Recent Advances in Biological Control of Submersed Aquatic Weeds

J. P. CUDA[1], R. CHARUDATTAN[2], M. J. GRODOWITZ[3], R. M. NEWMAN[4],
J. F. SHEARER[3], M. L. TAMAYO[5] AND B. VILLEGAS[6]

ABSTRACT

The submersed aquatic plants hydrilla (*Hydrilla verticillata* [L.f.] Royle), Eurasian watermilfoil (*Myriophyllum spicatum* L.),and Brazilian egeria (*Egeria densa* L.) are three of the worst invasive aquatic weed problems in the U.S., with millions of dollars spent annually to control large infestations in all types of waterbodies. Historically, various control technologies have been used to manage infestations of these submersed species, including biological control. During the past five years, there has been renewed interest in biological control of submersed aquatic weeds nationally, primarily in response to the discovery in Florida of several hydrilla biotypes that have developed resistance to the herbicide fluridone. This paper summarizes the current status of biological control activities in North America during the past 10-15 years. It includes a preferred definition of biological control and describes the different approaches currently used by practitioners in the field. It also covers the types of natural enemies commonly used as biological control agents and the various abiotic, biotic, and technical factors that have contributed to project successes and failures. Finally, priority areas are identified where more resources are needed for research and outreach programs to increase the effectiveness and acceptance of biological control technology for managing submersed aquatic weeds in the future.

Key words: Brazilian egeria, hydrilla, Eurasian watermilfoil, limiting factors, natural enemies.

INTRODUCTION

"One of the success stories revealed in the catalogue [by Julien and Griffiths] is the biological control of several major water weeds; yet 40 years ago they were regarded as unpromising targets."—*in* Forward by D. F. Waterhouse, Julien and Griffiths (1998: vi).

This review is not intended to be a comprehensive treatment of biological control methods for all aquatic weeds. Instead, it will: (1) focus on the use of arthropods (mainly insects), fish, and pathogens, both introduced and naturalized, for biological control of submersed aquatic weeds; (2) examine the factors contributing to the repeated and often predictable control of certain aquatic weeds as well as identify possible reasons for failure; and (3) discuss biological control research and outreach priorities for the most invasive submersed aquatic plant species.

For general information on the theoretical and practical aspects of weed biological control, consult recently published references (Harris 1991, Harley and Forno 1992, Center et al. 1997a, Deloach 1997, Julien and White 1997,

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[1]Entomology and Nematology Department, Institute of Food and Agricultural Sciences, P.O. Box 110620, University of Florida, Gainesville, FL 32611-0620, e-mail: jcuda@ufl.edu.

[2]Plant Pathology Department, Institute of Food and Agricultural Sciences, P.O. Box 110680, University of Florida, Gainesville, FL 32611-0680.

[3]U.S. Army Engineer Research and Development Center, CEERD-EE-A, 3909 Halls Ferry Road, Vicksburg, MS 39280.

[4]Fisheries, Wildlife and Conservation Biology, 120 Hodson Hall, 1980 Folwell Ave., University of Minnesota, St. Paul, MN 55108-6124.

[5]Washington Cooperative Fish and Wildlife Research Unit, School of Aquatic and Fishery Sciences, Box 355020, University of Washington, Seattle, WA 98195-5020.

[6]California Department of Food and Agriculture, Biological Control Program, 3288 Meadowview Rd., Sacramento, CA 95832. Received for publication June 21, 2006 and in revised form February 21, 2007.

McFadyen 1998, Bellows and Headrick 1999, Goeden and Andres 1999, Isaacson and Charudattan 1999, Rosskopf et al. 1999, Van Driesche et al. 2002, Randall and Tu 2003, Coombs et al. 2004, Cuda 2004). In addition, the following reviews, devoted exclusively to aquatic weed biological control, are highly recommended (Charudattan 1990, 2001, Harley and Forno 1990, Pieterse 1990, Buckingham 1994, Center 1994, Barreto and Evans 1996, Madsen 1997, Cofrancesco 1998, Center et al. 1990, 2002, Barreto et al. 2000, Balciunas et al. 2002, Johnson and Blossey 2002).

DEFINITION AND SCOPE OF BIOLOGICAL WEED CONTROL

Defining biological control in the context of other pest management practices is like differentiating between a wetland and an aquatic weed. The transition from one to the other is often difficult to distinguish. Recent advances in the field of biological control, particularly in the area of biotechnology (Nordlund 1996), and changes in public policy in the last two decades have generated a new definition of biological control, which states ". . . the use of natural or modified organisms, genes, or gene products to reduce the effects of undesirable organisms (pests), and to favor desirable organisms such as crops, trees, animals, and beneficial insects and microorganisms" . . . (Anonymous 1987). Regrettably, this expanded all-inclusive definition of biological control advocated by the U.S. Committee on Science, Engineering, and Public Policy (COSEPUP) fails to capture the "natural enemy" component that is the foundation of the discipline of biological control.

Although there are numerous definitions of biological control, we follow the definition proposed by DeLoach (1997) because it preserves the natural enemy aspect that sets biological control apart from other methods of weed control. He defines biological control of weeds as ". . . the planned use of undomesticated organisms (usually insects or plant pathogens) to reduce the vigor, reproductive capacity, or density of weeds . . . it excludes cultural controls (grazing management, crop rotation, etc.) and natural control (the action of organisms without human intervention) . . ." The intentional use of grass carp for aquatic weed control is consistent with this definition. However, the application of biotechnology to genetically modify organisms as well as cultural practices like hand weeding, plant competition, allelopathy, and other management practices that alter the biotic balance of the soil are not included in this definition.

Three different approaches are currently used in the biological control of aquatic weeds: classical (importation), nonclassical (augmentation), and conservation (habitat manipulation) (Julien and White 1997, McFadyen 1998, Goeden and Andres 1999). The classical approach is by far the most common method and typically involves the planned introduction of natural enemies from their native range to control a nonnative invasive species. Researchers travel to the native range of the invasive plants to find natural enemies of the unwanted species. These organisms are tested initially for efficacy and host-specificity in their native range; successful candidates are then imported under permit into approved containment laboratories for final host range testing. Before scientists can re-

lease a natural enemy into the U.S. for classical biological control of an invasive aquatic plant, the potential agent must undergo rigorous testing in quarantine to ensure it will not harm nontarget species. The candidate agent is exposed to a series of carefully chosen test plants in no-choice and multiple-choice replicated trials to determine if the natural enemy is safe to release. The U.S. Department of Agriculture Animal and Plant Health Inspection Service, Plant Protection Quarantine unit (APHIS PPQ) controls the release approval process (Buckingham 1994, Scoles et al. 2005). A voluntary multiagency Technical Advisory Group reviews information provided by the requesting scientist prior to making a recommendation to APHIS PPQ concerning the release of an agent (Buckingham 1994, Scoles et al. 2005).

