Miscellaneous Paper A-97-5
September 1997

US Army Corps of Engineers
Waterways Experiment Station

Aquatic Plant Control Research Program

Release and Establishment of *Hydrellia balciunasi* (Diptera: Ephydridae) for the Biological Control of the Submersed Aquatic Plant *Hydrilla verticillata* (Hydrocharitaceae) in the United States

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Prepared for Headquarters, U.S. Army Corps of Engineers

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Final report

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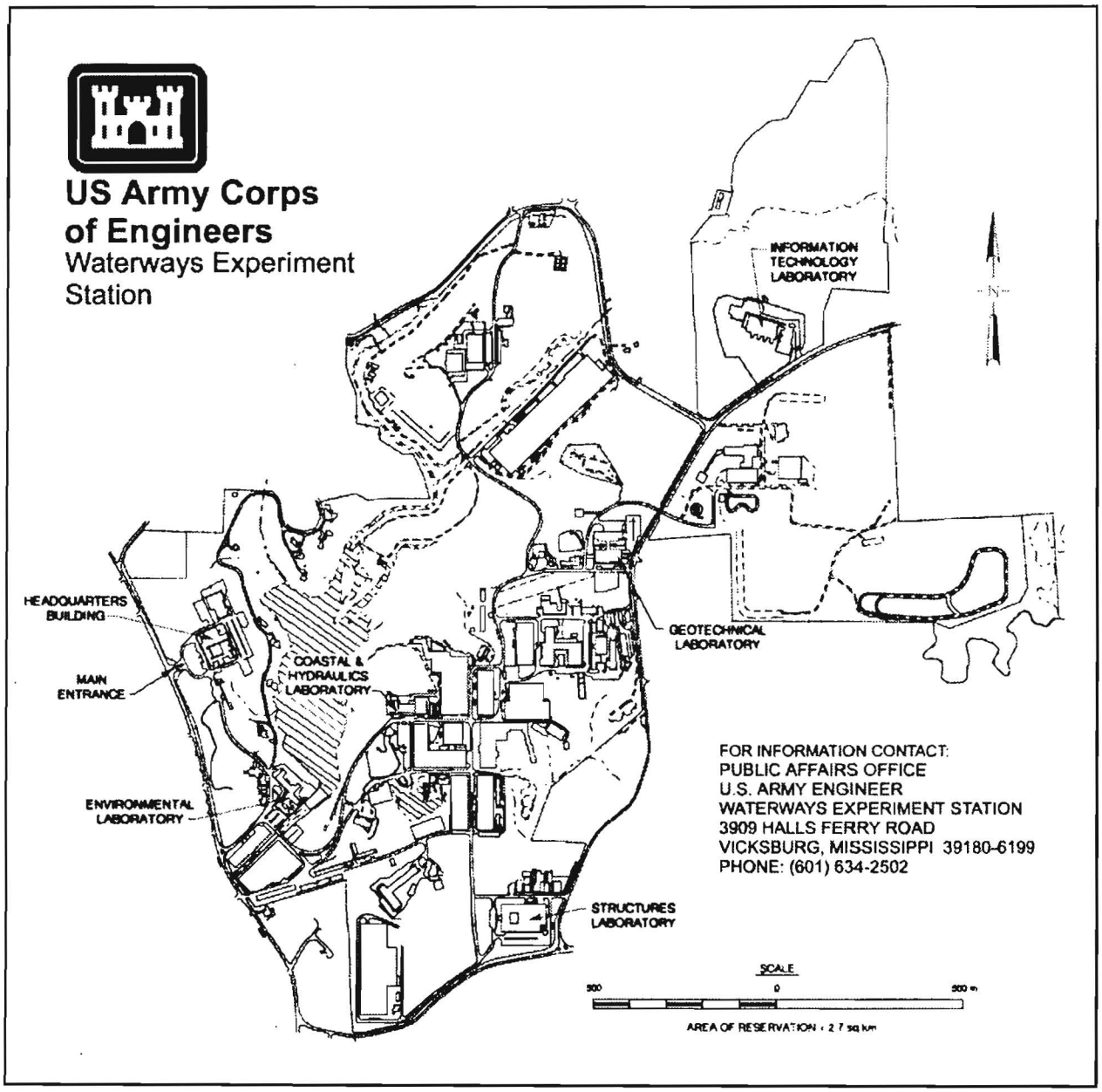
Prepared for U.S. Army Corps of Engineers
Washington, DC 20314-1000



Waterways Experiment Station Cataloging-in-Publication Data

Release and establishment of *Hydrellia balciunasi* (Diptera: Ephydridae) for the biological control of the submersed aquatic plant *Hydrilla verticillata* (Hydrocharitaceae) in the United States / by Michael J. Grodowitz ... [et al.] ; prepared for U.S. Army Corps of Engineers.

14 p. : ill. ; 28 cm. -- (Miscellaneous paper ; A-97-5)

Includes bibliographic references.

1. Hydrellia -- Biological control. 2. Insects. 3. Hydrilla -- Management -- United States. 4. Aquatic plants -- Management -- United States. I. Grodowitz, Michael Jay. II. United States. Army. Corps of Engineers. III. U.S. Army Engineer Waterways Experiment Station. IV. Aquatic Plant Control Research Program (U.S. Army Engineer Waterways Experiment Station) V. Series: Miscellaneous paper (U.S. Army Engineer Waterways Experiment Station) ; A-97-5.

TA7 W34m no.A-97-5

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 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 Center for Aquatic Plant Research and Technology (CAPRT), Dr. John W. Barko, Director. Mr. Robert C. Gunkel was Assistant Director for the CAPRT. Program Monitor during this study was Ms. Denise White, HQUSACE. Additional funding and/or logistical support were provided by the U.S. Army Engineer District, Galveston, and the Texas Department of Parks and Wildlife.

The Principal Investigator for this study was Dr. Alfred F. Cofrancesco, Aquatic Ecology Branch (AEB), Ecological Research Division (ERD), EL, WES. The report was prepared by Dr. Michael J. Grodowitz, Dr. Cofrancesco, and Ms. Jan E. Freedman, AEB; and Dr. Ted D. Center, U.S. Department of Agriculture, Agricultural Research Service. Technical reviews were made by Dr. Judy Shearer and Mr. Brent Harrel, AEB. Results of this study were first published as an article in *Biological Control* (Vol 9, 1977), reprints of which were provided for use in preparation of this report.

