7 HYDRILLA

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PEST STATUS OF WEED

Hydrilla verticillata (L.f.) Royle (hereafter, referred to as "hydrilla") (Fig. 1) is a submersed, rooted aquatic plant that forms dense mats in a wide variety of freshwater habitats (canals, springs, streams, ponds, lakes, rivers, and reservoirs) (Langeland, 1990). Plants grow from the substrate to the water's surface in both shallow and deep water (0-15 m in depth) (Langeland, 1990; Buckingham, 1994). This plant is listed on the 1979 federal noxious weed list (USDA-NRCS, 1999) and also is identified in the noxious weed laws of Florida (FDEP, 2000), Louisiana (LDWF, 2000), Texas (TPWD, 2000), California (CDFA, 2000a), South Carolina (SCDNR, 2000), North Carolina (NCAWCA, 2000), Oregon (OSDA, 2000), Washington (WSDA, 2000), and Arizona (ERDC 2001b). In addition, the states of Alabama, Georgia, Maryland, Mississippi, Tennessee, and Virginia, have programs for the control of this invasive plant (Eubanks, 1987; Earhart, 1988; Zattau, 1988; Bates, 1989; Henderson, 1995; Center *et al.*, 1997).

Figure 1. Growing tip of hydrilla. Note crowded internodes at tips.

Nature of Damage

Economic damage. In the United States, hydrilla often dominates aquatic habitats causing significant economic damage (Fig. 2). Hydrilla interferes with a wide variety of commercial operations. Thick mats hinder irrigation operations by reducing flow rates by as much as 90% (CDFA, 2000a) and impede the operation of irrigation structures (Godfrey *et al.*, 1996). Hydroelectric power generation also is hindered by fragmented plant material that builds up on trash racks and clogs intakes. During 1991, hydrilla at Lake Moultrie, South Carolina shut down the St. Stephen powerhouse operations for seven weeks resulting in $2,650,000 of expenses due to repairs, dredging, and fish loss. In addition, during this repair period, there was an estimated $2,000,000 loss in power generation for the plant (letter from Charleston District Engineer to Commander, South Atlantic Division, dated March 8, 1993).

Figure 2. Heavy infestation of hydrilla at Rodman Reservoir (August 2, 1978). The rapid underwater growth "pushes" a portion of the mat above the water, giving the reservoir a field-like appearance.

Boat marinas have been reported closed for extended periods on the Potomac River, Virginia; Lake Okeechobee, Florida; Santee Cooper Reservoirs, South Carolina; and Clear Lake, California. Propeller driven boats are hampered by thick mats of hydrilla that form at the water's surface, requiring frequent cleaning to progress short distances. The fragmented plant material removed from the propellers can easily colonize new areas. In the late 1980s, hydrilla populations at Lake Guntersville, Alabama increased rapidly. Henderson (1995) examined the economic impact of aquatic plant control programs on recreational use of this lake between 1990 and 1994. He found that the greatest economic value for recreation ($122 million annually) occurred when vegetation levels were 20% of the total lake area, and that revenue declined as hydrilla acreage increased.

Although California does not consider hydrilla established, the state has, for decades, aggressively pursued an eradication program that seeks to rapidly eliminate new infestations as they are discovered. California officials have stated that if infestations are not contained and treated promptly, hydrilla will spread throughout the state and cost millions of dollars annually to manage (CDFA, 2000b).

Ecological damage. Native plants act as the primary producers in most ecosystems (Drake *et al.*, 1989; Pimm, 1991). In the United States, hydrilla frequently forms large monocultures that displace native vegetation (Haller, 1978), reducing biodiversity and altering native ecosystems. These alterations also affect the primary and secondary consumers in affected communities (Westman, 1990; Frankel *et al.*, 1995; Schmitz and Simberloff, 1997). Massive amounts of hydrilla can alter dissolved oxygen, pH, and other water chemistry parameters (Smart and Barko, 1988). The portion of the water column occupied by aquatic plants also influences the presence and size distribution of fish (Killgore *et al.*, 1993; Harrel *et al.*, 2001). In dense hydrilla mats, feeding by certain predatory fish is hampered, and small insectivores predominate, reducing community diversity. (Dibble *et al.,*1996).

Extent of losses. Hydrilla is a major aquatic weed problem throughout the southeastern United States (Center *et al.*, 1997). It was introduced to North America in 1951 or 1952 by an aquarium plant dealer who discarded six bundles of hydrilla into a canal near his business in Tampa, Florida (Schmitz *et al.*, 1991). Since then, it has spread explosively because it can reproduce from very small fragments (Langeland and Sutton, 1980). Apparently, recreational boaters and fishermen quickly spread hydrilla to new locations when fragments of hydrilla are transported on boats, motors, and trailers. Once an aquatic site is infested, eradication of hydrilla is very difficult. It produces specialized asexual, reproductive 'buds' on stems (referred to as turions) and on the underground stolons (tubers). These tubers and turions assist hydrilla in reinfesting a site after a drought, or after application of herbicides. Langeland (1990) reported that the annual control cost to manage 7,600 ha of hydrilla in Florida exceeds $5 million. The U.S. Army Corps of Engineers spends more than one million dollars per year to suppress hydrilla populations in the Jacksonville District and more than $400,000 annually to treat infestations of this plant at Lake Seminole, a 30,000-acre lake located on the borders of Florida, Alabama, and Georgia. Since 1989, millions of dollars have been spent to introduce the triploid grass carp into the Santee Cooper Reservoirs (70,000 ha) for the management of more than 17,000 ha of hydrilla (Morrow *et al.,* 1997; Kirk *et al.,* 1996, Kirk *et al.,* 2000). Grass carp populations have reduced the infestation levels of hydrilla; however, additional stocking may be needed to maintain the current level of control (Kirk *et al.,* 2000), which will also add to the management costs of this program.

Hydrilla was first reported in California in 1976, and at that time the state established an eradication management plan. This program has eradicated hydrilla from various sites in ten counties. At some sites, treatment of hydrilla continued for six to eight years before eradication was achieved. Funding for this program has gradually increased over time, and during the last three years, California has spent more than $5.39 million (nearly $1.8 million annually) to eradicate hydrilla infestations in that state (CDFA, 2000a).

Geographical Distribution

Hydrilla is now almost cosmopolitan in its distribution. Antarctica and South America are the only continents from which it has not been recorded. It is very common on the Indian subcontinent, many of the Middle East countries, Southeast Asia, and northern and eastern Australia. Based on C. D. K. Cook's (pers. comm.) list of herbarium specimens, hydrilla is found in the Southern Hemisphere as far south as the North Island of New Zealand (approximately 40° S). In the

Northern Hemisphere hydrilla is found as far north as Ireland, England, Poland, Lithuania and Siberia. The Lithuanian sites, at about 55° N latitude, are the furthest from the equator that hydrilla is known to occur. Since virtually the entire continental United States, except Alaska, lies below a latitude of 48°, hydrilla is climactically suited for growth in any of the contiguous states as well as Hawaii. Even Alaska cannot be considered entirely safe from invasion by hydrilla since places such as Juneau are at approximately the same latitude as the hydrilla infestations in Lithuania and Siberia (Balciunas and Chen, 1993).

The female form of dioecious hydrilla arrived in Florida in the early 1950s (Schmitz *et al.*, 1991) and quickly spread throughout the southeastern United States. Although the monecious biotype of hydrilla was not detected in the United States until the late 1970s (Haller, 1982; Steward *et al.,* 1984), it too is now spreading rapidly, especially into northern states. Monecious hydrilla has now been detected as far north as the Columbia River in Washington state in the western United States, and in Pennsylvania and Connecticut in the eastern United States (Madeira *et al.,* 2000). An excellent color map showing the current U.S. distribution of both biotypes of hydrilla can be found in Madeira *et al.* (2000).

BACKGROUND INFORMATION ON PEST PLANT

Taxonomy

The following description is compiled primarily from Cook and Lüönd (1982), Sainty and Jacobs (1981), and Godfrey and Wooten (1979). Hydrilla is a perennial, submerged, rooted, vascular plant. Roots are long, slender, and simple and are whitish or light brown in appearance. They are usually buried in hydrosoil, but also form adventitiously at nodes. Stems are long, usually branching, growing from the hydrosoil and frequently forming dense, intertwined mats at the surface of the water. Detached portions of hydrilla plants remain viable and are a common mode for infestation of new areas. Below the hydrosoil, the stems are horizontal, creeping, and stoloniferous. Leaves are verticillate, and along most of the stem, usually number three to five per node. Apical portions of the stem usually have the nodes tightly clustered, with each verticil bearing up to eight leaves. The leaves are usually strongly serrated with the teeth visible to the naked eye, and each leaf terminates in a small spine. The midvein is sometimes reddish in color, and is usually armed with an irregular row of spines. The squamulae intravaginales (nodal scales) are small (ca. 0.5 mm long), paired structures at the base of the leaves and are lanceolate, hyaline, and densely fringed with orange-brown, finger-like structures called fimbrae. Flowers are imperfect (unisexual), solitary, and enclosed in spathes. The female flower is white, translucent, with three broadly ovate petals, about 1.2 to 3.0 mm long; the three petals alternate with the sepals that are much narrower and slightly shorter; the three stigmas are minute; the ovary is at the base of a long (1.5 to 10+ cm) hypanthium. The male flower is solitary in leaf axils. Mature flowers abscise and rise to the surface. Sepals and petals are similar in size and shape to those of female flowers. Each of three stamens bears a four-celled anther that produces copious, minute, spherical pollen. Hydrilla plants occur as two biotypes. They can be either dioecious, with flowers of only one sex being produced on a particular plant, or monecious, with flowers of both sexes on the same plant. Fruits are cylindrical, about 5 to 10 mm long, usually with long, spine-like processes. Seeds are smooth, brown, usually five or less, 2 to 3 mm long and borne in a single linear sequence. Two types of hibernacula are produced—a brown, bulb like type is produced at the ends of the stolons (Fig. 3), while a green, conical form is found in axils of branches. In the United States, the first type is usually called tubers and the latter turions.