The augmentation approach involves the mass rearing and periodic releases of resident or naturalized aquatic weed biological control agents to increase their effectiveness. This approach is used primarily with pathogens, but it also can extend to other types of natural enemies, such as insects (Grodowitz 1998, Jester et al. 2000, Hairston and Johnson 2001) and fish (Cassani 1996, Sutton and Vandiver 1998). For instance, augmentative releases of native or naturalized insects have been proposed for biological control of water hyacinth (*Eichhornia crassipes* [Mart.]) Solms; Center and Hill 2007), hydrilla (*Hydrilla verticillata* [L.f.] Royle; Cuda et al. 2002, Wheeler and Center 2007), and Eurasian watermilfoil (*Myriophyllum spicatum* L.; Jester et al. 2000).

The conservation approach involves identifying and manipulating factors to enhance the abundance of potentially effective native or introduced natural enemies of aquatic weeds. Although conservation strategies have rarely been exploited (Harris 1993), their importance in the biological control of aquatic weeds is now gaining recognition (MacRae et al. 1990, Creed and Sheldon 1995, Sheldon and O'Bryan 1996, Newman et al. 1998, Creed 2000, Tamayo et al. 2000, 2004, Newman 2004).

The "new association" approach is a variation of classical biological control first proposed by Pimental (1963) and later by Hokkanen and Pimental (1984). They contend that natural enemies from closely related plant species growing in similar climates but different geographical areas from the target plant are potentially more damaging than co-evolved natural enemies. The target weed is more likely to be damaged by the new associate because presumably it lacks the appropriate defense mechanisms to resist attack. This approach, more recently referred to as "neoclassical biological control" (Lockwood 1993), differs from classical biological control in that the natural enemies have not played a major role in the evolutionary history of the host plant, and are therefore considered new associates (Hokkanen and Pimentel 1984). The neoclassical approach for selecting plant-feeding insects as biological control agents has been actively supported by some practitioners of biological weed control (Dennill and Moran 1989, DeLoach 1995), and vigorously criticized by others (Goeden and Kok 1986). Because organisms used in neoclassical biological control are not by definition entirely host specific, they also represent a threat to nontarget congeners of the weed. Therefore, this approach is appropriate only in those cases where the target weed has few or no native relatives in the area of introduction.

In a broad sense, the term neoclassical biological control could be applied to those cases where a native organism develops a new association with a non-native weed species. For example, a number of studies have demonstrated the native milfoil weevil (*Eurychiopsis lecontei* [Dietz]) is an important biological control agent of the non-native Eurasian watermilfoil in the U.S. and Canada (Creed et al. 1992, Creed and Sheldon 1993, 1994, 1995, Sheldon and Creed 1995, Sheldon 1997, Creed 1998, Engel and Crosson 2000, Jester et al. 2000, Newman and Biesboer 2000, Cofrancesco et al. 2004, Newman 2004). The milfoil weevil is native to North America and attacks milfoils (*Myriophyllum* spp. Haloragaceae). Recent studies have shown that weevils reared on Eurasian watermilfoil not only develop faster and survive better on the introduced milfoil (Newman et al. 1997, Roley and Newman 2006), but also will preferentially attack the non-native species over its natural host plant northern water milfoil (*M. sibiricum* Komarov) (Solarz and Newman 1996, 2001).

TYPES OF BIOLOGICAL CONTROL AGENTS

Three major groups of organisms are commonly used in biological control of aquatic weeds: arthropods (insects and mites), fish (primarily grass carp), and pathogens (fungi and bacteria).

Arthropods

In an early review article on biological control of weeds, Wilson (1964) stated that . . . "no insects have yet been used for the biological control of aquatic weeds, . . . it may be that in the fresh-water environment the relatively small numbers of species of plants and phytophagous insects, and perhaps the domination of this environment by fish, have caused in aquatic phytophagous insects a level of host specialization much lower than that occurs in the species-rich terrestrial environment." A very different viewpoint is presented in later review articles on the same topic (Andres and Bennett 1975, McFadyen 1998). Biological control of aquatic weeds with insects has been remarkably successful since it was first attempted in the U.S. against alligatorweed (*Alternanthera philoxeroides* [Mart.] Griseb.) in 1964 (Hawkes et al. 1967), which was coincidentally the same year Wilson's article was published. Complete or substantial biological control of the floating macrophytes water hyacinth, water lettuce (*Pistia stratiotes* L.), salvinia (*Salvinia molesta* D.S. Mitch.), and red water fern (*Azolla filiculoides* Lamarck) by insects has been achieved in most countries where it has been attempted (Julien and Griffiths 1998, Hill 1999). Although biological control of many introduced weeds is not always effective (Crawley 1989), the success rate for the control of aquatic weeds is much higher. A cursory examination of the various projects (e.g., see Julien and Griffiths 1998, McFadyen 1998, Hill 1999) suggests this high success rate may be associated with the growth form of the weeds, the insect taxa used as biological control agents, susceptibility to disease-causing pathogens, fluid nature of the aquatic environment, or some combination of these elements.

Historically, it was thought that herbivory on aquatic macrophytes was uncommon and unimportant (Lodge 1991)

and that insects would not be effective in controlling aquatic weeds (Wilson 1964). Some authors still support this notion. For instance, Jolivet (1998) stated that, "Insects rarely eat aquatic plants, more often eating subaquatic ones . . . It seems that the variety of deterrents . . . reduces the chances for specialization. This is one of the reasons why it is so difficult to find specific herbivores to use to control introduced aquatic weeds . . ." However, studies conducted over the past several decades indicate many instances of successful control of aquatic weeds worldwide by insects (Andres and Bennett 1975, Julien and Griffiths 1998, McFadyen 1998, Hill 1999). Furthermore, the extensive recent literature on specialized insects that mine and feed on the living tissues of the submersed macrophytes hydrilla and Eurasian watermilfoil clearly demonstrates the biological control potential of aquatic insects (MacRae et al. 1990, Balciunas and Purcell 1991, Newman 1991, Buckingham and Okrah 1993, Kangasniemi et al. 1993, MacRae and Ring 1993, Creed and Sheldon 1994, Allen and Center 1996, Balciunas and Burrows 1996, Buckingham and Bennett 1996, Grodowitz et al. 1997, 2004, Wheeler and Center 1997, 2001, 2007, Buckingham 1998, Johnson et al. 1998, Cuda et al. 1999, 2002, Bennett and Buckingham 2000, Epler et al. 2000, Johnson et al. 2000, Newman 2004).