The investigation was performed under the general supervision of Dr. Edwin A. Theriot, Chief, AEB; Dr. Conrad J. Kirby, Chief, ERD; and Dr. John Harrison, Director, EL.

At the time of publication of this report, Director of WES was Dr. Robert W. Whalin.

This report should be cited as follows:

> Grodowitz, M. J., Center, T. D., Cofrancesco, A. F., and Freedman, J. E. (1997). "Release and establishment of *Hydrellia balciunasi* (Diptera: Ephydridae) for the biological control of the submersed aquatic plant *Hydrilla verticillata* (Hydrocharitaceae) in the United States," Miscellaneous Paper A-97-5, U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS.

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BIOLOGICAL CONTROL 9, 15–23 (1997)
ARTICLE NO. BC970513

Release and Establishment of *Hydrellia balciunasi* (Diptera: Ephydridae) for the Biological Control of the Submersed Aquatic Plant *Hydrilla verticillata* (Hydrocharitaceae) in the United States

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Received May 24, 1996; accepted January 30, 1997

The Australian leaf-mining fly, *Hydrellia balciunasi*, was first released as a biological control agent for the management of the submersed aquatic plant, *Hydrilla verticillata* in August 1989 in southern Florida. Since then, 284,684 individuals have been released at seven Florida and four Texas sites. Despite this effort, populations have persisted at just two Texas sites: Sheldon Reservoir near Houston and Lake Raven in Huntsville State Park. *H. balciunasi* levels have remained relatively low at these sites, averaging, at best, less than 1000 immatures/kg wet plant weight. All other collections were substantially lower. Reasons for poor establishment are unknown but probably relate to a complex of factors, including competition with another introduced leaf-mining species, parasitism by native wasps, poor host quality, genetic differences between Australian and United States hydrilla resulting in mismatched physiological strains of agent and host plant, and possibly inbreeding depression in laboratory colonies used for releases. © 1997 Academic Press

KEY WORDS: **aquatic weed; Insecta; aquatic plant; insect releases; classical biological control; submerged weed.**

Hydrilla, *Hydrilla verticillata* (L. f.) Royle (Hydrocharitaceae), is a submersed aquatic plant that causes numerous problems for water resource management in the United States. Since its introduction into Florida through the aquarium trade in the early 1950s (Schmitz *et al.,* 1991), it has spread extensively throughout North America, even into temperate areas (Center *et al.,* 1989). Problems associated with extensive growth of hydrilla include hinderance of navigation, recreational use of waterbodies, flood control, and water distribution systems, and a loss of biodiversity in aquatic ecosystems (Haller, 1978).

The only currently practiced methods of hydrilla management involve the use of herbicides (Schmitz *et al.,* 1991) and to a lesser extent a herbivorous fish (Sutton and Vandiver, 1986). However, these are costly, short-term solutions. This prompted federal and state agencies to initiate searches for host-specific insect biological control agents.

Surveys in India and Pakistan during the 1970s yielded several potential agents (Baloch and Sana-Ullah, 1974; Baloch *et al.,* 1980; Sankaran and Rao, 1972). Two of these, *Bagous affinis* Hustache and *Hydrellia pakistanae* Deonier (Buckingham *et al.,* 1989; Bennett and Buckingham, 1991), were later released in the United States but only the latter has established (Center *et al.,* 1997). Other surveys in Australia, conducted during the 1980s, yielded several additional agents (Center *et al.,* 1989). One of these was the leaf-mining ephydrid fly, *Hydrellia balciunasi* Bock, which is similar in morphology and habit to *H. pakistanae* (Deonier, 1978; Bock, 1990; Buckingham *et al.,* 1991; Balciunas and Burrows, 1996). *H. balciunasi* was brought into quarantine in 1988 and released in Florida in 1989 and in Texas in 1991.

H. balciunasi is a small gnat-like fly in the family Ephydridae. Buckingham *et al.* (1991) have described the laboratory biology of this species. In summary, the adults rest on various species of emergent or floating vegetation where eggs are laid singly at or near the water surface. Females oviposit an average of 36 eggs which hatch after 3 days (at 27°C). The emerging larvae descend into the water to locate suitable feeding material. They then burrow through the leaf epidermis of a hydrilla plant and feed on internal leaf tissues. A single larvae can feed and damage 9 to 12 leaves during the three larval stadia. The larvae pupate within the cuticle of the third instar. The cuticle is hardened into a protective covering known as the puparium. The cigar-shaped puparia are often located in the leaf axils and resemble axillary leaf buds. The pupal stage lasts from

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6 to 15 days. The adult ecloses from the puparium enveloped in a bubble of air. It then floats upwards within the bubble, to be released on the surface as the bubble bursts. This paper describes attempts to establish *H. balciunasi* populations in the United States.

MATERIALS AND METHODS

Insects. *H. balciunasi* was imported from several locations in Australia including North Pine Dam, near Petrie in southern Queensland, and the Alice River and the Bohle River, near Townsville in northern Queensland. These shipments provided the insects for quarantine testing as well as stock for initial colonies used in later field releases. To minimize loss of viability in laboratory-reared individuals destined for release, colonies were purged after about 20 generations and replaced with new insects derived from United States field-collected or overseas insect stock. Colonies were maintained at the U.S. Department of Agriculture, Agriculture Research Service (ARS), Aquatic Plant Control Research Unit in Fort Lauderdale, Florida, and the Waterways Experiment Station (WES), U.S. Army Corps of Engineers, Vicksburg, Mississippi.

The primary method used for rearing *H. balciunasi* was that of Buckingham *et al.* (1989) for *H. pakistanae,* modified slightly as described in Center *et al.* (1997). Briefly, for larval rearing, egg-laden hydrilla sprigs were placed in 2- to 4-liter jars containing about 100 g of fresh hydrilla. Adults were transferred to oviposition chambers consisting of wooden boxes with clear plastic or glass covers. Fly handling in the oviposition chamber was accomplished through entry portals covered by cloth sleeves. Adults were fed solutions of sucrose and yeast hydrolysate either separately or in a mixture (4 g yeast hydrolysate, 7 g sucrose, and 10 ml of water). Partially submerged hydrilla sprigs held within 9- or 14-cm-diameter petri dishes provided an oviposition substrate. After oviposition, the egg-laden hydrilla sprigs were transferred to new containers to perpetuate the rearing process.