Figure 3. Subsoil turions (tubers) at the end of horizontal stolons. These asexual reproductive structures are resistant to herbicides, and allow for rapid recolonization of a treated site by hydrilla.

Biology

Although the female biotype of hydrilla quickly became widespread throughout the southeastern United States, it was not until 1976 that a male flower was observed in the United States (Vandiver *et al.*, 1982). The female flowers can only be pollinated in the air. The female flower reaches the water surface by elongation of the hypanthium (flower "stalk"). The petals and sepals of the female flower form an inverted bell with an air bubble when growing to the surface, and if after reaching the surface the flower becomes submerged, the petal and sepals revert to this position, and enclose an air bubble thus preventing wetting of the stigmas and ensuring air pollination. The male flower lacks a hypanthium, and reaches the surface by detaching from the plant and floating up as a ripe, air-filled bud. The perianth segments recurve towards the water surface and eventually the anthers dehisce, explosively scattering pollen in a radius of about 10 cm around the flower. Where male hydrilla flowers are present, the water surface frequently becomes visibly greenish-white due to the floating pollen grains and discarded male flowers.

Hydrilla is usually a gregarious plant that frequently forms dense, intertwined mats at the water's surface. Approximately 20% of the plant's biomass is concentrated in the upper 10 cm of such a mat (Haller and Sutton, 1975). The plants grow and spread quickly. Small fragments of the plant, containing but a single node, can quickly develop adventitious roots and eventually produce an entire plant.

Hydrilla has very wide ecological amplitude, growing in a variety of aquatic habitats. It is usually found in shallow waters, 0.5 m or greater in depth. In very clear waters it can grow at depths exceeding 10 m. It tolerates moderate salinity – up to 33 percent of seawater (Mahler, 1979). While hydrilla flourishes best in calcareous ponds and streams, water quality rarely seems to be limiting, since it is found in both acidic and alkaline waters. It also grows well in both oligotrophic and eutrophic waters, and even tolerates high levels of raw sewage (Cook and Lüönd, 1982). Sediments with high organic content provide the best growth, although hydrilla also is found growing in sandy and rocky substrates.

While hydrilla does not grow well in deeply shaded areas, it is adapted to grow under very low light conditions (Bowes *et al.,* 1977), and this may account for its rapid growth and quick dominance over native vegetation.

Analysis of Related Native Plants in the Eastern United States

While hydrilla can assume widely different forms when growing in different environments, all are now considered to be a single species of *Hydrilla verticillata* (Cook and Lüönd, 1982). There are no other species in the genus *Hydrilla*, which is placed in the frog's bit family, Hydrocharitacae. There are eight other genera from this family in the eastern United States, two of which (*Halophila* and *Thalassia*) are native "marine grasses" that grow in shallow coastal waters (Godfrey and Wooten, 1979). The other native Hydrocharitacae, all of which grow in shallow freshwaters, include *Blyxa aubertii* Rich., *Elodea* (two species, *Elodea canadensis* Michaux and *Elodea nutallii* [Planch.] St. John), *Limnobium spongia* (Bosc.) Steud., and *Vallisneria americana* Michx. (Godfrey and Wooten, 1979). There also are three additional introduced Hydrocharitaceae in the United States: *Egeria densa* Planch., *Hydrocharis morus-ranae* L., and *Ottelia alismoides* (L.) Pers. The two native *Elodea* species, and the introduced *Egeria densa*, are difficult to distinguish readily from hydrilla. Hydrilla, however, is unique in having nodal scales (squamulae intravaginales) and specialized, asexual reproductive organs – tubers and turions.

HISTORY OF BIOLOGICAL CONTROL EFFORTS IN THE EASTERN UNITED STATES

Area of Origin of Weed

The area of origin of *Hydrilla verticillata* is not clear, but appears to be a broad region encompassing a large part of the Eastern Hemisphere and adjacent areas. Cook and Lüönd (1982), along with many other botanists, indicate that "its centre of origin lies in the warmer regions of Asia." However, hydrilla has been in central Africa for a long time — it was collected by Speke during his 1860 to 1863 expedition to find the sources of the Nile (Speke, 1864) — and some botanists believe that it originated there (Tarver, 1978). Mahler (1979) is even more precise, stating "…with a center of distribution or origin in southeastern Uganda and northwestern Tanzania." Hydrilla is also considered by some to be native to Australia (Sainty and Jacobs, 1981). The first records from Australia are from the early nineteenth century, soon after the arrival of European settlers.

A recent DNA analysis of hydrilla collections from around the world (Madeira *et al.*, 1997) supports the hypothesis of multiple introductions into the United States. The authors found that dioecious samples from the southern United States are more closely aligned with those from the Indian subcontinent, while the monoecious samples most closely resembled those from South Korea.

Domestic Surveys and Natural Enemies Found

Prior to initiating a biological control project, it is recommended that the target weed be surveyed to determine what natural enemies are already associated with it in the invaded area. Native insects or pathogens might be suppressing a target weed at some sites, or non-native natural enemies may have been introduced accidentally. The Army Corps of Engineers Waterways Experiment Station funded thorough faunistic surveys of U.S. hydrilla populations by University of Florida entomologist, Joe Balciunas. Between 1978 and 1980, he made 289 collections of hydrilla at 75 sites, 58 of which were in Florida (Balciunas and Minno, 1984). More than 17,000 insect specimens, comprising nearly 200 species, were collected and identified (Balciunas and Minno, 1984), but of these only 15 were feeding on hydrilla (Balciunas and Minno, 1985). Among the most damaging of the insects found in Florida was the introduced Asian moth *Parapoynx diminutalis* Snellen. This moth was first detected in south Florida (Delfosse *et al.*, 1976), but dispersed rapidly to additional areas, at some of which it caused heavy damage to hydrilla (Balciunas and Habeck, 1981).

Other researchers (Cuda *et al.*, 1999, in press; Epler *et al.*, 2000) have commented on the feasibility of using the midge *Cricotopus lebetis* Sublette (Diptera: Chironomidae) as a biological control agent for hydrilla.

The feasibility of using native pathogens to control hydrilla also has been investigated. In the fall of 1987 and 1988, surveys were conducted in 15 lakes and 3 rivers in southeastern United States for pathogens of hydrilla (Joye and Cofrancesco, 1991). Nearly 200 fungal and 27 bacterial isolates were collected from hydrilla foliage. An endemic fungal pathogen originally identified as *Macrophomina phaseolina* (Tassi) Goid. and later determined to be *Mycoleptodiscus terrestris* (Gerd.) Ostazeski was collected from hydrilla growing in Lake Houston, Texas in 1987 (Joye, 1990; Shearer, 1996). Field and laboratory studies have shown that the fungus can significantly reduce hydrilla biomass after inoculation compared with untreated plants (Joye, 1990; Shearer, 1996). Disease symptoms appear in 5 to 7 days after inoculation as interveinal chlorosis followed by a complete loss of color. Within 10 to 14 days, plants treated with *M. terrestris* begin to disintegrate (Joye, 1990; Shearer, 1996). Transmission electron microscopy studies have shown that the fungus attaches to lower epidermal cells of hydrilla leaves within eight hours postinoculation and penetration through the cell wall is completed within 40 hours (Joye and Paul, 1992). The fungus then completely colonizes the host, resulting in collapse of the entire plant. While not currently available as a product, *M. terrestris* is undergoing evaluation for its potential as a bioherbicide for hydrilla management. As an initial step in the process, the U.S. Army Engineer Research and Development Center Environmental Laboratory (ERDC), Vicksburg, Mississippi and the USDA, ARS National Center for Agricultural Utilization Research in Peoria, Illinois are studying fermentation methods that will yield high concentrations of effective propagules at a low cost. SePro Inc. (Carmel, Indiana) also is involved as a cooperator in the project. The goal is to produce a bioherbicide that can be competitive with chemical herbicides.

