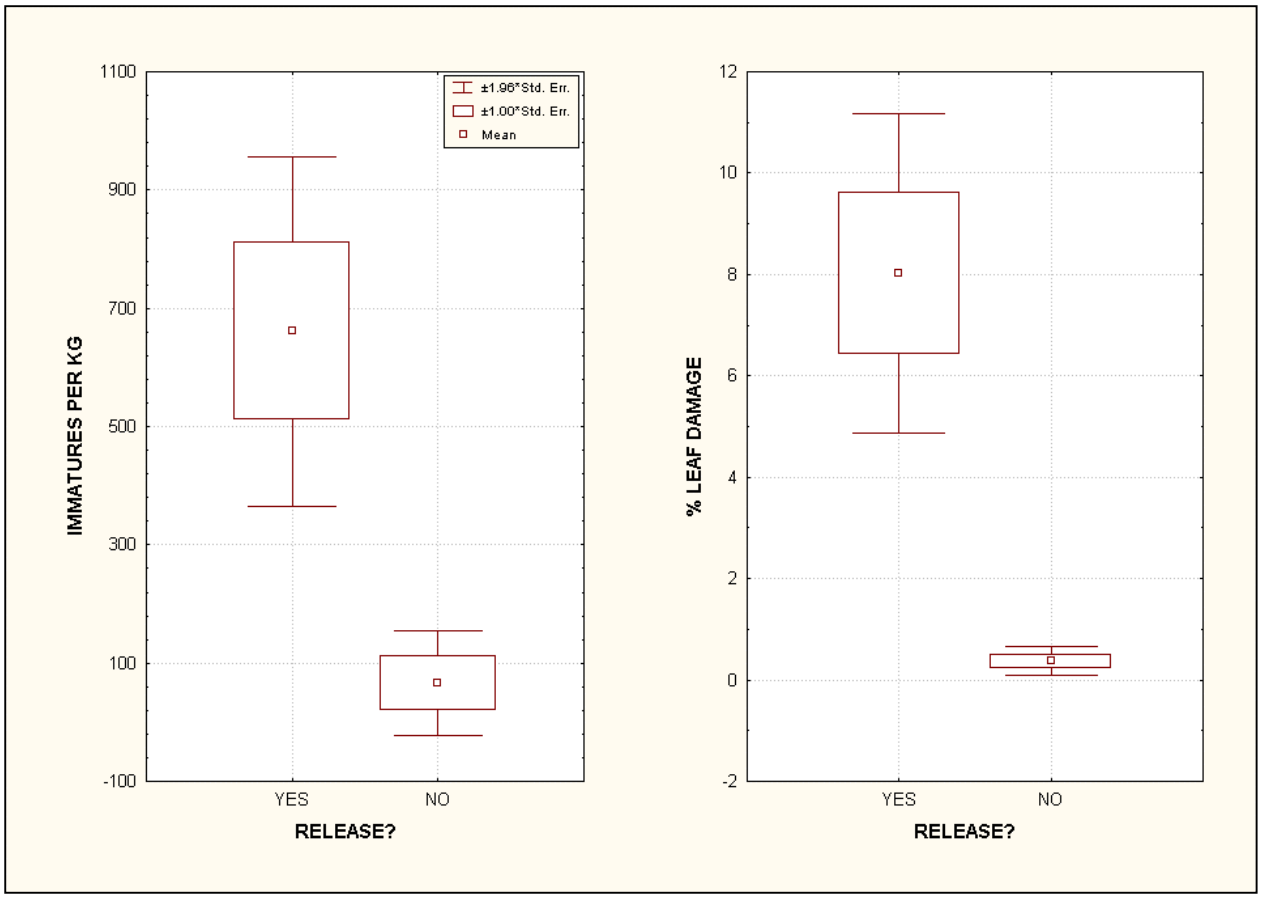

Figure 26. *Hydrellia pakistanae* and *H. balciunasi*, immatures per kilogram at release sites and nonrelease sites, and the percent leaf damage at release sites and nonrelease sites

A successful biocontrol insect-rearing program is one that produces large quantities of uniform insects for field release and establishment. For *Hydrellia* spp. to be a success, large quantities of leaf-mining flies need to be released and become established in the field. From results seen at Coleto Creek, TX, Choke Canyon, TX, Lake Cypress Springs, TX, Lewisville, TX, Lake Seminole, FL, and Guntersville, AL, the WES *Hydrellia*-rearing program has been very successful. Populations of insects are impacting plants and are providing a control and management tool for hydrilla (Grodowitz et al. 1999).