Releases. The strategy employed to establish *H. balciunasi* was based on experience with *H. pakistanae* and involved continued releases at each site using relatively large numbers of individuals for each release. Egg-laden hydrilla sprigs were transferred from oviposition chambers to 14-cm-diameter petri dishes and held for 10 to 14 days to obtain second and third instars. Previous experience with *H. pakistanae* suggested that lower mortality is associated with releasing older immatures (Center *et al.,* 1997). We placed the plant material harboring the larvae into shallow, floating cages (described by Center *et al.,* 1997) at the release site. The cages only detained the insects, thereby slowing dispersal and increasing the likelihood that emerging adults would encounter one another. The cages also clearly demarcated release locations, thereby facilitating recovery of successive generations of flies and verification of establishment. Also, the shallow cages provided the adults with protection from some predators, such as odonates, by limiting their maneuverability. The cages also protected the plant material from larger herbivores.

Assessment of establishment. Sites were monitored for the presence of *H. balciunasi* by a variety of qualitative and quantitative sampling methods. Because of the possibility of the presence of the closely related species, *H. pakistanae,* which may have been released as a colony contaminant, adults were carefully identified.

Quantitative sampling methods for each site included the assessment of numbers of immatures and the percentage of leaves damaged. This was accomplished by randomly collecting from throughout the site 25 to 50 hydrilla sprigs, each measuring 10 to 20 cm in length, from the upper portion of the plant beds (i.e., canopy). Each sprig was examined microscopically and numbers of larvae, pupae, and damaged leaves were determined. Hydrilla sprigs were then individually weighed to express the insect counts as number per kilogram of fresh plant weight.

Unfortunately, *Hydrellia* immatures cannot be identified to species. Since quantitative sampling methods only assessed number of unidentified immatures, qualitative methods for adult collection were used to ascertain the species present. Qualitative sampling included hand collection of adults from the water surface, partial rearing of adults from field-collected hydrilla, and adult capture using "soap dish" techniques. The latter method employed 21-cm^2 (diameter) plastic containers surrounded by a 50-cm^2 (diameter) styrofoam platform for floatation. The containers were filled with a dilute solution of dishwashing detergent to reduce surface tension. Adult *H. balciunasi* trapped in the soap solution were removed after 3 to 5 h and preserved in 70% ethanol for later identification. Qualitative techniques were also used to determine the presence or the absence of immatures in those cases where quantitative sampling was not warranted. These techniques included simple examination of stem pieces and extraction of immatures and adults using Berlese funnels. We considered *H. balciunasi* to be established when we consistently recovered adults over a period of about 4 months (i.e., about 5 generations) by either qualitative or quantitative means.

Voucher specimens were preserved and verified by Dr. R. L. Deonier (University of Florida, Department of Entomology and Nematology, Gainesville, Florida). Specimens have been deposited in the Florida State Collection of Arthropods, Gainesville, Florida.

Statistical analysis. Differences between means using ANOVA as well as basic descriptive statistics were

calculated using the statistical program "Statistica," developed by the StatSoft company (StatSoft, Inc., 1994). Means are portrayed graphically as whisker plots to show ±1 and ±2 standard errors of the mean. The ±1 standard error of the mean provides a visual estimate of variation, while ±2 standard errors of the mean estimate the 95% confidence limits and thereby elucidate statistical differences between pairs of means.

RESULTS AND DISCUSSION

H. balciunasi has been released in two states at 11 separate locations (Table 1). In Florida, nearly 50,000 individuals were released at 7 sites and in Texas close to 240,000 individuals have been released at 4 different locations. The first release of *H. balciunasi* was made on 1 August 1989 at the Orangebrook Golf Course in southern Florida. Since then, over 100 releases have been made in the United States. The highest number released at a single site was at Lake Raven, where nearly 76,000 individuals were released in 15 separate introductions. This is followed by the Sheldon Reservoir site, where about 75,000 individuals were released. The largest releases in Florida were at Wilson Cypress Stand site, where close to 20,000 immatures were released in 3 introductions.

Currently, established populations of *H. balciunasi* in the United States persist at one, and possibly at two, Texas locations: Sheldon Reservoir near Houston and Lake Raven in Huntsville State Park. Establishment at the Sheldon Reservoir site was documented during September 1992. At that time adults, larvae, and associated damage were easily observed throughout the hydrilla infestation using qualitative sampling methods. This widespread evidence of establishment prompted us to begin quantitative monitoring.

Beginning in October 1992, the number of immatures and associated levels of leaf damage were determined approximately monthly at the Sheldon Reservoir site (Fig. 1). More immatures and leaf damage were noted during the first 2 years of sampling than during later years. The highest number of immatures were noted during this period, with about 1000 individuals/kg occurring on October 1992 and July 1993. This corresponded to about 5% leaf damage, far below the damage threshold (≥40%) needed to induce a decline (Greg Wheeler, USDA, ARS, Aquatic Plant Control Lab., Ft. Lauderdale, Florida, personal communication; Grodowitz, unpublished data). The number of immatures and the level of leaf damage on other sampling dates were substantially lower. In general, fewer immatures were noted during the 1994 and 1995 growing seasons. This is summarized in Fig. 2, where averages for each growing season (i.e., June through October) were determined. Immature populations declined drastically from 1992 through 1993 and 1994. Based on the consistent collection of low numbers of both immatures and adults over four growing seasons, we conclude that *H. balciunasi* is established at Sheldon Reservoir. We expect the population to persist, but it is unlikely to attain levels sufficient to control hydrilla.