Overseas Areas Surveyed and Natural Enemies Found

Determining the native range of a weed is extremely important in biological control programs since the center of origin is usually considered to be the best area to begin searches for natural enemies. In its native range, the weed should have a greater array of natural enemies that coevolved with it. Since evidence to pinpoint hydrilla's evolutionary origin was lacking, searches have been made in several regions, including Africa, Asia, and Australia.

Opportunistic surveys began in India in 1968, and since that time surveys have been conducted in at least 15 additional countries. A time-line and list of overseas research to develop biocontrol agents for hydrilla is presented in Table 1. Only the major overseas projects will be discussed here, as it is beyond the scope of this chapter to completely review the results of all the surveys noted in Table 1. For a more complete review of the history of foreign exploration

Table 1. Chronology of foreign searches for insect enemies of hydrilla (*Hydrilla verticillata*)

Year	Search
1971	CIBC initiates search for insect enemies of hydrilla in Pakistan.
1973	Varghese begins studies of insect enemies of hydrilla in Malaysia.
1973	Baloch et al. (1972) present preliminary report on natural enemies of hydrilla in Pakistan. Of the eight insects and two snails found, only the ephydrid fly *Hydrellia* sp., the moth *Parapoynx diminutalis,* and the weevil *Bagous* sp. nr. *limosus* Gyllenhal are considered to be promising biological control agents.
1975	Delfosse et al., (1976) discover *Parapoynx diminutalis* Snellen in Fort Lauderdale, Florida. This Asian species was probably introduced in a shipment of aquarium plants.
1975	George Allen (USDA, ARS, Gainesville, Florida) searches in Africa and Indonesia for insect enemies of hydrilla. Results not reported.
1976	Varghese and Singh (1976) present final report on studies in Malaysia. Only two insect enemies were recorded, an aphid and a moth, probably *Parapoynx diminutalis.*
1976	Baloch et al. (1980) submit final report on insect enemies of hydrilla in Pakistan. Species discussed included a *Bagous* sp. weevil that feeds on hydrilla tubers, *Parapoynx diminutalis,* and a leaf-mining *Hydrellia* sp.
1976	Pemberton (1980) and Lazor conduct surveys in Africa for insect enemies. Hydrilla not found until late in three-month survey and only one possible enemy, the larvae of a midge (Chironomidae), probably in the genus *Polypedilum*, is observed.
1978	Sanders and Theriot discover a moth, later identified as P*arapoynx* sp. nr. *rugosalis* (prev. *P. rugosalis*), damaging hydrilla and Najas (Balciunas and Center, 1981).
1979	Balciunas and Center (1981) study *Parapoynx* prob. *rugosalis* in Panama and find that it feeds primarily on hydrilla and Najas.
1980	Buckingham receives permission to bring Panamanian *Parapoynx* into quarantine facilities in Gainesville for further testing. However, the species tested by Balciunas and Center can no longer be located in Panama.
1981	CIBC begins search for insect enemies of hydrilla in East Africa.
1981	Balciunas (1982) spends four months searching for natural enemies of hydrilla in tropical Asia. Most of the species previously recorded on hydrilla in Asia are found.
1982	Habeck and Bennett made two unsuccessful trips to Panama searching for *Parapoynx* sp. nr. *rugosalis* (prev. P. *rugosalis*) and the *Parapoynx* sp. tested by Balciunas and Center (Habeck pers. comm.).
1982	Balciunas (1983) spends six months searching for natural enemies of hydrilla in Kenya, India, Southeast Asia, and northern Australia. Several new moth species are found damaging hydrilla, along with approximately 15 new species of *Bagous* weevils.
1982	Balciunas sends *Bagous* spp. weevils from India to Gainesville quarantine.
1983	Markham (CIBC) (1986) begins studies of insects attacking hydrilla in Burundi, Rwanda, and Tanzania.
1983	CIBC scientists in India send several shipments of *Bagous affinis* Hustache to Gainesville quarantine.
1983	Balciunas (1984) spends five months searching for natural enemies of hydrilla in the Philippines, Borneo, Malaysia, Bali, Papua New Guinea, northern Australia, Myanmar, and India. Weevils including *Bagous* spp. were again collected along with pyralid moths from the genus *Parapoynx* and ephydrid flies from the genus *Hydrellia*.
1985	Balciunas sets up a laboratory in Townsville and another in Brisbane (Queensland, Australia) to collect and evaluate biological control candidates.
1985	The leaf-mining fly *Hydrellia pakistanae* Deonier is first shipped to Gainesville quarantine.

Table 1. Chronology of foreign searches for insect enemies (continued)

Year	Search
1987	First shipment of the hydrilla stem borer weevil *Bagous hydrillae* O'Brien from Australia to the Gainesville quarantine facility in Florida.
1987	First field release of *Hydrellia pakistanae* in Florida.
1987	First field release of *Bagous affinis* in Florida.
1988	First shipment of the hydrilla leaf-mining fly *Hydrellia balciunasi* Bock from Australia to the Gainesville quarantine in Florida.
1988	USDA establishes the Sino-American Biological Control Laboratory (SABCL) in Beijing, China, to search for and evaluate temperate biological control agents of hydrilla.
1989	Balciunas (1990) and Buckingham, along with cooperating scientists from SABCL, begin annual surveys in China for insects on hydrilla and Eurasian watermilfoil. A new species of *Hydrellia,* later identified as *Hydrellia sarahae* var. *sarahae* Deonier, is found and shipped to the Gainesville quarantine for evaluation.
1989	University of Florida biological control laboratory in Australia becomes a USDA facility, called the Australian Biological Control Laboratory (ABCL); Balciunas appointed director.
1989	First field release of *Hydrellia balciunasi* in Florida.
1991	First field release of *Bagous hydrillae* in Florida.
1991	Buckingham and Pemberton (Buckingham 1993) survey hydrilla in Korea and Japan. A new, undescribed species of *Hydrellia* from Japan is sent to Florida, but a colony is not established.
1992	Dale Habeck (1996) spends five months studying stream-dwelling moths in north Queensland, Australia. Two of these moths, *Theila siennata* Warren (prev. *Aulacodes sienatta*) and *Ambia ptolycusalia* Walker (prev. *Nymphula eromenalis*), are sent to quarantine facilities in the United States.
1996	Balciunas et al. (1996a) present final report on Australian surveys. Four Australian insects exported, and two of these released in the United States.
1997	Scientists from the USDA, ARS Invasive Weed Lab, along with cooperators from Australia (ABCL) and Thailand's National Biological Control Research Center (NBCRC) begin surveys for hydrilla biocontrol agents in Thailand and Vietnam; several new insects are found and some are sent to Florida (Table 2) for further evaluation (Buckingham pers. comm., Center pers. comm.).

for hydrilla agents, readers should consult Balciunas (1985), Buckingham (1994), and Balciunas *et al.,* (1996a).

Many of the overseas surveys consisted of either brief trips to one or more countries, or efforts in which hydrilla was added as a target to a larger, ongoing project in a specific region. While these opportunistic surveys frequently noted potential agents, as of 2000, none of these had been approved or released in the United States. The most productive overseas studies have been intensive, multi-year projects concentrating on hydrilla natural enemies in a particular region. The first of these was the USDA-sponsored project in Pakistan from 1971 to 1976, conducted by scientists from CIBC (Commonwealth Institute of Biological Control). Ten insects were studied (Baloch and Sana-Ullah, 1974), but only three were recommended for importation into the United States (Baloch *et al.,* 1980). Unfortunately, these recommendations were not acted upon, possibly because there was no USDA scientist or facility available at that time to work on hydrilla insects.

In 1981, Joe Balciunas, a University of Florida entomologist, began systematic, intensive world-wide surveys to locate potential biocontrol agents for hydrilla. These surveys, funded by the Army Corps of Engineers (COE) Waterways Experiment Station (WES) and USDA, ARS, consisted of three, 5 to 6 month around-the-world trips. During these three trips, he visited 10 countries, made 180 collections, and found at least 45 different insects damaging hydrilla (Balciunas, 1985; Center *et al*., 1990) (Figure 4). His surveys had two immediate consequences. First, they resulted in the importation and quarantine

Figure 4. Joe Balciunas surveying for hydrilla agents at Lake Dal, Kashmir, India (August 1983). Of the four agents approved for release, two were shipped from India.

evaluation of four weevils and a leaf-mining fly (Table 2). Although all five of these insects had been previously studied in Pakistan, Balciunas's studies and shipments rekindled interest in these potential agents. The second outcome was that in 1985, Balciunas established a laboratory in Townsville, Australia, along with a substation in Brisbane, Australia, to further evaluate several promising insects that he had collected there during his worldwide surveys. Although hydrilla is widespread throughout tropical and eastern Australia, it seldom becomes abundant enough there, to be considered a problem.