Fish

One of the most controversial biological control agents currently used to control hydrilla and other submersed aquatic weeds is the grass carp (*Ctenopharyngodon idella* Val.) (Chilton and Muoenke 1992, Cassani 1996, Elder and Murphy 1997, Killgore et al. 1998). Native to cold and warm water regions of China and Russia (Sutton and Vandiver 1998), the fish is highly adaptable to a wide range of temperature extremes and has been introduced into many countries worldwide for aquatic weed control (Julien and Griffiths 1998). Although interest in expanding the use of grass carp for controlling hydrilla has increased following the recent discovery of herbicide resistance in some Florida hydrilla populations (Michel et al. 2004, Netherland et al. 2005), the widespread use of grass carp for aquatic weed control has been questioned because of concerns about its negative impact on water quality and nontarget species (McKnight and Hepp 1995). Unlike host-specific arthropods and pathogens typically used in biological weed control programs, grass carp are nonselective grazers that can potentially alter entire freshwater ecosystems and may be unsuitable for biological control of aquatic weeds in some water bodies (Bain 1993, Kirk and Socha 2003, Kirkagac and Demir 2004). Consequently, this fish may be regarded as unsuitable for biological control of aquatic weeds in some water bodies and is illegal to release in some states (e.g., Minn., Vt., Wisc.) (Getsinger et al. 2004).

Consumption of aquatic plants by grass carp depends on a variety of factors (Pine and Anderson 1991, Sutton and Vandiver 1998). Generally, grass carp tend to feed in relatively shallow areas and near the surface of a water body, preferring to graze on the soft tips of tender submersed aquatic plants. In Florida, large fish preferentially consume hydrilla over other non-native species such as Brazilian egeria (*Egeria densa* Planch.), hygrophila (*Hygrophila polysperma* [Roxb.] T.),

and Eurasian watermilfoil (Sutton and Vandiver 1998). However, small fish exhibit a clear preference for native muskgrasses (*Chara* spp.) over hydrilla where the plants occur together (Sutton and Vandiver 1998).

Because of fears about grass carp's potential for reproducing and possible negative impacts on native fisheries, early research focused on developing a nonreproductive fish (Cassani 1996, Sutton and Vandiver 1998). Sterile fish are now produced routinely by shocking fertilized eggs with hot or cold water, or with pressure. Eggs that are shocked retain an extra set of chromosomes (triploids) that causes sterility. To ensure that all stocked fish are incapable of reproducing, each fish is screened by scanning the blood cell nuclei with a Coulter Counter™ (Cassani 1996). This instrument is used to measure the diameter of the nuclei, which is larger in triploid fish.

Grass carp are difficult to remove from a body of water after they have been introduced. Consequently, rigid barriers capable of confining the fish while maintaining unrestricted movement of water are usually installed on culverts or canals to prevent grass carp from escaping into other areas. Because the life span of grass carp can be 20 or more years, appropriate methods of removal must be considered prior to stocking the fish (Sutton and Vandiver 1998). Draining the water body or using a fish toxicant like rotenone are normally used to remove grass carp (Sutton and Vandiver 1998), but these methods are nonselective and can be ecologically disruptive. The development of Grass Carp Management Baits (GCMB; a floating alfalfa-based pellet laced with rotenone) may help to alleviate some of these problems (Mallison et al. 1994). Although GCMBs can selectively remove up to 80% of the grass carp from a water body with minimal effects on nontarget fish species (Mallison et al. 1994), the sudden appearance of dead grass carp on the surface of a water body could create a public relations problem. The public perception of fish kills is generally negative, regardless if they are intentional or a natural occurrence.

Pathogens

Generally, pathogens with a capacity for rapid secondary reproduction (i.e., having the potential to cause secondary infections and disease spread) and capable of causing high levels of damage to the weed's vegetative or reproductive parts are most suitable as classical biological control agents. Several factors contribute to the effectiveness of these pathogens, including host-pathogen disjunction (i.e., lack of host-pathogen homeostasis), presence of a target weed population that is predominantly or wholly susceptible (i.e., lacking in genetic diversity), and high levels of virulence and acceptable levels of host specificity of the pathogen. Presence of dense weed populations and environmental conditions conducive for epidemic build-up also are required. Currently, only one pathogen has been deployed as a classical biological control agent of an aquatic weed anywhere in the world. This fungal agent, *Cercospora piaropi* Tharp (= *C. rodmanii* Conway; Tessman et al. 2000), was imported into South Africa from Florida and released against water hyacinth (Morris et al. 1999). Surveys for pathogens of hydrilla and Eurasian watermilfoil with classical biological control potential were carried

out in the 1990s in Asia and Europe (Harvey and Varley 1996, Harvey and Evans 1997, Shearer 1997). Although the biological control potential of several promising isolates from these surveys has been evaluated (Harvey and Varley 1996, Shearer 1999a), further studies are needed to demonstrate the safety and efficacy of these pathogens.

Pathogens indigenous to a region and those that cause endemic diseases are ideal candidates for development as nonclassical (augmentative or inundative) biological control agents. Generally, inundative biological control agents are industrially developed and registered as bioherbicides by governmental agencies such as the Environmental Protection Agency (EPA). These pathogens must have high levels of virulence to be capable of inflicting acceptable damage. Host specificity is not a major concern with these pathogens because their effectiveness is contingent on inundative application, and in the absence of such applications, the pathogens cease to spread or do not cause a prolonged or escalating epidemic. Bioherbicide pathogens should be easily cultured to produce infective propagules, and the propagules should have good viability and shelf life. They also should be capable of causing infection and disease cycles over a range of environmental conditions. Pathogens that have high levels of genetic stability are desirable for the sake of long-term safety. Currently, there are no registered bioherbicides to control any submersed aquatic weed, but several promising candidates have been the subject of numerous investigations.