Limited numbers of *H. pakistanae* have also been collected at the Sheldon Reservoir site. *H. pakistanae* was evidently released as a contaminant in *H. balciu-*

TABLE 1

Numbers of *Hydrellia balciunasi* Released in Florida and Texas with Information Regarding Release Dates, Number of Releases, and Current Establishment Status

Site	County	Release dates	No. of releases	Quantity released		Unquantified releases made?	Establishment?
				Immatures	Adults		
Florida							
Orangebrook Golf Course	Broward	8/89–7/90	26	13,420	0	No	No
Wilson Cypress Strand	Collier	3/91–5/91	3	19,434	0	No	No
Big Gant Lake	Sumter	8/91–12/92	3	?	0	Yes	No
Lake Panasoffkee	Sumter	9/91–5/92	8	8,867	0	Yes	No
Naples Manor	Collier	11/91	1	1,066	0	No	No
Sawgrass Lake	Brevard	1/92–4/92	5	4,021	0	Yes	No
Lake Okahumpka	Lake	5/92–6/92	2	?	50	Yes	No
Florida totals		8/89–12/92	48	46,808	50		
Texas							
Sheldon Reservoir	Harris	8/91–5/92	25	74,903	0	No	Yes
Lake Raven	Walker	10/92–12/94	15	76,531	0	No	Yes?
Coleto Creek Reservoir	Victoria	12/92–5/93	11	33,560	0	No	No
Choke Canyon	McMullen-Live Oak	8/93–1/94	12	52,802	0	No	No
Texas totals		8/91–12/94	63	237,796	0		
Grand totals			111	284,604	50		

Note. The numbers of *H. balciunasi* released may actually be substantially lower due to unquantified contamination with the closely related species *H. pakistanae.*

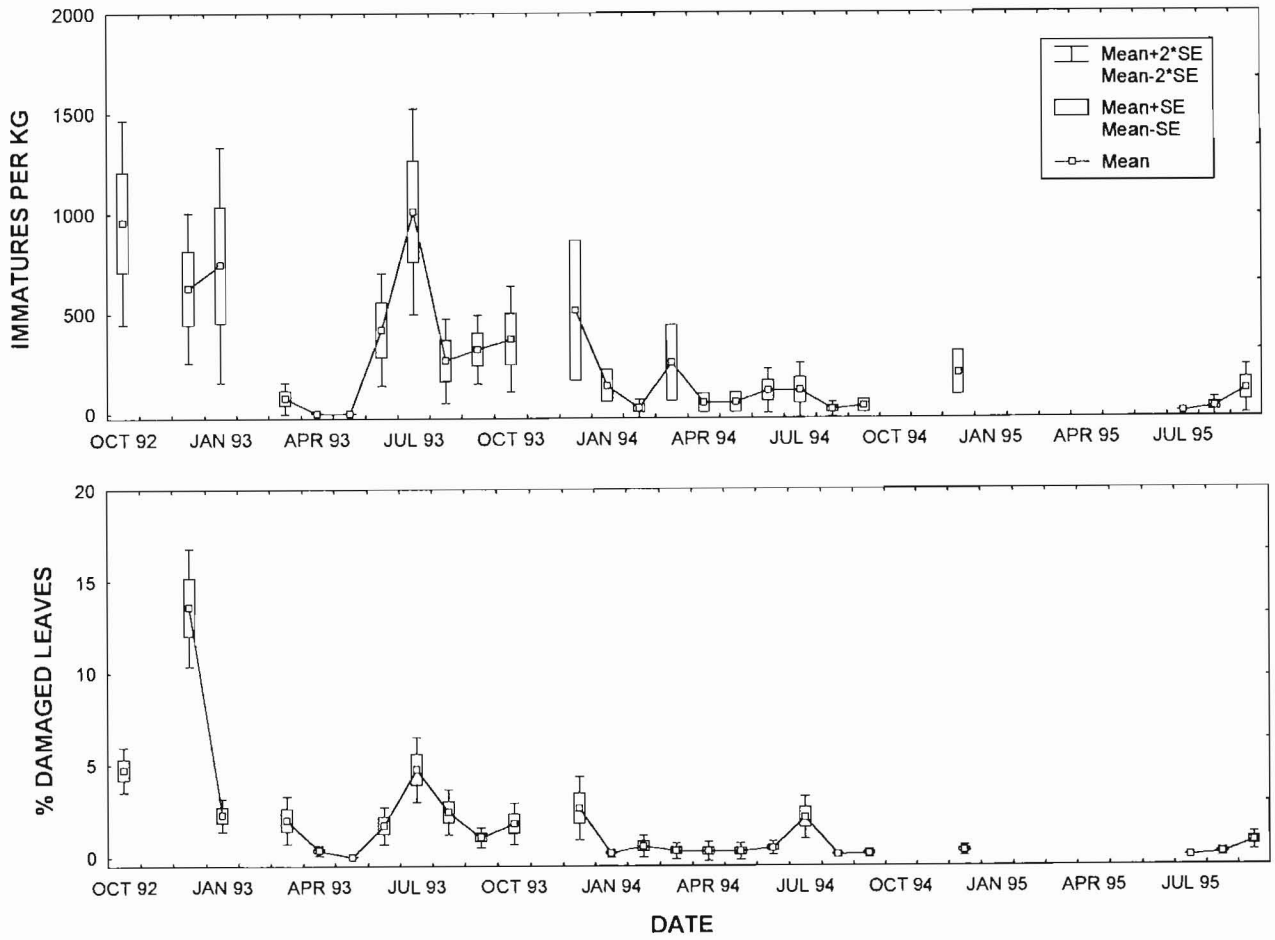

FIG. 1. Average number (±1 and ±2 standard errors of the mean) of immature *Hydrellia balciunasi*/kg wet plant weight and corresponding percentage of leaf damage for Sheldon Reservoir beginning in October 1992 and continuing through September 1995. The means for number of immatures/kg are significantly different at $F = 4.598$, $P < 0.00001$, and $df = 24,1274$. The means for percentage of leaf damage are significantly different at $F = 25.610$, $P < 0.001$, and $df = 24,1274$.

nasi colonies. Initial proportions of *H. pakistanae* in samples of adults collected at the site were comparable to colony contamination proportions (only about 2%) but only for a few months after the last release (up to January 1993). Thereafter, no additional *H. pakistanae* were found.