Between 1985 and 1992, Balciunas and his Australian staff made more than 100 non-quantitative collections and 588 quantitative collections of hydrilla at 70 sites in Australia (Balciunas *et al.*, 1996a). In order to ascertain the field host range of the potential agents, he and his team also made 1,007 quantitative collections of 47 other aquatic plant species from 27 families (Balciunas *et al.,* 1996a). Balciunas and his team evaluated six insects for their potential as biological control agents for hydrilla. Four of these were exported to the Florida quarantine for further evaluation (Table 2), and two were eventually released.

In 1989, Balciunas joined USDA, ARS, and for three years headed a project, based at the Sino-American Biological Control Laboratory (SABCL), to find new agents for both hydrilla and Eurasian milfoil, *Myriophyllum spicatum L.*, in temperate parts of China. Since then, the USDA, ARS Invasive Plant Laboratory in Ft. Lauderdale, Florida has led the searches in China for hydrilla natural enemies, and has expanded the surveys to Thailand and Vietnam (Table 1). Staff of this laboratory have been assisted in these surveys not only by SABCL scientists, but by other scientists from the United States and the Australian Biological Control Laboratory (ABCL). The most promising insects identified during the past decade are listed in Table 2.

Overseas pathogens for controlling hydrilla also have been investigated, but far less extensively than the insects. During a three-month period in 1971 and 1972, surveys were conducted in India for pathogens of hydrilla (Charudattan, 1973). Of 40 fungi and 15 bacteria isolated and screened for pathogenicity, only two species, a *Pythium* sp. and a *Sclerotium* sp., were found to be damaging. Charudattan *et al. (*1980) reported that a pathogen, *Fusarium roseum* (Link ex Fr.) var. *culmorum* Snyd. and Hans. found on diseased *Stratiotes aloides* L. in The Netherlands, was efficacious on hydrilla. Staff of the Sino American Biological Control Laboratory also conducted surveys in the People's Republic of China in 1994 and 1995 for pathogens of hydrilla. All isolates were subsequently deposited at the USDA, ARS quarantine facility located at Fort Detrick, Frederick, Maryland. Following identification of the isolates, they were subjected to pathogenicity screening on the host. Six isolates (an unidentified Moniliaceous hyphomycete, an unidentified Coelomycete, *Phoma* sp., *Colletotrichum gloeosporioides* [Penz.] Penz. and Sacc. in Penz., and *M. terrestris* were found to induce disease symptoms on hydrilla. Additional pathogenicity testing on rooted plants has yet to be completed. If potential biological control candidates are found among the isolates they will have to undergo intense host specificity testing because some have been reported on other hosts (Farr *et al.,* 1989).

Host Range Tests and Results

The host range tests on the more than two dozen non-U.S. species of insects or pathogens that have been considered as potential biological control agents for hydrilla have been recorded in more than a hundred (mostly unpublished) reports. In Table 2, we summarize the primary test results for these potential agents. Only a few agents were tested extensively

Table 2. Candidate biological control agents evaluated for use against hydrilla.

Potential Agent	Primary Damage to Hydrilla	Country and Year First Collected	Where Tested	Test Results	References
Coleoptera: Chrysomelidae, subfamily Donaciinae					
Donacia australasiae Blackburn	larvae feed externally on stems	Australia 1985	Australia	no adults emerged; testing incomplete	Balciunas et al., 1996a
prob. *Donacia* sp.	larvae feed externally on stems	Vietnam 1996	Florida		Buckingham, pers. comm.
prob. *Macroplea* sp. 1	larvae feed externally on stems	China 1992	Florida	unable to rear adults from quarantine; additional field information needed	Buckingham, 1998
Coleoptera: Curculionidae					
Bagous affaber Faust (prev. *B.* sp. nr. *limosus* Gyllenhal, and *B. dilgiri* Vazirani*)*	larvae bore stems; adults feed on submersed stems and leaves	India 1982	Pakistan Florida	reproduced on *Potomogeton nodosus*; lab colony destroyed	Baloch et al., 1980 Balciunas, 1985 Buckingham and Bennett, 1998
Bagous affinis Hustache	larvae bore and develop inside tubers	Pakistan 1971	Pakistan India Florida	sufficiently host specific; released in Florida in 1987	Baloch et al., 1980 Balciunas, 1985 Buckingham, 1988 Buckingham and Bennett, 1998
Bagous hydrillae O'Brien	larvae bore stems; adults feed on submersed stems and leaves	Australia 1982	Australia Florida	narrow laboratory host range, and Australia field data confirming lack of impact on other hosts allows approval and release in 1991	Balciunas, 1985 Balciunas and Purcell, 1991 Buckingham, 1994 Balciunas et al., 1996b
Bagous laevigatus O'Brien and Pajni	larvae bore and develop inside tubers	Pakistan 1971 misidentified and tested with *B. affinis*	Pakistan Florida	prefers sago pondweed (*Potomogeton pectinatus* L.) tubers; lab colony destroyed	Baloch et al., 1980 Buckingham, 1994 O'Brien and Pajni, 1989 Bennett and Buckingham, 1991
Bagous latepunctatus Pic	larvae tunnel in stems; adults feed on submersed stems and leaves	India 1982 (mixed with *B. affinis*) Thailand 1997	Florida	completed life cycle on hydrilla and Najas in laboratory; further testing needed	Bennett and Buckingham, 2000
Bagous subvittatus O'Brien and Morimoto	larvae tunnel in stems; adults feed on submersed stems and leaves	Thailand 1997	Florida	broad host range in laboratory; additional data on field host range needed	Bennett and Buckingham, 2000
Bagous vicinus Hustache(prev., *B.* sp. nr. *lutulosus* Gyllenhal)	larvae feed on dessicating hydrilla; adults feed on submersed stems and leaves	Pakistan 1971	Pakistan Florida	since larvae damages only dessicating hydrilla, dropped from future consideration as a potential agent	Baloch et al., 1972 Baloch and Sana-Ullah, 1974 Baloch et al., 1980 Bennett, 1986 Buckingham, 1994
Bagous n. sp. (Thailand)	larvae bore stems; adults feed on submersed stems and leaves	Thailand 1997	Florida	broad host range in laboratory; additional data on field host range needed	Bennett and Buckingham, 1999

Table 2. Candidate biological control agents evaluated for use against hydrilla (continued).

Potential Agent	Primary Damage to Hydrilla	Country and Year First Collected	Where Tested	Test Results	References
Diptera: Chironomidae					
Polypedilum sp.	burrows into stem tips	Tanzania (Lake Tanganyika) 1977	Florida	unable to rear under laboratory conditions	Pemberton, 1980 Markham, 1986
Polypedilum dewulfi Goetghebuer and *Polypedilum wittae* Freeman	burrows into stem tips	Burundi 1990	Florida	unable to rear under laboratory conditions	Buckingham, 1994
Diptera: Ephydridae					
Hydrellia balciunasi Bock	larvae mine leaves	Australia 1982	Australia Florida	specific to hydrilla; released in Florida in 1989	Balciunas, 1985 Balciunas and Burrows, 1996 Buckingham et al.,1991
Hydrellia pakistanae Deonier	larvae mine leaves	Pakistan 1971	Pakistan Florida	hydrilla preferred host; released in Florida in 1987	Baloch et al., 1980 Balciunas, 1985 Buckingham et al., 1989
Hydrellia sarahae sarahae Deonier (prev., *Hydrellia* n. sp. CH-1, and "silver-faced Hydrellia")	larvae mine leaves	China 1989	China India Florida	host range appears broad; more field data needed	Balciunas, 1990 Krishnaswamy and Chacko, 1990 Bennett, 1993 Bennett and Buckingham, 1999
Hydrellia n. sp. (Japan)	larvae mine leaves	Japan 1991		laboratory colony not established	Buckingham, 1994
Hydrellia n. sp. (Korea)	larvae mine leaves	Korea 1991		laboratory colony not established	Buckingham, 1994
Hydrellia n. sp. (Thailand)	larvae mine leaves	Thailand 1997	Florida	testing incomplete	Bennett and Buckingham, 1999
Lepidoptera: Pyralidae					
Ambia ptolycusalia Walker (prev., *Nymphula eromenalis* Snellen)	larvae eat leaves, defoliating the stems	Australia 1982	Australia Florida	laboratory colony not established; research incomplete	Balciunas et al., 1989 Buckingham, 1994
Margarosticha repetitalis Warren (prev., *Strepsinoma repititalis* Walker)	larvae eat leaves, defoliating the stems	Australia 1982	Australia	present on other hosts in the field in Australia, not recommended for use as biological control agent	Balciunas et al., 1989 Balciunas et al., 1996a
Parapoynx diminutalis Snellen (prev., *Nymphula dicentra* Meyrick)	larvae eat leaves, defoliating the stems	India 1971 Pakistan 1971	India Malaysia Pakistan Phillipines Florida	host range determined too broad for release, but was later discovered to have immigrated to Florida	Rao, 1969 Baloch and Sana-Ullah, 1974 Varghese and Singh, 1976 Chantaraprapha and Litsinger, 1986 Buckingham and Bennett, 1996

Table 2. Candidate biological control agents evaluated for use against hydrilla (continued).