Typically, a variety of microorganisms, including common plant-associated saprophytes, plant parasites, and general members of the microbial community, reside on submerged plants such as hydrilla. For instance, in one Florida study by Shabana and Charudattan (1996), 458 different microorganisms (211 bacteria, 202 fungi, 44 actinomycetes, and 1 cyanobacterium) were recovered from 48 samples taken from the ponds. Another 287 pathogens (132 bacteria, 154 fungal isolates, and 1 cyanobacterium) were recovered in 25 samples collected from the two lakes. Fungi belonging to several plant pathogenic genera, including *Botryosporium, Cercosporidium, Chaetophoma, Diplodia,* and *Pyrenochaeta,* were found mainly on hydrilla and in soil samples. The frequency and diversity of the microorganisms isolated confirmed the occurrence of a rich microbial flora associated with hydrilla (Shabana and Charudattan 1996, Shabana et al. 2004); this condition should be typical in any body of water infested with a submersed aquatic weed.

Despite this rich microbial biodiversity, no practical microbial herbicide has been developed thus far for hydrilla. One reason is the lack of understanding of the epidemiological principles involved in underwater diseases. For instance, little information exists on the mode of inoculum dispersal, settlement, and early infection processes in underwater pathosystems. Recent studies have attempted to address this deficiency. Smither-Kopperl et al. (1998, 1999a) studied the epidemiology of disease caused by an isolate of *Fusarium culmorum* (Wm.G.Sm.) Sacc. originally obtained from water soldiers (*Stratiotes aloides* L.) but shown to be pathogenic to hydrilla (Charudattan and McKinney 1978). The process of deposition and attachment of spores in the hydrilla—*F. culmorum* pathosystem is quite complex (Smither-Kopperl et al. 1999a) and must be understood in relation to spore dispersal

in water. Smither-Kopperl et al. (1998) investigated the dispersal of spores of *F. culmorum* in still and moving aquatic systems. They found that the physical components of dispersal of *F. culmorum* spores in a still aquatic system were defined by rapid lateral dispersal and sinking due to gravity. In moving water, the dynamics of water movement were superimposed over the other two factors, which complicated the movement dynamics of the spores.

Another pathogen with bioherbicide potential is *Plectosporium tabacinum* (van Beyma) Palm et al., the anamorph of *Plectosphaerella cucumerina* (Lindfors) Gams. This pathogen was isolated in 1996 from naturally diseased hydrilla shoots (Smither-Kopperl et al. 1999b). In the laboratory, *P. tabacinum* was pathogenic to hydrilla shoots maintained in aqueous solutions in test tubes. Koch's postulates (establishing a causal relationship between a causative microbe and a disease) were fulfilled in several replicated experiments. Infected shoots became slightly chlorotic within 24 h and the leaves became flaccid. There was also an increase in disease severity as inoculum concentration increased from 10^5 to 10^7 conidia ml^{-1} and the disease developed over a range of temperatures from 15 to 30 C. This fungus clearly has potential as a biological control agent for hydrilla.

An isolate of *Mycoleptodiscus terrestris* (Gerdemann) Ostazeski, first reported as a pathogen of Eurasian watermilfoil by Gunner (1983), is capable of causing disease in hydrilla (Joye and Paul 1992, Verma and Charudattan 1993). Prototype formulations of *M. terrestris* tested against dioecious hydrilla in laboratory and field studies showed that hydrilla was susceptible to infection by the fungus (Joye 1990, Joye and Cofrancesco 1991). Incorporating the fungus into a patented biocarrier, Biocar™ 405, produced more recent formulations. Initial test-tube studies demonstrated that both granular and caplet formulations induced severe disease or death of excised hydrilla shoots 2 weeks after inoculation. Low, medium, and high dosage rates of the granular formulation applied to rooted hydrilla in 12-L columns reduced shoot biomass at 4 weeks after application by 87.7, 94.8, and 99.2% respectively compared to untreated controls (Shearer 1998). In microcosm studies, a granular formulation reduced shoot biomass of hydrilla grown in 1700-L tanks by 97.5% at 4 weeks after application (Shearer 1996, 1998). However, initial field trials of *M. terrestris* formulated with Biocar™ 405 failed because the company changed the ingredients in the carrier that inadvertently killed the fungus (Shearer 1999b). Further development and registration of a Texas isolate of *M. terrestris* as a bioherbicide are anticipated now that some hydrilla populations in Florida have become resistant to fluridone (Michel et al. 2004). Current research is focusing on better ways of processing the fungus for commercialization (J. F. Shearer, pers. comm.).

In the late 1970s, *M. terrestris* was isolated from Eurasian watermilfoil plants collected in Massachusetts (Gunner 1983). Preliminary greenhouse and laboratory studies established the effectiveness of the fungus in reducing milfoil biomass (Stack 1990, Gunner et al. 1991, Smith and Winfield 1991). A small-scale field trial using fungal mycelia in Stockbridge Bowl, Massachusetts, supported the laboratory findings by inducing a 16-fold reduction in shoot biomass in treated versus untreated plots (Gunner 1987).

EcoScience Corporation, Worcester, Massachusetts, developed a prototype formulation of *M. terrestris*, named Aqua-Fyte™, for potential registration and commercial use. Verma and Charudattan (1993) tested the prototype formulation on several aquatic and terrestrial plants and found a number of species (including hydrilla) to be susceptible to infection with the Gunner (1983) isolate applied as Aqua-Fyte™.

Aqua-Fyte™ was effective in controlling milfoil in growth chamber studies when water temperatures were between 20 and 28 C, the optimum disease-inducing range for this fungus. Successful tests in laboratory, pool, and pond experiments gave impetus for further evaluations on a field population of Eurasian watermilfoil in Guntersville, Alabama, Lewisville, Texas (Shearer 1994). However, the mycoherbicide was ineffective in reducing aboveground biomass of milfoil under natural conditions at these sites (Smith and Winfield 1991). A reevaluation of the formulation was deemed necessary to understand the reduced levels of efficacy between laboratory and field trials. Using naturally infected plant material from Florida, Shearer (2002) recently examined how *M. terrestris* could become pathogenic when milfoil is stressed.

Morris et al. (1999) recorded the occurrence of a bacterial disease of parrotfeather (*Myriophyllum aquaticum* [Vell.] Verdc.), in South Africa. Diseased aerial shoots of parrotfeather plants were found in most areas infested with this weed in that country. The disease was characterized by the wilting of scattered, individual aerial shoots from the tip downward for about 10 cm accompanied by a greying color. Microscopic examination revealed that the xylem vessels of the stems and leaves were filled with bacterial cells. The causal bacterium was isolated in pure culture and identified as a strain of *Xanthomonas campestris* (Pammel) Dawson. Although natural infections seldom caused more than 1% of the aerial shoots to be affected, an inundative application of the bacterium at 10^8 colony-forming units per ml produced 100% shoot infection when the plants were sprayed in the morning when guttation droplets were still present on the leaves (Morris et al. 1999). Although all aerial parts of the plant were dead, about 6 weeks later new shoots appeared from the submersed stems and the plants recovered. Microscopic examination revealed that the bacterium did not invade the older underwater stems. Because of this inability to kill submersed biomass and the ability of the plant to replace killed shoots, the bacterium was not considered an effective bioherbicidal agent (Morris et al. 1999). However, it may prove to be more effective if used in combination with an approved herbicide (see Integration of control tactics).