We recently discovered that *H. balciunasi* may have established at a second Texas location, Lake Raven, a small water body located in Hunstville State Park about 100 km north of Houston. We released *H. balciunasi* at Lake Raven from fall 1992 until December 1994 but saw no evidence of establishment. Limited numbers of immatures, but no confirming adult specimens, were collected through 1994 (Fig. 3). However, during a routine sampling trip in late June 1994 small numbers (i.e., <10) of adult *H. balciunasi* were hand-collected. While no damage by immatures was noted, small numbers of adults were consistently hand-collected at Lake Raven during the next 6 months. Quantitative estimates of immature population levels and leaf damage have been minimal, never exceeding 200 immatures/kg and 2% leaf damage (Fig. 3). No statistical differences ($F = 0.781$, $P = 0.731$, and $df = 19,644$) in mean number of immatures were detected among sampling dates. Qualitative sampling during July 1996 failed to reveal the presence of adults or associated leaf damage. Unfortunately, the hydrilla beds at the release sites were disrupted by major flooding and herbicide applications during 1995. These events may have extirpated the incipient *H. balciunasi* populations. If *H. balciunasi* persists at Lake Raven, it does so at very low levels. The consistent collection of adults during 1993 and 1994, however, suggests that a population might still be present and mandates additional sampling.

H. balciunasi releases were made at two additional Texas sites; Coleto Creek Reservoir, about 100 km north of Corpus Christi, and Choke Canyon Reservoir, about 50 km north of Corpus Christi, beginning December 1992 and August 1993, respectively (Table 1). More than 30,000 individuals were released at Coleto Creek

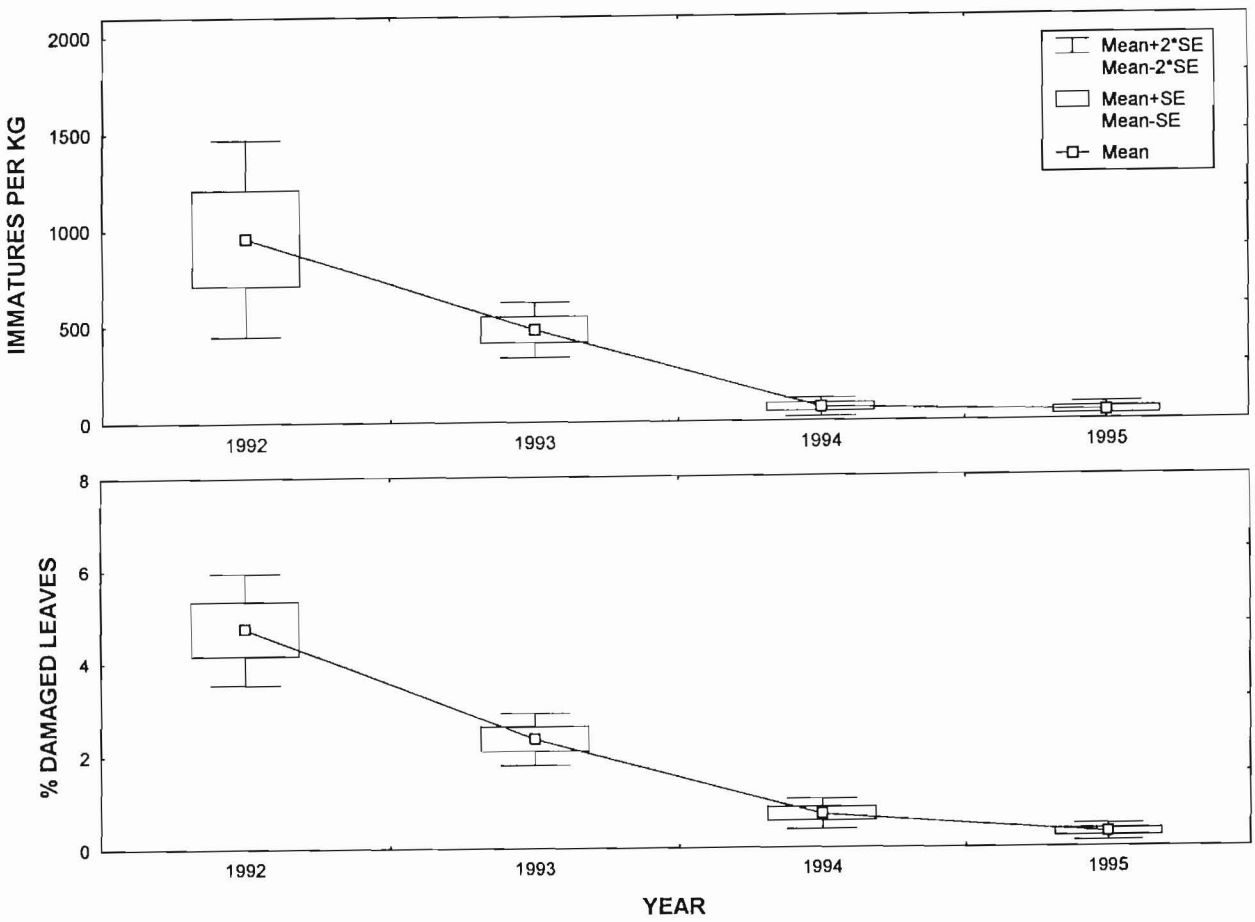

FIG. 2. Average number (±1 and ±2 standard errors of the mean) of immature *H. balciunasi*/kg wet plant weight and corresponding percentage of leaf damage for each of the sampled growing seasons (i.e., 1992, 1993, 1994, and 1995) for Sheldon Reservoir. The growing season was defined as that period from June through October. The means for number of immatures/kg were significantly different at $F = 21.066$, $P < 0.0001$, and $df = 3,645$. The means for percentage leaf damage are significantly different at $F = 32.368$, $P < 0.00001$, and $df = 3,645$.

Reservoir and nearly 50,000 at Choke Canyon Reservoir. Substantial collecting efforts using a variety of quantitative and qualitative sampling methods have failed to detect the presence of *H. balciunasi* at these sites.