Potential Agent	Primary Damage to Hydrilla	Country and Year First Collected	Where Tested	Test Results	References
Lepidoptera: Pyralidae (continued)					
Parapoynx sp. nr. *rugosalis* (prev., *P. rugosalis*)	larvae eat leaves, defoliating the stems	Panama 1977	Panama	larvae prefer hydrilla and Najas; tests attempted but *P.* sp. nr. *rugosalis* could not be recollected in Panama (completely replaced by *P. diminutalis*)	Balciunas and Center, 1981 Buckingham and Bennett, 1996 Habeck, pers. comm.
Theila siennata Warren (prev., *Aulacodes siennata* Warren)	larvae eat leaves, defoliating the stems	Australia 1982	Australia Florida	laboratory colony not established; research incomplete	Balciunas et al., 1989 Buckingham, 1994 Balciunas et al., 1996a
Pathogens					
Fusarium roseum (Link ex Fr.) var. *culmorum* Snyd. and Hans. (Hyphomycetes)		The Netherlands	Florida		Charudattan and McKinney, 1977 Charudattan et al., 1980 Charudattan et al., 1984

overseas, and their host range tests subsequently published in refereed journals, e.g., Balciunas and Center (1981), Balciunas and Burrows (1996), and Balciunas *et al.* (1996b). Nearly 20 hydrilla insect species were shipped to the quarantine facility in Gainesville, Florida for evaluation (Table 2). The testing there was conducted by Gary Buckingham, USDA, ARS, and University of Florida cooperators. Heightened concern for safety has increased the number of plant species tested, and the hydrilla agents eventually approved for release were tested on more than 60 species of plants in 30 families (Buckingham, 1994). Although a few species were conclusively ruled out as having too broad a host range, testing of many remains incomplete. Eventually, however, sufficient laboratory and field data was gathered to gain approval for release of two weevils and two leaf-mining flies. Although none of these four insects were strictly monophagous, hydrilla was greatly preferred, and the risk to the few other alternate hosts was considered very minimal.

Releases Made

Many of the natural enemies identified during overseas surveys still have not been fully evaluated to judge their safety as potential biological control agents for hydrilla. Only four hydrilla insects have been released in the United States: The tuber attacking weevil *Bagous affinis* Hustache (Coleoptera: Curculionidae) and the leaf mining fly *Hydrellia pakistanae* Deonier (Diptera: Ephydridae) were both released in 1987; another leaf-mining fly *H. balciunasi* Bock (Diptera: Ephydridae) was released in 1989; and the stem-mining weevil *B. hydrillae* O'Brien (Coleoptera: Curculionidae) was released in 1991 (Buckingham, 1994).

The leaf-mining flies have been the most extensively released species. *Hydrellia pakistanae* has been released at more than 50 sites in Alabama, California, Florida, Georgia, Louisiana, and Texas (Center *et al.*, 1997). About 1.2 million individuals were obtained, mainly from greenhouse colonies maintained at the U.S. Army Engineer Research and Development Center in Vicksburg, Mississippi and various USDA, ARS facilities, along with an additional two million insects from a Tennessee Valley Authority pond-based rearing facility (Grodowitz and Snoddy, 1995). These releases ended in 1995. Recently (September 2000), releases resumed using *Hydrellia*-containing hydrilla obtained from ponds at the Lewisville Aquatic Ecosystem Research Facility, Lewisville, Texas with more than 300,000 immatures being released in Lake Raven in Huntsville State Park, Texas.

Although considerably less effort went into the release of *H. balciunasi*, still close to one million individuals were released at 11 sites in Florida and Texas only (Grodowitz *et al.*, 1997).

Bagous affinis was extremely difficult to maintain under mass-rearing conditions. This was due primarily to the high demand of tubers for larval feeding. However, over 10,000 individuals were released in three states (i.e., California, Florida, and Texas) at more than 10 locations (Godfrey *et al.*, 1994; Grodowitz *et al.*, 1995).

A larger effort went into the release of the stem-feeding weevil, *B. hydrillae*. For example, close to 300,000 individuals have been released in four states (Florida, Texas, Georgia, and California) at more than 15 locations (Grodowitz *et al.*, 1995).

No overseas pathogens have yet been approved for release to control hydrilla.

BIOLOGY AND ECOLOGY OF KEY NATURAL ENEMIES

Hydrellia pakistanae - "Asian Hydrilla Leaf Mining Fly" and *Hydrellia balciunasi* - "Australian Hydrilla Leaf Mining Fly" (Diptera: Ephydridae)

Hydrellia pakistanae and *H. balciunasi* are small leaf-mining ephydrid flies. *Hydrellia pakistanae* (Fig. 5) is an Asiatic species, first released in the United States on Lake Patrick, Florida in 1987 (Buckingham *et al.*, 1989). It is very similar in habit and appearance to another introduced ephydrid, *H. balciunasi*, an Australian species first released in the United States in 1991 (Buckingham *et al.*, 1991). Both species are small, about 2 mm in length, and live almost exclusively on or near hydrilla infestations. The introduced *Hydrellia* spp. are apparently not strong flyers and appear to hop along the water surface from one resting place to another (Deonier, 1971).

Adult *H. pakistanae* and *H. balciunasi*, the two introduced *Hydrellia* spp. can be difficult to identify because of their small size, lack of obvious distinguishing characters, and similarity to other native species of *Hydrellia* (including *H. bilobifera* Cresson and *H. discursa* Deonier). Examinations of reproductive organs are frequently required for positive identification. Adult male *H. pakistanae* can be distinguished from other commonly collected native *Hydrellia* spp. and *H. balciunasi* by several characters, including the length of the thorax in comparison to the abdomen length, the presence of crossed or cruciate macrochaetae, and the shape and size of the macrochaetae (ERDC 2001a, b).

Figure 5. Adult female *Hydrellia pakistanae* on hydrilla leaf (photograph courtesy of USDA, ARS).

To separate the introduced *Hydrellia* spp. from native individuals, the size of the abdomen and the shape and position of the macrochaetae are used. The abdomen in both species of introduced *Hydrellia* is relatively short and is roughly the same size as the thorax (Fig. 6). In contrast, for males of all the commonly encountered native *Hydrellia*, the abdomen is 1.5 to 2 times the length of the thorax. In addition, both *H. pakistanae* and *H. balciunasi* have crossed or cruciate macrochaetae (Fig. 6).

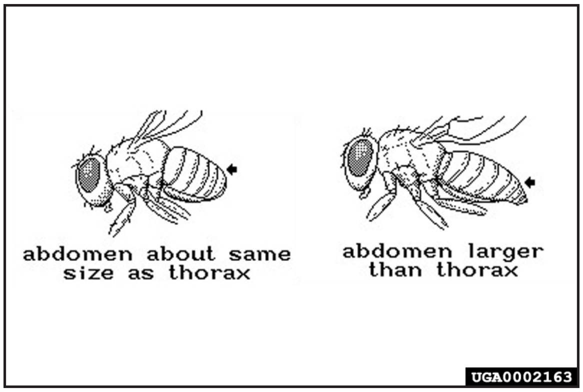

Figure 6. Diagram of relative sizes of the abdomen in both the native *Hydrellia spp.* (right) and the introduced species (left).

The only way to accurately separate *H. pakistanae* from *H. balciunasi* is by the shape and size of the macrochaetae, which are small hair-like structures associated with the male external reproductive structures and are thought to be responsible for holding the female in place during copulation (Deonier, 1971). In both introduced species of *Hydrellia*, the macrochaetae are crossed or cruciate, but in *H. pakistanae* they are small and more distinctly needle-shaped, while those of *H. balciunasi* are larger and appear flattened at the tip (Fig. 7).

Female *Hydrellia* are distinguished from native and other introduced *Hydrellia* by the morphology of the genitalia, especially the shape of the cerci (ERDC 2001a, b). The cerci are hooked or L-shaped in *H. pakistanae* as compared to arrow- or diamond-shaped in *H. balciunasi* (Fig. 8).

The larvae are cream colored and relatively nondescript. There are few morphological differences between the species; the most notable being in the feeding apparatus and spiracular peritreme (Deonier, 1971).

Eggs are laid on hydrilla or almost any emergent aquatic vegetation near hydrilla infestations (Buckingham *et al.*, 1989; Buckingham *et al.*, 1991). Females lay eggs singly, and each female can produce several hundred eggs during her reproductive period. Eggs hatch in three to four days, depending on temperature. Larvae tunnel or mine hydrilla leaves, feeding and destroying about nine to 12 leaves during the three larval stages. Late third instars pierce the stem tissues with portions of the spiracular peritremes, which are modified into two needle-like projections that subsequently provide oxygen to the pupae (Deonier, 1971). Pupae are formed within a puparium, and the pupal stage lasts six to 15 days attached to the stem typically in the leaf axils, after which the adult floats to the surface in an air bubble after emerging from the puparium. Total development time is from 20 to 35 days. The overwintering stage is unknown but larvae have been found on hydrilla throughout the entire winter. The total number of generations per growing season appears to be highly variable and related to geographic area but may be as high as seven.