Brazilian egeria and its congener *Egeria najas* Planc. are two submersed species native to Brazil that have become serious weeds in hydroelectric reservoirs in the southern part of this South American country. Because the use of chemical herbicides not only is impractical but prohibited in these reservoirs, biological control studies were initiated by Nachtigal and Pitelli (2000). This research resulted in the discovery of a *Fusarium* sp. (tentatively identified as *F. graminearum*; R. A. Pitelli, pers. comm.) from naturally diseased shoots of the two egeria species. Pathogenicity studies proved that this fungus caused a disease characterized by stem necrosis and foliar chlorosis that intensified progressively until a complete breakdown of the

plant tissues occurred. Propagation of the fungus on sterilized rice grains was the most suitable method for inoculum production. The rice-grown inoculum was highly efficacious in killing egeria plants at the rate of 0.5 g/L, and it could be stored for more than 8 months at 4 C. The specificity of the fungus was tested on 14 cultivated species and 11 aquatic plants, but only hydrilla and the two egeria species developed symptoms. The biological control potential of this fungus needs to be investigated further (Nachtigal and Pitelli 2000).

FACTORS CONTRIBUTING TO PROJECT SUCCESSES AND FAILURES

Defining success in biological control of weeds is usually subjective and highly variable. A project may be considered successful in an ecological sense when a weed biological control agent establishes and negatively changes the weed's equilibrium density (Crawley 1990, Grodowitz et al. 2004). However, the type of damage inflicted by the biological control agent may not cause the desired level of economic control (Ehler and Andres 1983). Forno and Julien (2000) also make a clear distinction between biological success and impact success. Biocontrol agents can be biologically successful in establishing and sustaining high population densities on the target weed but may not provide the desired level of control or impact on the weed.

Julien (1997) argues that the use of short descriptive terms to define success, such as complete, substantial, or negligible, as proposed by Hoffman (1995), oversimplifies reality because variations in time and space are not taken into account. For example, in those countries where alligatorweed has been introduced, biological control can range from complete to negligible depending on the season, geographic area, and habitat. However, Hoffman's system is more meaningful from an operational perspective because it equates the degree of biological control with the extent to which other control measures (e.g., harvesters, aquatic herbicides) must be used. The advantage of this approach is that it describes success in practical terms that are more readily understood by aquatic plant managers and bureaucrats. For example, biological control is defined by Hoffmann (1995) as complete when no other control method is required, substantial when other methods such as herbicides are still required but at reduced level, and negligible when other control methods are necessary for managing the weed problem. Measuring biological control success in economic terms (e.g., reduced herbicide applications) has an additional benefit. Funding agencies are more inclined to continue supporting biological control when they can see a return on their investment.

The most recent comprehensive listing of aquatic weed biological control programs worldwide was published by Julien and Griffiths (1998). Alligatorweed, water hyacinth, water lettuce, salvinia, and red water fern have been predictably controlled using the classical approach (McFayden 1998, 2000, Hill 1999). An interesting pattern emerges when the weed and natural enemy attributes associated with these successes are examined. (1) All the aforementioned weeds are free-floating, or produce floating mats in the case of alligatorweed. This plant growth form is strongly affected by wave action and currents that are inherent in aquatic systems. (2) Reproduction in these weeds is primarily by rapid vegetative growth (Hoyer et al. 1996). Crawley (1989, 1990) suggests that high genetic uniformity usually associated with vegetative reproduction is a necessary prerequisite for successful biological control, although its importance has been questioned (Chaboudez and Sheppard 1995). (3) These weeds are highly susceptible to secondary infection. Aquatic plants that have sustained damage by insects or disease will rot and disintegrate very rapidly (Buckingham 1994). (4) Beetles, especially weevils, have been responsible for most of the control. Numerous successes in weed biological control have been associated with this group of insects (Crawley 1989, 1990, O'Brien 1995). These agents also tend to remain above the water, which may reduce fish predation pressure (Newman 2004).

Classical biological control programs targeting submersed aquatic weeds such as hydrilla have been less predictable (Bennett and Buckingam 2000, Forno and Julien 2000; see also Cuda et al. 1999, 2000, 2002, Doyle et al. 2002, Grodowitz et al. 2003, 2004, Owens et al. 2006). Success or failure can be attributed to a variety of factors that may be grouped into three general categories: physical, biological, and technical. These factors, working alone or in combination, can affect the population dynamics of the biological control agents as well as the weeds.

ABIOTIC FACTORS

Climate and Weather

Several independent studies have shown that approximately one-half of the failures in weed as well as insect biological control programs is climate and weather related (Stiling 1993, Cullen 1995). Climate matching should be an important consideration when planning releases of biological control agents (Buckingham 1994, McFadyen 1998, Newman et al. 1998), but its importance to aquatic weed biological control is not well understood (Buckingham 1994). Climate matching may be less important in aquatic systems because the thermal capacity of water dampens temperature fluctuations, and relative humidity in aquatic systems is more or less constant.

However, physical factors may have an effect in some aquatic systems. For example, dense mats of hydrilla increase the surface water temperature considerably, creating unfavorable conditions for a biocontrol agent. For instance, the Asian hydrilla leaf-mining fly (*Hydrellia pakistanae* Deonier) was introduced as a biological control agent of hydrilla in 1987, and establishment has been confirmed at most locations in the southeastern U.S. where hydrilla occurs (Center et al. 1997b, Balciunas et al. 2002). However, laboratory studies by Buckingham and Okrah (1993) showed that a constant water temperature of only 36 C prevented adult emergence. Water temperatures in excess of 40 C for extended periods are not uncommon in hydrilla canopies during the summer months in Florida. Growth chamber studies simulating midsummer hydrilla mat surface temperatures in Florida support the high temperature mortality hypothesis (Cuda et al. 1997). Therefore, it appears that high temperatures occurring in the hydrilla mats in the summertime are probably detrimental to *H. pakistanae*. Regression analysis of the density of

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H. pakistanae larvae with the maximum surface water temperature showed a significant negative correlation (Cuda et al. 1997). Further evidence for the high temperature-mortality hypothesis is supported by the seasonal abundance of *H. pakistanae* in north Florida. Similar patterns of low density in midsummer and progressively higher densities later in the season when water temperatures are cooler were observed in experimental ponds and lakes with established populations of *H. pakistanae* (Cuda et al. 1997, Wheeler and Center 2001). Heavy rainfall and cold weather also have been observed to cause high mortality of leaf-mining flies (D. L. Deonier, pers. comm. in Buckingham 1994; Wheeler and Center 2001).