It is interesting to note that *H. pakistanae* is evidently established throughout Coleto Creek Reservoir. When we concluded that *H. balciunasi* would not establish at this site, we made a single release of approximately 34,000 immature *H. pakistanae,* obtained from field locations in southern Florida, at the original *H. balciunasi* release area during May 1995. Quantitative estimates, which were again initiated in June 1995, revealed an increasing population of *H. pakistanae* (data not shown), but no *H. balciunasi* (Fig. 4). The peak numbers of *H. pakistanae* immatures occurred during July 1995 when estimates reached 2320 immatures/kg and a corresponding leaf damage of nearly 20% (data not shown). These values are over twofold higher than levels observed anywhere for *H. balciunasi.* The immatures are assumed to be *H. pakistanae* because no adult *H. balciunasi* were collected using the floating trays, hand-collection, or Berlese funnel extraction techniques; all adults collected from these sites have been *H. pakistanae.* Qualitative examinations made during July 1996 also revealed the presence of a flourishing *H. pakistanae* population throughout the reservoir. *H. pakistanae* immatures have subsequently been found at sites up to 2.5 km from the nearest point of release (data not shown).

Similarly, despite extensive efforts, establishment failed to result at the south Florida sites. Shortly after the release of *H. balciunasi* at Orangebrook Golf Course in southern Florida, qualitative examinations revealed the presence of *H. pakistanae,* not *H. balciunasi,* as expected. Inspection of the *H. balciunasi* colony from which the releases were made revealed a high level of contamination (90%) by *H. pakistanae.* As a result, we are unsure of the numbers of *H. balciunasi* actually released there (Table 1). The entire contaminated colony was released at the Orangebrook site shortly after this was discovered. New laboratory colonies were then

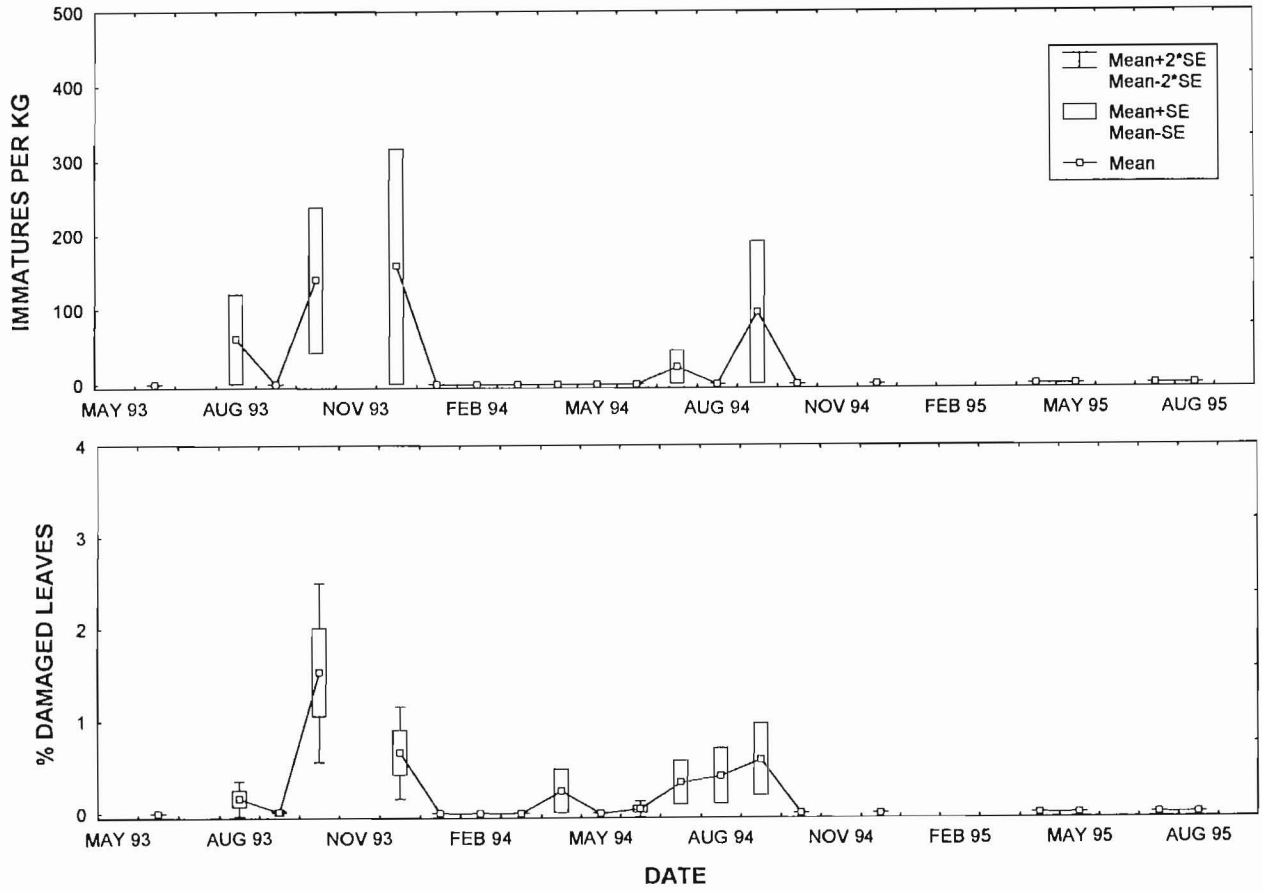

FIG. 3. Average number (±1 and ±2 standard errors of the mean) of immature *H. balciunasi*/kg wet plant weight and corresponding percentage of leaf damage for Lake Raven (Huntsville State Park) beginning in June 1993 and continuing through September August 1995. The means for number of immatures/kg are not significantly different at $F = 0.781$, $P = 0.731$, and $df = 19{,}644$. The means for percentage leaf damage are significantly different at $F = 3.777$, $P < 0.00001$, and $df = 19{,}644$.

established from overseas material. *H. pakistanae* was becoming widely established throughout southern Florida at the time (Center *et al.,* 1997) and field-collected hydrilla which undoubtedly contained *H. pakistanae* was being used to sustain the *H. balciunasi* colonies. Hence, contamination of laboratory colonies was a continual problem. However, we instituted careful monitoring procedures and thereafter successfully minimized contamination levels prior to initiating additional releases.