From a distance, a hydrilla mat containing large numbers of *Hydrellia* spp. appears brown, and upon close examination, one can observe clusters of leaves along the stem where feeding has occurred. Damage

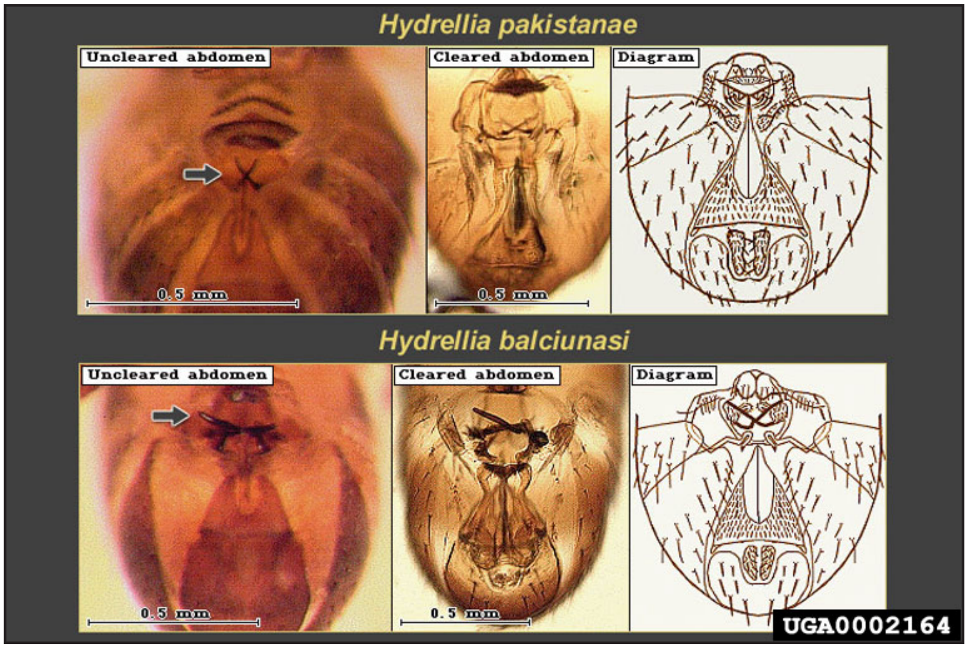

Figure 7. Ventral views of the abdomens' of both *H. pakistanae* and *H. balciunasi* showing the morphology of the external male genitalia. Note the cruciate or crossed macrochaetae in both species, a configuration that is not found in native *Hydrellia* species. The primary difference between the two introduced species is the size and shape of the macrochaetae. In *H. pakistanae*, the macrochaetae are smaller and needle-like in comparison to *H. balciunasi* where the macrochaetae are larger and spoon-shaped at the ends. (Photographs courtesy of ERDC 2001a, b.)

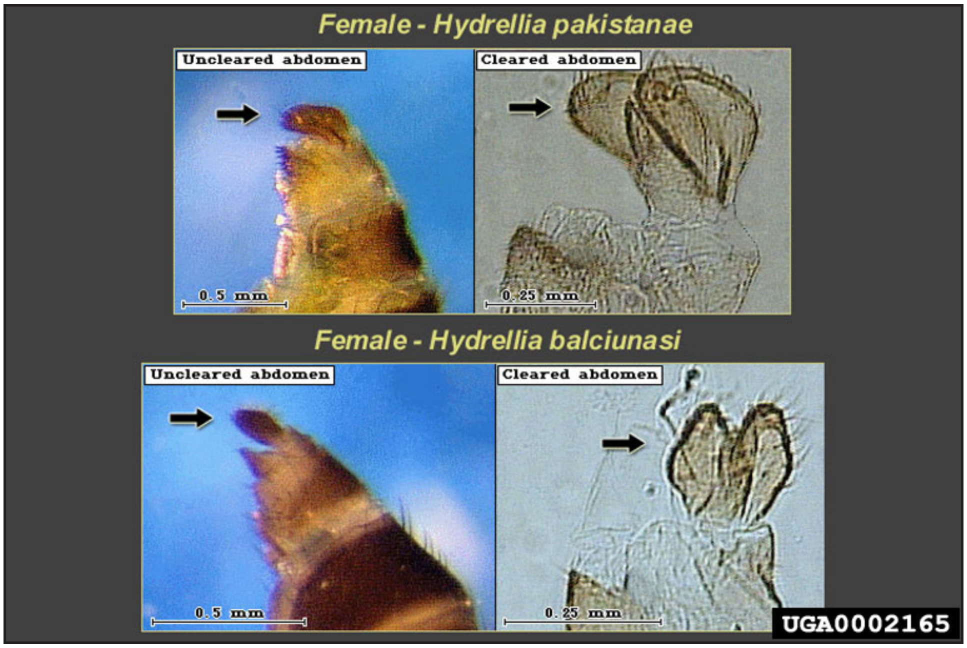

Figure 8. The cerci, located at the posterior end of the abdomen, are used to identify female *Hydrellia*. In *H. pakistanae,* the cerci are distinctly L-shaped in contrast to *H. balciunasi,* where the cerci are roughly triangular. (Photographs courtesy of ERDC 2001a, b.)

to hydrilla is probably due to a reduction in total photosynthetic area caused by the leaf damage (Doyle *et al*., 2002), which reduces growth and vigor and leads to a decrease in the competitiveness of the affected plants. In addition, some evidence suggests that feeding may reduce the buoyancy of the plant and allow the stem to become more brittle in areas of heavy feeding, leading to stem fragmentation (Grodowitz *et al.,* 1999). Limited field observations suggest that *Hydrellia* feeding may predispose the plant to infection by fungi and other pathogens.

Bagous affinis - "hydrilla tuber weevil" (Coleoptera: Curculionidae)

Adult weevils are brown to dark brown, and frequently have a mottled appearance (Fig. 9) (ERDC 2001a, b; Bennett and Buckingham,1991). Unlike the hydrilla stem-feeding weevil, the tuber weevil cannot live if submerged for extended periods. Adults are relatively long-lived, surviving under laboratory conditions from 55 to 225 days. Females are known to produce upwards of 650 eggs throughout their reproductive period. Eggs are roughly spherical and creamy white. Eggs are laid on hydrilla stems, tubers, or moist wood and apparently not on any submersed material. Eggs hatch after three to four days, and the emerging larvae crawl through the drying sediment in search of tubers. There are three larval instars and they are non-descript and typically creamy-white. The larvae can be found on or within the hydrilla tubers, where they burrow and feed. The larvae pupate within the tubers but also can pupate in nearby moist wood. The duration of the larval stage is anywhere from 14 to 17 days. The pupal stage lasts four to six days.

While the adults feed on the tubers, their damage is minimal compared to the destructiveness of the larvae. The larvae can attack and destroy tubers deep within the sediment. High weevil populations have been reported from hydrilla-infested ponds in the insect's native range.

Bagous hydrillae - "hydrilla stem weevil" (Coleoptera: Curculionidae)

Adult *B. hydrillae* are dark brown with a distinctly mottled body appearance (Fig. 10) (ERDC 2001a, b; Balciunas and Purcell, 1991). In many individuals, two to four light spots can be seen on the posterior portion of the elytra. There are three larval instars, each lasting from three to four days. The pupa is naked, with no cocoon or other protective structure. Total development time ranges from 2.5 to 3 weeks (Buckingham and Balciunas, 1994).

Adults can be found on submersed hydrilla as well as on hydrilla that washes up on the shoreline. Adults feed externally on leaf and stem tissues of

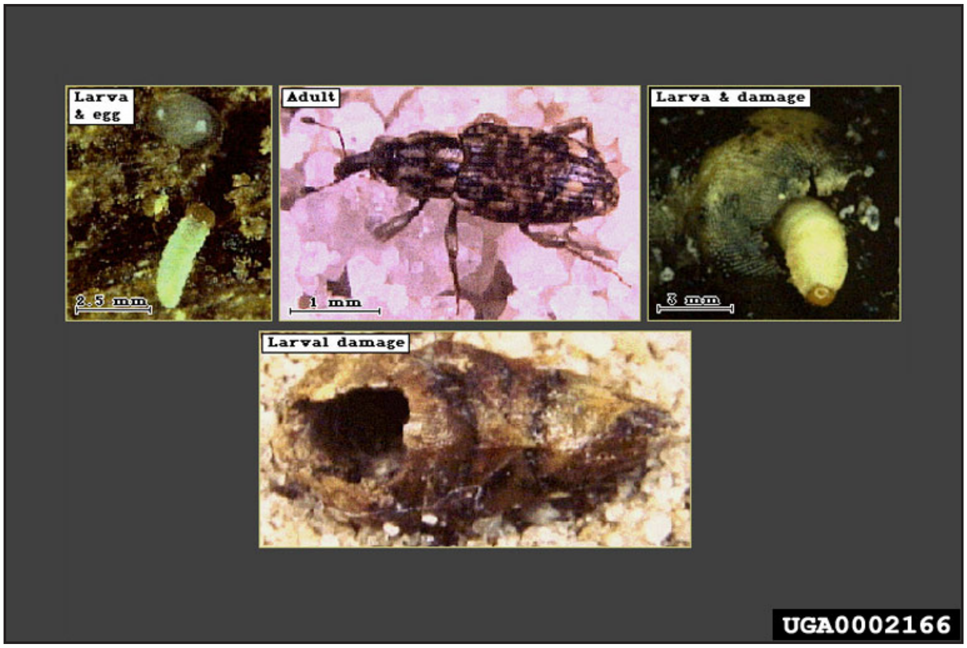

Figure 9. The life stages and feeding damage of *Bagous affinis.* (Photographs courtesy of ERDC 2001a and b and USDA, ARS.)