Yet some aquatic insects have been able to adapt to extreme weather conditions. For instance, overwintering temperatures do not appear to be a limiting factor for the milfoil weevil in Minnesota (Newman et al. 2001), which is not surprising because the weevil is native to northern North America. High summer water temperatures (>35 C) probably precludes establishment of the weevil in southern lakes (Newman 2004).

Habitat Conditions

Lack of fluctuating water levels and drought conditions can affect the establishment or survival of some insect biological control agents of aquatic and semi-aquatic weeds. For instance, larvae of the Indian weevil (*Bagous affinis* Hustache) and the Australian weevil (*B. hydrillae* O'Brien) severely damage the tubers and stems, respectively, of hydrilla in its native range (Balciunas and Purcell 1991, Buckingham 1994). However, these insects failed to become permanently established following their release in the United States because they were unable to complete their development entirely on submersed hydrilla (Buckingham 1994, Godfrey et al. 1994). Three additional *Bagous* weevils from Thailand that failed to complete their life cycles on submersed hydrilla in quarantine studies were dropped from further consideration (Bennett and Buckingham 1999, 2000).

For insects that overwinter on the shoreline, both shoreline and in-lake habitat can be important. Milfoil weevil densities have been correlated with shoreline development (Jester et al. 2000) and plant cover (Tamayo 2003); the weevils need dry sites with good duff to overwinter successfully (Newman et al. 2001). Within a lake, weevil densities appear highest in large beds of watermilfoil in shallower water (Jester et al. 2000, Johnson et al. 2000, Tamayo et al. 2000). Plants in deeper water may be harder to find by adult weevils, are subjected to wave action that may displace the insects, and also are more accessible to fish that feed on the insects (Newman 2004).

Other Control Practices

Depending on the circumstances, mechanical harvesting operations may disrupt or enhance the effectiveness of aquatic weed biological control agents. For example, the density of the weevil *E. lecontei* was reduced significantly after its host plant Eurasian watermilfoil was subjected to harvesting (Sheldon and O'Bryan 1996). Conversely, there is anecdotal evidence indicating that new shoot growth stimulated by harvesting hydrilla in Crystal River, Florida, may increase

the number of sites available for larval development of the hydrilla stem tip midge (*Cricotopus lebetis* Sublette) (J. P. Cuda, pers. observ.).

Applying pesticides to control biting flies and aquatic weeds also may affect the density and performance of certain biological control agents. In Florida, for example, insecticides used for controlling mosquitoes are routinely applied to areas in close proximity to water bodies with established populations of aquatic weed biological control agents (J. P. Cuda, pers. observ.). Drift from the aerial application of mosquito adulticides and larvicides is unavoidable due to the density of these waterbodies in peninsular Florida. In a recent laboratory study, the Asian hydrilla leaf-mining fly *H. pakistanae* was found to be highly susceptible to aerial application of malathion at rates typically used for controlling adult mosquitoes in Florida (N. Tietze, unpubl. data). The mosquito larvicides temephos and methoprene also are extremely toxic to the larvae of *H. pakistanae* (J. P. Cuda, unpublished data). This discovery led to the use of these insecticides in manipulative laboratory and field studies where the effect of this classical biological control agent on hydrilla was evaluated experimentally by using these pesticides to chemically exclude the insect from control tanks or ponds (Cuda et al. 1997). Likewise, the commercially available microbial mosquito larvicide *Bacillus sphaericus* is toxic to the Indian moth *Parapoynx diminutalis* (Snellen), an adventive natural enemy of hydrilla (Haag and Buckingham 1991).

Biological control agents are more likely to come into direct contact with herbicides used for aquatic plant control. In most cases, aquatic herbicides, when applied at recommended field rates, are regarded as harmless to fish and arthropods used as biological control agents. Under laboratory conditions, larval mortality of the Asian leaf-mining fly *H. pakistanae* was attributed to loss of habitat rather than to direct toxicity following exposure to the herbicides endothall, fluridone and diquat (Haag and Buckingham 1991). The hydrilla tuber weevil (*B. affinis*) also was not adversely affected by direct contact with these same herbicides (Haag and Buckingham 1991). However, herbicides also can have a negative effect on biological control agent populations by removing too much of their food supply. For example, reduced feeding activity was observed in the grass carp after hydrilla was treated with diquat or fluridone, suggesting the plant's food quality or palatability was altered by exposure to the herbicides (Kracko and Noble 1993). Center (1994) showed repeated herbicide treatments that eliminate or reduce the host plant can eliminate weed biocontrol agents (see also Newman et al. 1998). Although a combination of herbicides and natural enemies often is suggested as an integrated approach for managing aquatic weeds, more research is needed on a case-by-case basis to determine the compatibility of these two methods.

BIOTIC FACTORS

Host Quality

Texture and nutrient content of aquatic plants are two of the more critically studied aspects of host plant quality because they directly affect palatability of the plants and consumption by the natural enemies. Variations observed in the

texture of aquatic plants can be due to interspecific, intraspecific, or induced differences. For instance, the grass carp will preferentially consume hydrilla over other submersed aquatic plant species. Plant texture is cited as the primary reason for this preference (Sutton and Vandiver 1998). Apparently, the soft tips of young tender hydrilla plants are more palatable to grass carp than other submersed plant species including Brazilian egeria, hygrophila, and Eurasian watermilfoil. Likewise, growth and survival of the Asian leaf-mining fly *H. pakistanae* and the Australian stem mining weevil *B. hydrillae* were enhanced on hydrilla plants with soft apical leaves or stems (Wheeler and Center 1996, 1997).