Subsequent attempts to establish *H. balciunasi* in Florida were made at sites at least 100 km away from known infestations of *H. pakistanae.* Nonetheless, *H. pakistanae* was soon discovered at all *H. balciunasi* release sites and throughout the state. Apparently this can be attributed to the rapid dispersal of *H. pakistanae* throughout south and central Florida (Center *et al.,* 1997). Although some *H. balciunasi* adults were recovered shortly after termination of the releases, subsequent sampling produced only *H. pakistanae* and the native *Hydrellia* species; *H. bilobifera* Cresson, *H. discursa* Deonier, and a few unidentified *Hydrellia* spp. Small populations of *H. balciunasi* may have persisted and may still exist in Florida, possibly having gone undetected due to the overwhelming numbers of *H. pakistanae* present. This was a major consideration in our decision to discontinue releases in Florida in favor of releasing *H. balciunasi* in Texas.

H. balciunasi has been confirmed to persist at fewer than 10% of the sites in both Texas and Florida where releases have been made. This is in contrast to the closely related *H. pakistanae,* for which 70% or more of the release attempts have produced long-term or permanent establishment (Center *et al.,* 1997). Not only have establishment rates been low, population numbers have never increased significantly. For example, at Sheldon Reservoir population levels remained static or declined during 3 of the 4 years sampled (i.e., 1993, 1994, and 1995, Fig. 2). This is also in contrast to *H. pakistanae,* whose populations have increased rapidly, at many sites reaching levels 10 times greater than those of *H. balciunasi* (Grodowitz, unpublished data).

The reasons for failure of *H. balciunasi* to establish at most sites are unknown but likely result from a

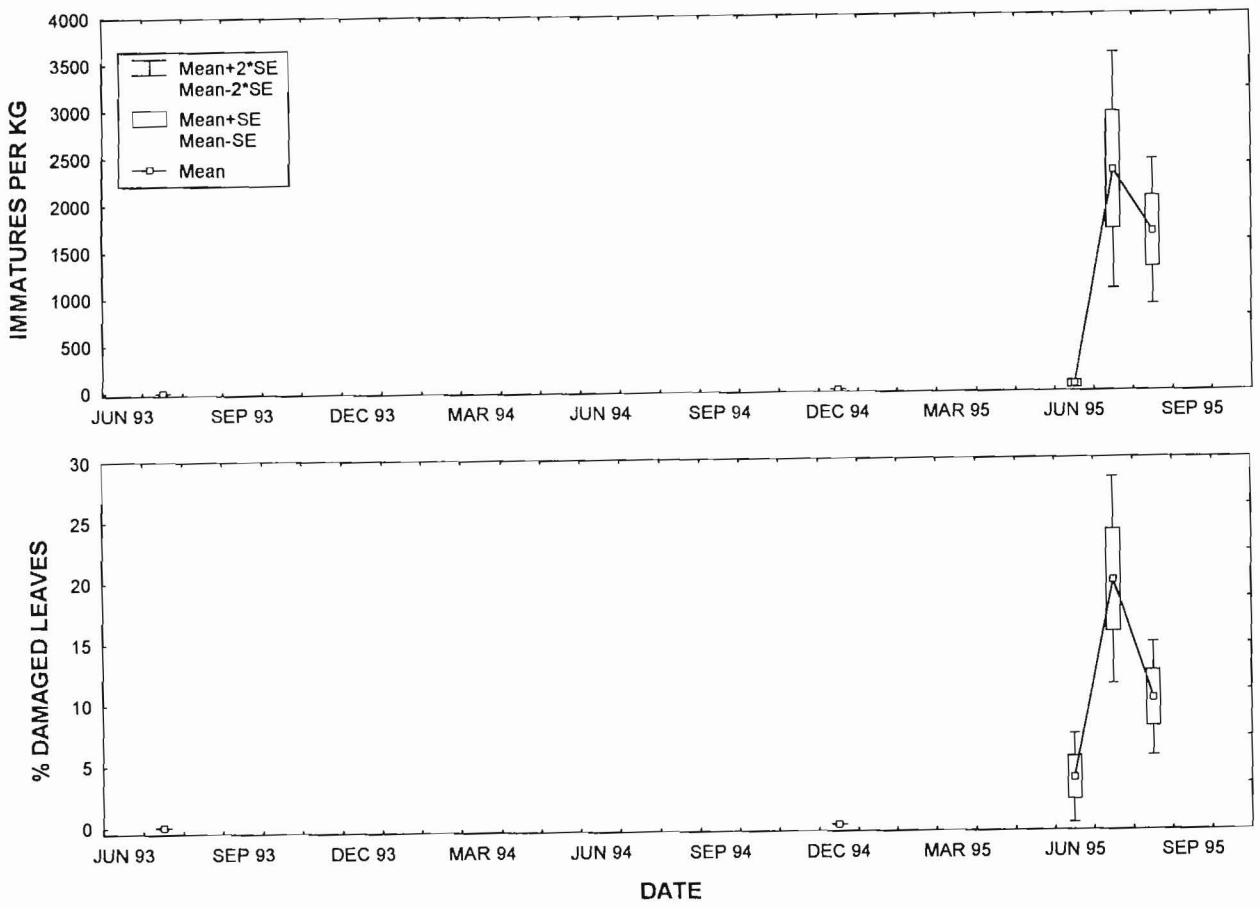

FIG. 4. Average number (±1 and ±2 standard errors of the mean) of immature *H. balciunasi* and *H. pakistanae* per kg wet plant weight and corresponding percentage of leaf damage for Coleto Creek Reservoir beginning in July 1993 and continuing through August 1995. The means for number of immatures per kilogram are significantly different at $F = 17.095$, $P < 0.00001$, and $df = 4,144$. The means for percentage of leaf damage are significantly different at $F = 21.803$, $P < 0.000001$, and $df = 4,144$.

combination of factors. These may include differences in climatic conditions where the various strains originated compared to their actual release sites, poor viability in released individuals, high parasitism rates, genetic and physiological differences in hydrilla from the United States compared to that from Australia (the home range of *H. balciunasi*), and competition from the closely related *H. pakistanae.*

It is well known that rearing in and of itself can have profound effects on the viability of insect biocontrol agents destined for introduction into new areas (Bush and Neck, 1977). Such effects are difficult to avoid, but our rearing program was designed to minimize inbreeding depression of the reared individuals. This was accomplished mainly by continually replacing entire colonies with fresh overseas material after rearing only a few generations. In addition, large spacious containers were used to minimize flight degeneration. Such measures apparently resulted in successful establishment of *H. pakistanae.* Rearing procedures identical to those used for *H. balciunasi* have enabled field colonization of *H. pakistanae* from laboratory-bred individuals, albeit with some difficulty (Center *et al.,* 1997). Hence, the viability of laboratory-reared individuals was probably not a primary factor limiting establishment of *H. balciunasi.*