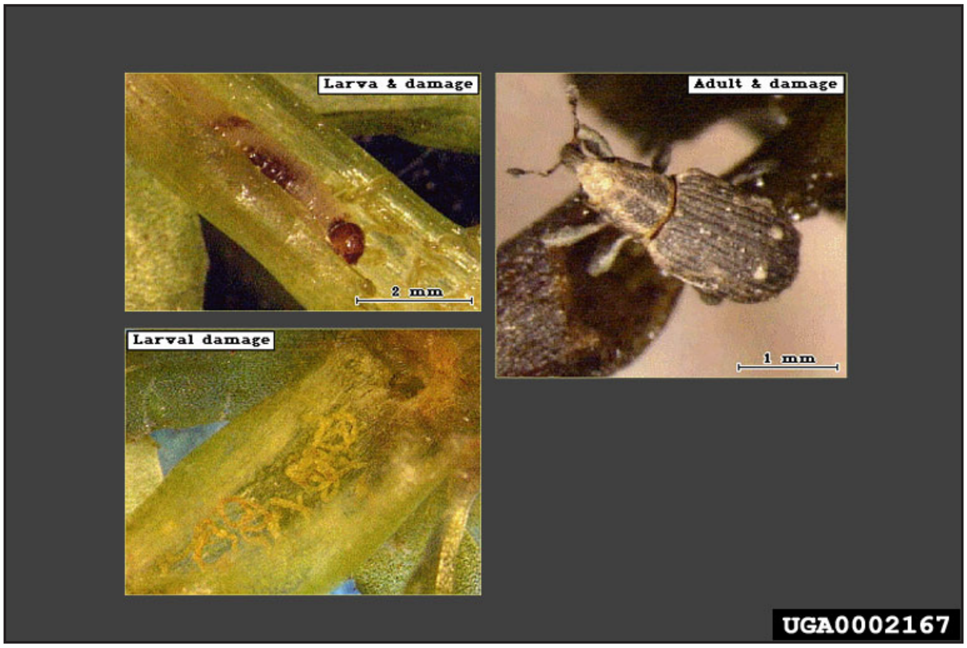

Figure 10. *Bagous hydrillae* adult and larvae, and associated larval damage. (Photographs courtesy of ERDC 2001a and b and USDA, ARS.)

drying or submersed hydrilla, apparently preferring the stem tissue at the internodes. Eggs are laid within stem tissues usually at the leaf nodes. Eggs hatch in three to four days and larvae feed throughout internal stem tissues. Larval feeding subsequently fragments the stem, which floats to the shoreline where the third instars exit and subsequently pupate within soil or drying hydrilla. Pupation must take place under relatively dry conditions. The pupal period lasts from three to four days depending on the ambient temperature.

Since no permanent populations of *B. hydrillae* exist in the United States, large-scale damage has not been observed; however, researchers in Australia have indicated that larval feeding by *B. hydrillae* causes the plants to have a mowed appearance due to the removal of the hydrilla from the surface to a depth of 100 cm (Balciunas and Purcell, 1991).

EVALUATION OF PROJECT OUTCOMES

Establishment and Spread of Agents

Although four insects have been released, neither of the weevils appears to have established, and *H. balciunasi* has only been recovered from a few sites in east Texas (Bennett and Buckingham, 1999; Grodowitz *et al.,* 2000a). However, *H. pakistanae* established and dispersed readily and is now found throughout Florida; north to Muscle Shoals, Alabama; west to Austin, Texas; and south to the lower Rio Grande Valley (Center *et al.,* 1997; Grodowitz *et al.,* 1997; Grodowitz *et al.,* 2000a). Populations of both species, but especially *H. pakistanae*, have expanded in distribution considerably since they were first released. For example, *H. pakistanae* was released in the early 1990s at only one location, Lake Boeuf in extreme southern Louisiana, but surveys conducted in 2000 revealed its presence at several locations up to 300 km west and north of the original introductions (Freedman and Grodowitz, unpub.). In Florida, *H. pakistanae* is found associated with a majority of sites containing hydrilla infestations, indicating considerable range expansion (Center, 1992; Center, pers. comm.). In Texas, populations of *H. pakistanae* and *H. balciunasi* also have increased considerably from the four original release sites. One of the most interesting findings has been the discovery of *H. pakistanae* in the extreme south central portion of Texas on the Rio Grande, more than 250 km from the nearest release site (Grodowitz *et al.,* 1999). Such range extensions are surprising since the introduced *Hydrellia* spp. are relatively weak fliers with short adult life spans. In addition, the non-contiguous lake systems in both Texas and Louisiana should have hampered range extension for these species. At many sites throughout the country, especially non-release sites, *Hydrellia* spp. population levels appear minimal with less than 200 immature insects/kg wet weight of hydrilla and leaf damage not exceeding 2%.

Bagous affinis was originally described from India and Pakistan and was first released in the United States in Florida in 1987 (Bennett and Buckingham, 1991). As of the spring of 2001, no permanent populations were known to exist in the United States. Because of its strict environmental requirement for distinct wet/dry periods to allow access to buried tubers, this species has not been released at many sites. Releases in California, at locations where water levels can be controlled, have indicated that this weevil can successfully establish and, with appropriate water level management, overwinter (Godfrey *et al.,* 1996). Unfortunately, because of the hydrilla eradication program in California, the hydrilla at the California site was destroyed soon after verifying overwintering. The use of biological control in conjunction with an aggressive eradication program is counterproductive.

Bagous hydrillae was first released in the United States in Florida in 1991 (Grodowitz *et al.,* 1995), but no established populations have been confirmed. Extensive surveys were initiated, however, no weevils have been recovered at actual release sites even after extended periods. *Bagous hydrillae* adults have only been collected after suspension of releases at one site, Choke Canyon Reservoir, Texas during 1993 and 1994 (Grodowitz et al., 1995). However, soon after the termination of releases *B. hydrillae* adults were no longer observed at Choke Canyon Reservoir.

Suppression of Target Weed and Recovery of Native Plant Communities

Impact of the introduced *Hydrellia* spp. has apparently been observed at several release sites in Georgia, Florida, and Texas. For example, significant changes have been observed in the hydrilla status at Lake Seminole, Georgia, over the last few years, following the release of more than 1.5 million *H. pakistanae* in 1992 (Grodowitz et al., 1995; Grodowitz, Cofrancesco, Stewart, and Madsen, unpub.). For the first several years following this large release, numbers of *H. pakistanae* in Lake Seminole remained at low but detectable levels based on the presence of immatures on randomly selected stem pieces and Berlese funnel extraction of plant material. Beginning in 1997, hydrilla populations began to decline in various areas of the lake and increases in plant diversity were observed that appeared related to increasing *H. pakistanae* populations. In 1999, large numbers of *H. pakistanae* adults were observed throughout large areas of the lake and these correlated with significant decreases in hydrilla populations and increases in other native plants, including several species of *Potamogeton* and *Najas*.

Quantitative sampling of *Hydrellia* immatures based on stem counts and quantification of number of leaves damaged in September 1999 revealed the presence of more than 2,000 immatures per kg wet weight of hydrilla and close to 20% of the total number of leaves damaged. Quantitative plant sampling conducted during November showed significant reductions (ca. four-fold) in tuber numbers and three-fold increases in species richness in areas significantly affected by *H. pakistanae* feeding as observed in September.