Current rearing methods used at WES were not a deterrent to the quality of *Hydrellia* spp. produced; however, the cost and extensive maintenance were a drawback. Mass-rearing is being viewed as a solution to the high cost of generating *Hydrellia* spp., and future techniques will allow for the production of high quality insects at a fraction of the current cost.

Other Mass-Rearing Techniques

Hydrellia spp. are in a group of insects that lend themselves to being adequately mass-reared (Leppla and Ashley 1989). Data have shown that releasing large numbers of insects results in greater establishment success. Therefore, producing large quantities of leaf-mining flies is essential for having an effective insect biocontrol program.

One experimental mass-rearing technique evaluated in the greenhouse facility required the use of large, open polypropelene trays. The trays, ca. 4 by 8 by 10 in. deep, were divided in half by a screen attached to a polyvinyl chloride frame. Water circulators were plumbed to the trays to maintain cooler temperatures in summer. Large amounts of hydrilla were placed in both halves of the tray with enough deionized water to cover the plants. Hydrilla on one side of the tray was inoculated with *H. pakistanae* with the idea that, once established, the insects would move to the other side containing healthy hydrilla.

Much time and effort was made to implement this tray-type mass-rearing technique; however, it never proved successful. Failure could have been due to fly dispersal in the greenhouse. Also, a layer of filamentous algae developed and eventually covered the entire tray. Attempts to remove the algae resulted in removing eggs deposited on plant surfaces. Other problems included high temperatures in the trays that affected insect development. Although the trays were plumbed to water circulators, the outtake hoses would frequently become clogged, hindering water flow. Large amounts of hydrilla were required for this mass-rearing method, which at times was difficult to maintain. Fly populations increased several times, but then would suddenly decline. After many attempts, this plan was abandoned.

Another highly successful mass-rearing technique utilized small ponds at an abandoned fish hatchery at the Tennessee Valley Authority reservation, Muscle Shoals, AL (Grodowitz and Snoddy 1995). *Hydrellia pakistanae* individuals were released into a series of small 0.0405-ha ponds for use as a rearing facility for introducing the flies at sites on Lake Guntersville. The hydrilla in two thirds of a single 0.0405-ha pond was harvested, and the resulting plant material was moved to Lake Seminole, GA. This single harvest yielded over 1.5 million flies and resulted in fly establishment throughout Lake Seminole and more recently widespread impact including significantly lower tuber production (unpublished data, Dr. Grodowitz). Actual production costs are unknown, but it was significantly lower than the $0.50 per fly costs associated with the greenhouse-rearing techniques. Similar hydrilla harvests from ponds located at the Lewisville Aquatic Ecology Research Facility, Lewisville, TX, yielded over 300,000 individuals from only minimal collections. Cost estimates for collecting pond insects were calculated to be ca. $0.023 per fly, a major reduction from greenhouse-reared flies.

Because of the success with using ponds, the Environmental Laboratory is in the process of constructing a pond facility at WES for use in mass-rearing *Hydrellia* spp. The facility will contain two conditioning ponds 40 by 80 by 8 ft and ten rearing ponds 20 by 20 by 6 ft (Figure 27). The conditioning ponds will

be used to store and condition water to be used in the 10 study ponds. Conditioning ponds will consist of concrete sides and bottoms, and the first of these will be used to treat water as it comes directly from Brown's Lake located at WES. After the water is conditioned in the second pond, it will be used to fill the 10 study ponds. The 10 study ponds will also have concrete sides and bottoms and will have a standing pipe drainage system for regulating water depth, a water intake from the second conditioning pond, and a gravity flow drainage system. The pond facility will allow for the production of large numbers of *Hydrellia* spp. for field releases as well as to conduct nutritional studies of hydrilla and associated effects on the flies.