Parasitism of *H. balciunasi* may also be an important factor limiting establishment. Observations on *H. pakistanae* has shown the potential for high parasitism by the native wasp species *Trichopria columbiana* (Ashmead) in the family Diapriidae (personal communication E. Reeves, Guntersville, Alabama). Parasitism rates as high as 90% were observed when sentinel pupae were placed at field locations in northern Alabama. While it is unknown whether *T. columbiana* will parasitize *H. balciunasi,* there is no reason to believe that it will not, especially considering the close similarity in the behavior and habitat requirements for the two species. In addition, it is well documented that high parasitism occurs for many native species of *Hydrellia* (Deonier, 1971). More research is needed on the population-level effects of parasites on *H. balciunasi.*

Another important reason may be related to genetic differences in the hydrilla originating from Australia and that found in the United States. Recent work on the phenetic relationships among international accessions of hydrilla using RAPD analysis indicates that the dioecious form of hydrilla in the United States is phenetically more similar to Asian rather than Australian plants (P. T. Madeira, Ft. Lauderdale, Florida, personal communication). Hence, more research is needed to quantify the effects of host races on the performance of both *H. balciunasi* and *H. pakistanae.*

Plant quality has also been suggested as a possible reason for lack of establishment by *H. balciunasi.* Observations made on the growth and development of *H. balciunasi* while in quarantine and during mass rearing procedures have suggested that larval *H. balciunasi* are sensitive to the hardness of hydrilla, more so than *H. pakistanae.* Decreased performance is apparently associated with tougher leaves (G. Buckingham, USDA, ARS, Gainesville, Florida, personal communication). Wheeler and Center (1996) have found that hydrilla leaf texture (i.e., hardness) and tissue nitrogen concentrations affect *H. pakistanae* larval survival, developmental times, and adult size.

Another explanation for the lack of establishment in Florida is the rapid dispersal of *H. pakistanae.* Large increases in *H. pakistanae* populations throughout south and central Florida may have competitively excluded *H. balciunasi.* However, this conclusion is premature, especially considering the limited establishment of *H. balciunasi,* even with the considerable effort spent in Texas, where *H. pakistanae* was not present to any great extent. Also, the accidental release and temporary presence of *H. pakistanae* compared to the long-term presence of *H. balciunasi* at Sheldon Reservoir suggests that the former species does not always preempt the latter.

In summary, the release program has resulted in the introduction of nearly 300,000 *H. balciunasi* in two states and 11 locations. Despite repeated attempts, it is established permanently at just one, and possibly two, Texas locations, where populations have remained low. This suggests that *H. balciunasi* will have minimal utility as a biological control agent for hydrilla.

ACKNOWLEDGMENTS

The tests described and the resulting data presented herein, unless otherwise noted, were obtained from research conducted under the Aquatic Plant Control Research Program of the U.S. Army Corps of Engineers by the U.S. Army Engineer Waterways Experiment Station. Permission was granted by the Chief of Engineers to publish this information. We thank Dr. Judy Shearer and Mr. Brent Harrel for their critical review and constructive criticisms of the manuscript. Thanks are also given to Ms. Ramona Warren, Mr. Harvey Jones, Mr. Rhett Hobgood, Mr. Bob Dew, Ms. Kate Hopkins, Ms. Sharon Martin, Mr. Jason Eakin, Mr. Brian Durham, Mr. Brent Harrel, Mr. Jason Courtney, Ms. Kai-Ching Cha, and Mr. Bradley Lewis for their technical assistance in many aspects of the project including field sampling, data input and analysis, and insect rearing. Thanks are also extended to the Texas Parks and Wildlife Department for technical assistance and use of their facilities, especially the personnel at Sheldon Reservoir and Mathis, Texas.

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REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503.

1. AGENCY USE ONLY *(Leave blank)*	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	September 1997	Final report

4. TITLE AND SUBTITLE

See reverse.

5. FUNDING NUMBERS

6. AUTHOR(S)

Michael J. Grodowitz, Ted D. Center, Alfred F. Cofrancesco, Jan E. Freedman

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

See reverse.

8. PERFORMING ORGANIZATION REPORT NUMBER

Miscellaneous Paper A-97-5

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Corps of Engineers
Washington, DC 20314-1000

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

Available from National Technical Information Service, 5285 Port Royal Road, Springfield, VA 22161.

12a. DISTRIBUTION/AVAILABILITY STATEMENT

Approved for public release; distribution is unlimited.

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*

The Australian leaf-mining fly, *Hydrellia balciunasi*, was first released as a biological control agent for the management of the submersed aquatic plant *Hydrilla verticillata* in August 1989 in southern Florida. Since then, 284,684 individuals have been released at seven Florida and four Texas sites. Despite this effort, populations have persisted at just two Texas sites: Sheldon Reservoir near Houston and Lake Raven in Huntsville State Park. *Hydrellia balciunasi* levels have remained relatively low at these sites, averaging, at best, less than 1,000 immatures/kilogram wet plant weight. All other collections were substantially lower. Reasons for poor establishment are unknown but probably relate to a complex of factors, including competition with another introduced leaf-mining species, parasitism by native wasps, poor host quality, genetic differences between Australian and United States hydrilla resulting in mismatched physiological strains of agent and host plant, and possibly inbreeding depression in laboratory colonies used for releases.

14. SUBJECT TERMS

Aquatic plant
Aquatic weed
Classical biological control
Insect releases
Insecta
Submersed weed

15. NUMBER OF PAGES

14

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
UNCLASSIFIED	UNCLASSIFIED		

4. (Concluded).

Release and Establishment of *Hydrellia balciunasi* (Diptera: Ephydridae) for the Biological Control of the Submersed Aquatic Plant *Hydrilla verticillata* (Hydrocharitaceae) in the United States

7. (Concluded).

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Aquatic Plant Control Research Laboratory
3205 College Avenue, Fort Lauderdale, FL 33314-7719