The role that plant nutrient content plays in the biological control of aquatic weeds has been examined extensively with insect natural enemies (Wheeler and Center 1996, Newman et al. 1998, Grodowitz et al. 2004). Applying fertilizer often increases the level of control by increasing host plant quality (Newman et al. 1998), but there may be a point of diminishing return. For instance, a study by Grodowitz et al. (2004) suggests that increasing nutrient loads, especially nitrogen, may enhance the performance of two species of *Hydrellia* flies that attack hydrilla. However, in an earlier tank study that examined the effects of a single generation of the Asian leaf-mining fly *H. pakistanae* on hydrilla, Wheeler and Center (2001) showed that high densities of the leaf miner reduced hydrilla biomass in plants subjected to a low fertilizer treatment but not in plants grown under high fertilizer conditions. Under higher nutrient conditions, the plants outgrew the leaf miner damage.

In a recent greenhouse study, Shearer et al. (2007) showed that the nutritional quality of a target weed also may influence the performance of fungal pathogens used as weed biological control agents. For example, hydrilla shoots obtained from plants grown in high-nutrient sediment and exposed to the fungal pathogen *M. terrestris* were impacted more by the fungus than shoots from low-fertility sediments.

Genotypes

Previous research has demonstrated that matching the correct biotype of a natural enemy with the variety or strain of the weed on which it evolved usually increases the likelihood the organism will establish and be an effective biological control agent in the weed's introduced range (Harley and Forno 1992). Retrospective studies of the origins of hydrilla and the performance of the two *Hydrellia* spp. released for control of this highly variable submersed aquatic weed in the U.S. support the "biotype matching concept." Using random amplified polymorphic DNA (RAPD) analyses, Madeira et al. (1997, 2004) were able to determine that U.S. accessions of dioecious and monoecious hydrilla are genetically similar to hydrilla plants from southern India and Korea, respectively. *Hydrellia pakistanae* is native to the same geographical region (Deonier 1993), and this natural enemy successfully established on the U.S. dioecious hydrilla (Center et al. 1997b). From laboratory studies, the U.S. monoecious biotype of hydrilla also appears to be a suitable host plant for *H. pakistanae* (Dray and Center 1996, Goodson 1997). These findings suggest *H. pakistanae* is capable of expressing its full reproductive potential on multiple biotypes of hydrilla that occur within its native range

(Goodson 1997). Conversely, *H. balciunasi* Bock, a related species native to Australia (Deonier 1993), failed to become widely established on dioecious or monoecious hydrilla in the U.S. (Grodowitz et al. 1997). The poor performance of *H. balciunasi* may be due to genetic differences between Australian and U.S. hydrilla because survival and development of the leaf miner was low on the U.S. strains of hydrilla when compared to the Australian biotype (Goodson 1997). The Australian biotype of hydrilla is genetically distinct from the Asian biotypes that are the source of the U.S. hydrilla (Madeira 1997). Biotype mismatching could account for the inability of *H. balciunasi* to become more widely established on the U.S. hydrilla. Competitive displacement by the better adapted *H. pakistanae* is another contributing factor (M. J. Grodowitz, pers. observ.).

Recently, Moody and Les (2002) suggested that newly discovered hybrids of Eurasian and northern watermilfoil may be more resistant to herbivory by the milfoil weevil. However, Roley and Newman (2006) recently found that weevil development and size at eclosion were identical on all three taxa, whereas juvenile survival was intermediate on the exotic Eurasian watermilfoil and the native northern watermilfoil hybrid. Different weevil populations also perform better on different plants (Tamayo and Grue 2004), suggesting an interaction between weevil and plant gentoypes. The discovery of hybrids and the identification of genetically distinct populations suggest that more consideration of both agent and plant genotype is warranted.

Carbohydrate Reserves

Knowledge of the phenology of an aquatic weed's carbohydrate allocation pattern may improve the effectiveness of biological control (Madsen and Owens 1998, Fox et al. 2002). Presumably, an aquatic weed would be most vulnerable to attack by a biological control agent when its total nonstructural carbohydrate (TNC) reserves are at their lowest. In other words, the plant's ability to regrow and recover from the herbivore's damage is dependent upon the stored TNCs (Madsen 1991). The TNC levels for the submersed aquatic weed hydrilla are at their lowest in June or July (Madsen and Owens 1998). However, the phenology of the hydrilla leafmining fly *H. pakistanae* in north central Florida indicates that high larval populations do not occur in mid-summer when hydrilla would be most susceptible to herbivore damage (Cuda et al. 1997, Wheeler and Center 2001). The mismatch between the phenology of hydrilla's TNC reserves and larval populations of *H. pakistanae* could explain the fly's apparent ineffectiveness in controlling hydrilla. However, herbivory by the weevil *E. lecontei* reduces the TNC stored in the roots of Eurasian watermilfoil and probably lowers the plant's overwintering survival and competitive ability (Newman et al. 1996, Newman and Biesboer 2000).

Biocontrol Agent Density

Successful biological control of a target weed is a function of the natural enemy's capacity to reproduce on individual plants, and to increase in abundance to critically damage a plant population (Gassmann 1996, Julien 1997). However, high population densities of an herbivore will not necessarily

guarantee success. Effective biological control may only occur when the weed is being stressed concurrently by local climatic conditions (Vogt et al. 1992, Cilliers and Hill 1996, Bellows and Headrick 1999), competing plants (Sheppard 1996, Newman et al. 1998, Van et al. 1998) or other natural enemies.

Another aspect of biological control agent density is self-evident. When biological control agents establish and increase in abundance in one geographical region, they are likely to attain this same level of abundance after they are introduced into ecoclimatically similar geographical regions (Maywald and Sutherst 1997, Byrne et al. 2004). High population densities of a weed biocontrol agent often observed soon after its release are usually attributed to an unlimited food supply and the absence of coevolved parasites and predators in the new environment (Gassmann 1996, Keane and Crawley 2002). This ecological concept is known as the enemy release hypothesis (ERH) and is fundamental to classical weed biological control (Williams 1954). The ERH is largely responsible for the numerous examples of successful biological control of giant salvinia, water hyacinth and water lettuce in various tropical and subtropical regions worldwide (Julien and Griffiths 1998).

PREDATION, PARASITISM, AND DISEASES

Although predators, parasitoids and diseases have been identified as important factors contributing to the failure of biological weed control (Stiling 1993, Cullen 1995), the effect of native parasitoids on weed biocontrol agents may be more important than previously thought (Hill and Hulley 1995, McFadyen and Jacob 2004). Great care is taken to ensure candidate weed biological control agents are released without their co-evolved natural enemies, but there is no way to prevent local natural enemies from exploiting this new resource. Herbivores introduced into new geographical regions as weed biocontrol agents often become prey items for resident natural enemies, usually generalist parasitoids or predators (Cornell and Hawkins 1993).