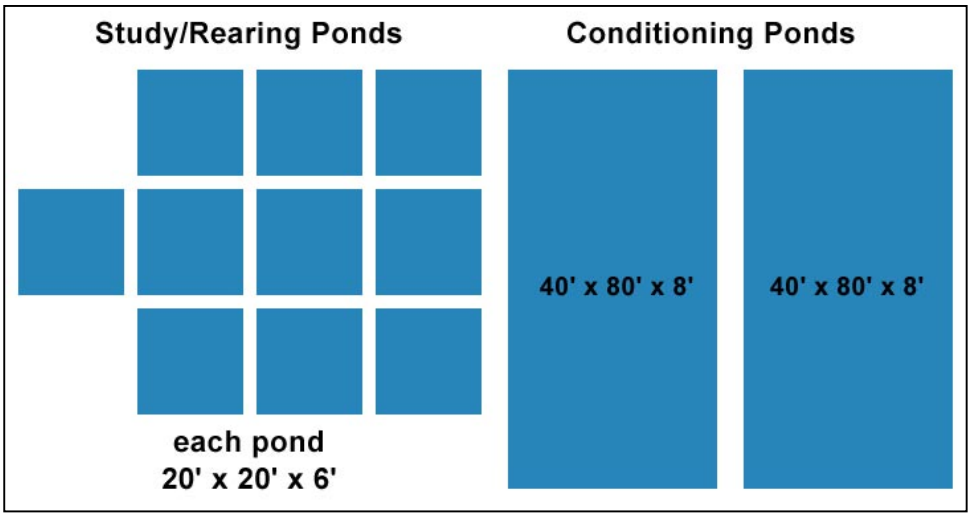

Figure 27. WES pond rearing schematic

Many benefits could arise from mass-rearing *Hydrellia* spp. Rearing greenhouse/laboratory insects is labor-intensive and expensive. Based on estimates calculated from labor and equipment costs at WES, the price per insect is ca. $0.50. The high cost of greenhouse-reared insects preempts their use by many state and federal agencies who have limited funding for aquatic plant management. If the flies were more affordable, agencies could purchase them and implement more active biocontrol programs. Insects would also be available for more extensive and detailed research studies.

4 Summary

For those involved in biocontrol research at both the applied and basic levels, this report is designed to elucidate the rearing process of *H. pakistanae* and *H. balciunasi*, biocontrol agents of *H. verticillata*. Proper equipment, frequent maintenance, and a constant supply of high nutritive hydrilla are essential in developing a successful rearing program. Mass-rearing techniques need to be perfected to decrease production costs while producing large numbers of uniform insects. Once these techniques have been implemented, extensive field releases can be made of *Hydrellia* spp. to be part of an integrated strategy to manage and control hydrilla.

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14. ABSTRACT

Two insect biocontrol agents of *Hydrilla verticillata*, *Hydrellia pakistanae* Deonier, from southern India, Pakistan, and northern China, and *H. balciunasi* Bock, from Australia, have been reared at the U.S. Army Engineer Research and Development Center, Waterways Experiment Station (WES), Aquatic and Wetlands Research and Development Support Facility, Vicksburg, MS, since 1990. Currently, *H. pakistanae* is still in production; however, the *H. balciunasi* colony was disbanded in 1994.

One main concern with greenhouse/laboratory-rearing procedures used at WES was whether they impacted insect quality. Various quality parameters measured included sex ratio, weight, female egg-laying, and developmental time. Quality data collected from 1990 to 1992 remained fairly uniform, indicating that the quality parameters did not seem to be affected by the rearing procedures. Production costs, however, became a major issue. Based on labor and materials, the cost of producing a single *Hydrellia* spp. was estimated to be $0.50 per insect. Large numbers of insects are needed for successful field establishment, and current fly production prices made it prohibitive for wide-scale production. Future plans for alternate rearing techniques include construction of a series of outdoor ponds. These ponds would allow for the creation of a large number of high-quality insects and decrease production costs associated with rearing *Hydrellia* spp.

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