While reductions in tuber numbers were surprising, such reductions have been substantiated during large-scale, long-term replicated tank studies conducted recently (Grodowitz *et al.,* 2000b; Doyle, Grodowitz, Smart, Owens, unpub.) and in short-term small container studies (Doyle *et al.,* 2002). In these studies, lower number of tubers and biomass occurred in biological control treatments where damage exceeded 40% of the leaves only for short durations. Similar reductions in hydrilla were observed at Coleto Creek Reservoir, Texas in 1999 and 2000. Reductions in hydrilla were first observed in the two original release sites in 1998 continuing through 1999. Currently, only small quantities of hydrilla persist at the original release sites and reductions in hydrilla have been observed in a nearby cove where fly densities and hydrilla status were quantified for many years to be used as a control. In 1999, higher fly levels were observed in the control cove followed by substantial hydrilla declines in 2000. Observations of the lake in 2000 have shown increasing fly numbers and associated damage throughout the entire reservoir. Sampling of stems during November 2000 demonstrated leaf damages in the 12 to 15 % range for hydrilla located in the extreme northern portion of the lake (Grodowitz *et al.,* 1999; Grodowitz, unpub.). Similar effects also have been observed in Sheldon Reservoir near Houston, Texas (Grodowitz *et al.,* 1999). In these situations, as the hydrilla declined, it was apparently replaced with a mixture of submersed plants, including Eurasian watermilfoil (*Myriophyllum spicatum* L.), star grass (*Heteranthera dubia* [Jacquin] MacM.), coontail (*Ceratophyllum demersum* L.), and various species of *Potamogeton* and *Najas*, as well as an emergent species, American lotus (*Nelumbo lutea* [Willd.] Pers.). Unfortunately, the causal relationship between fly establishment and decline in hydrilla is frequently difficult to document. Declines may only be partial and localized. Detailed data are not available to document high levels of larvae in leaves of hydrilla before declines, and natural fluctuations in densities of submersed aquatic plants, such as hydrilla, are common.

Economic Benefits

Economic benefits of the introduced leaf-mining flies in the genus *Hydrellia* cannot yet be evaluated. The effects of these species are just now becoming visible and ongoing evaluation programs will be needed to measure any economic benefits procured.

RECOMMENDATIONS FOR FUTURE WORK

There are four major areas that should to be considered for future work: 1) domestic surveys to evaluate the current expansion and effect of the *Hydrellia* spp. flies that are already established; 2) assessing the influence abiotic and biotic factors have on establishment success and population build-up of these species; 3) developing improved methods for their mass rearing; and 4) conducting overseas surveys to locate previously identified and new biological control agents, especially in regions not studied previously.

Continued field monitoring is needed to gain a clearer understanding of the potential impact of species of *Hydrellia* flies. This effort should include the development of lower cost, labor-efficient methods to measure hydrilla declines. Measuring changes in submersed plant populations has proven to be more difficult and costly than for terrestrial or floating plants. While range expansion of biological control agents is relatively easy to quantify, it is difficult to measure their impact since weed population changes occur over several growing seasons, with gradual replacement of hydrilla monocultures by mixtures of various native and non-native submersed plants (Grodowitz *et al.,* 1999). Such evaluations are made even more difficult by the patchy distributions of these flies, which also can vary greatly between years at single locations. Reasons for such variation is unknown but could possibly be related to a complex of abiotic and biotic factors including overwintering conditions, plant nutritional variation, parasite loads, etc. For example, Grodowitz et al. (1995) cited that unusually cold weather and the lack of large releases was apparently the cause of declines in *H. pakistanae* populations in 1994 in Muscle Shoals, Alabama ponds.

While many widespread releases of hydrilla biological control agents were made in the early 1990s, introductions into new areas have virtually ceased. Recent research indicates that population size of leaf-mining flies in a given water body is related to release status. For example, more than seven-fold higher numbers of immatures and percentage leaf damage was associated with actual release sites in Texas, Florida, and Georgia surveyed during 1998 and 1999 (Fig. 11). This strongly indicates the need for further releases of large numbers of individuals at sites that have never had releases previously.

However, rearing large numbers of flies is expensive, with costs per fly exceeding $0.50 per immature in greenhouse mass-rearing colonies (Freedman and Grodowitz, unpub.). Hence, a typical release of 50,000 individuals per site would cost more than $25,000 and be prohibitively expensive. Research to develop more cost effective rearing procedures is underway. For example, a mass-rearing facility based on the use of small ponds at an abandoned fish hatchery of the Tennessee Valley Authority Reservation in Muscle Shoals, Alabama, was highly successful (Grodowitz and Snoddy, 1995). A single harvest from a pond at this facility yielded more than 1.5 million flies and resulted in fly establishment throughout Lake Seminole, a large reservoir that borders both Florida and Georgia (Grodowitz, Cofrancesco, Stewart and Madsen, unpub.). While exact production costs are unknown it was significantly lower than the $0.50 per fly costs associated with greenhouse rearing techniques. Recently, a mass rearing system using a series of small ponds was implemented at the Lewisville Aquatic Ecosystem Research Facility in Lewisville, Texas. During 2000 and 2001 these ponds produced more than 600,000 individuals. Rearing costs were significantly lower, being less than $0.03 per immature (M. J. Grodowitz and R. Bare, unpub.). A similar, but smaller facility is currently under construction at the U.S. Army Engineer Research and Development Center in Vicksburg, Mississippi. Such facilities and procedures can significantly increase the number of sites at which releases can be made; however, local cooperation by state wildlife personnel and local water authorities is needed to facilitate the release of mass-reared flies.

Another area where more work is needed is in the understanding of the influence that abiotic and biotic factors have on fly establishment and population increase. Both laboratory and tank studies have quantified the influence of the plant's nutritional composition on growth of *Hydrellia* spp. flies (Wheeler and Center, 1996; Doyle, Grodowitz, and Smart, unpub.). Tissue nutritional components can significantly affect fly survival, development times, fecundity, and female weight (an indicator of overall

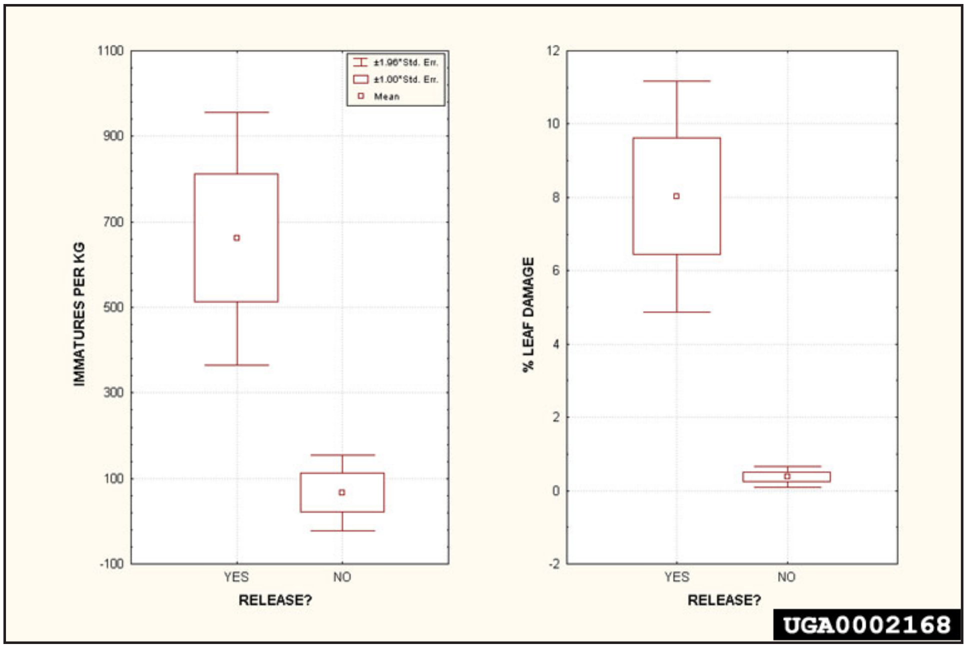

Figure 11. Mean number of immatures per kg and % leaf damage at release and non-release sites during 1999 and 2000 for sites in Texas, Georgia, and Florida.

health). Nutritional components that appear to be important include nitrogen content and possibly phosphorus content, with higher levels increasing the overall health and vigor of the flies. Preliminary field data has indicated higher fly damage at sites with higher nitrogen levels (Wheeler and Center 2001; Grodowitz and Freedman, unpub.) but further information is needed to verify relationships between establishment success and population increase with plant nutritional composition.

Among biotic factors of importance, more research is needed evaluating the impact of the pupal parasite *Trichopria columbiana* Ashmead, a diapriid wasp that attacks native *Hydrellia* species. Parasitism of the introduced *Hydrellia* species by *T. columbiana* can reach 30% by the end of the growing season in small ponds (Snell and Grodowitz, unpub.). However, the actual effect on fly population growth of removing 30% of the pupae from a given habitat is unknown. Also, *T. columbiana* may induce even higher mortality by probing pupae and hence causing mortality while searching for suitable oviposition sites (Bare and Grodowitz, unpub.).

Highest priority for additional research needs to be given to the collection and study of new agents from overseas locations that attack permanently submersed hydrilla. Complexes of organisms that feed on and damage a variety of plant tissues are frequently needed to effectively suppress a target plant. In the case of hydrilla only one part of the plant, the leaves, are affected by established biological control agents. For efficient suppression, other agents are needed that, for example, could damage stems, roots, apical tips, turions, and/or tubers. Foreign exploration should target areas of the world that have received only limited previous attention, such as Southeast Asia. For example, several weevil species with potential as hydrilla herbivores have previously been identified (Table 2) but were never examined in any great detail.

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