Parasitism by the semi-aquatic wasp *Trichopria columbiana* (Ashmead), a pupal endoparasitoid of native *Hydrellia* spp., may be reducing the effectiveness of the Asian hydrilla leaf miner *H. pakistanae* (Coon 2000, Wheeler and Center 2001, Doyle et al. 2002), and preventing widespread establishment of the Australian leaf miner *H. balciunasi* in the U.S. (Grodowitz et al. 1997). This parasitic wasp has been recovered from established populations of *H. pakistanae* in Alabama (Grodowitz et al. 1997), Florida (Cuda et al. 1997, Wheeler and Center 2001), and Texas (Doyle et al. 2002). Laboratory studies have confirmed that *T. columbiana* will effectively parasitize *H. balciunasi* (Coon 2000). Furthermore, predation by damselflies was suspected of reducing populations of *H. pakistanae* at release sites in Florida (Center et al. 1997b). An autoradiographic study was conducted by Cuda et al. (1997) to examine field predation on *H. pakistanae*. Labelling larvae of *H. pakistanae* with the radioactive isotope ^{35}S confirmed that aquatic naiads (immature stages, or nymphs) of the insect Order Odonata (dragonflies and damselflies) are voracious predators of larval *H. pakistanae*, whereas mosquitofish *Gambusia* sp. apparently do not feed on the larvae (Cuda et al. 1997).

Recently, the proteobacterium *Wolbachia* was isolated from *H. pakistanae* and the native parasitic wasp *T. columbiana* that attacks it in the U.S. (Jeyaprakash and Hoy 2000). *Wolbachia* are vertically transmitted reproductive parasites that can induce cytoplasmic incompatibility, parthenogenesis, feminization, or male killing in their hosts, and can be detrimental to the host's reproductive success (O'Neill et al. 1997). The origin of the *Wolbachia* and its effects on reproduction in *H. pakistanae* and *T. columbiana* are unknown. Further studies are needed to determine the implications of the *Wolbachia* infection on the population dynamics of the introduced hydrilla biological control agent and its native parasitoid.

Newman et al. (2001) concluded that parasitoids (not found) and pathogens were not important limiting factors for the milfoil weevil. However, microsporidians and gregarines were found at low levels, and the fungus *Beauvaria bassiana* (Balsamo) Vuillemin infected overwintering adults in the laboratory. Clearly, more work on the effects of parasitoids and pathogens on biocontrol agents is warranted.

Although difficult to document, predation by birds or fish is probably another important factor limiting the effectiveness of some insect biological control agents of aquatic weeds. For example, the adventive Asian hydrilla moth (*Parapoynx diminutalis* [Snellen]) severely damages cultivated hydrilla (Buckingham and Bennett 1996), but its effectiveness as a biological control agent in the field is limited due to predation, presumably by fish (Buckingham 1994, Center et al. 2002). Coots (*Fulica americana* [Gmelin]) and moorhens (*Gallinula chloropus* L.) initially prevented or interfered with the establishment of *H. pakistanae* at several release sites by selectively feeding on infested hydrilla placed at the sites (Center et al. 1997b). In Minnesota, predation by sunfish (*Lepomis* spp.) was identified as an important source of mortality for the weevil *E. lecontei*, a natural enemy of Eurasian watermilfoil (Sutter and Newman 1997, Ward and Newman 2006). Lakes with high densities of sunfish likely will not support adequate densities of control agents (Ward and Newman 2006). Newman (2004) suggested that predation by fish may be one reason for the comparatively lower success rate of biological control of submersed plants compared to the dramatic success on floating and emergent plants, where the biocontrol agents would be immune to fish predation.

TECHNICAL FACTORS

Establishing approved natural enemies on the target weed is a critical step in classical weed biological control programs. Clearly, natural enemies must establish in the new environment for the project to succeed. Establishment of newly released biocontrol agents depends not only on the aforementioned environmental factors that are beyond the investigator's control, but also on technical aspects of the project that can be influenced by the researcher, such as selection of release sites, release strategies, and the timing and size of releases (Buckingham 1994, Julien 1997, Center and Pratt 2004, Coombs 2004).

Climate matching should be given a high priority when planning releases (Buckingham 1994, Julien 1997). Natural enemies preadapted to the climatic conditions in the release area will have a better chance of surviving in the new envi-

ronment. Computer modelling programs such as CLIMEX, DYMEX (Maywald et al. 2000), and GARP (Stockwell and Peters 1999) are valuable tools for selecting release sites. These modelling packages use available meteorological and/or life table data to predict an organism's global geographic distribution and population dynamics relative to climate. For example, the CLIMEX model was used to predict locations in Asia, Australia, Africa, and Europe where the South American alligatorweed flea beetle is most likely to establish and successfully control alligatorweed (Julien et al. 1995).

Release Strategies

In the southeastern United States, the successful establishment of the Asian hydrilla leaf-mining fly *H. pakistanae* was thought to be directly related to the type of release (caged vs. open) and the stage of the insect released (Center et al. 1997b, Center and Pratt 2004). For example, in the early stages of the project, open releases of small numbers of eggs failed to establish persistent populations of *H. pakistanae*. However, establishment of the insect eventually succeeded when the release protocol was modified to include the use of cages for releasing large numbers of late instar larvae. Apparently, the eggs and first instar larvae of *H. pakistanae* suffer higher intrinsic mortality rates in comparison to later instars (Center et al. 1997b).

Formulation Issues

The fungal pathogen *M. terrestris* is a promising inundative biological control agent for hydrilla (Shearer 1996, 1998). This native pathogen, which is undergoing development as a bioherbicide (Shearer 1998, Shearer and Jackson 2003), effectively reduced the biomass of hydrilla in laboratory, glasshouse and small-scale field trials. It was recently discovered that the dilution and contact-time problems normally associated with applying a bioherbicide in an aquatic system could be overcome by formulating the pathogen in an EPA approved biocarrier that adheres to the plant (Shearer 1998). However, when production was scaled-up to meet the requirements for field-testing the new formulation, unanticipated changes in the formulation of the carrier itself rendered the fungus ineffective (Shearer 1999b). Current work is focusing on resolving the formulation problems, including developing new formulations that will not only stick to hydrilla but can be applied with conventional herbicide equipment (J. F. Shearer, pers. comm.).

Integration of Control Tactics

In general, the prospects for integrating biological control agents with herbicides are excellent. Several studies have demonstrated the successful integration of herbicides with insects for controlling floating aquatic plants (e.g., Center et al. 1999). Maintaining untreated refuge areas is important for sustaining sufficient densities of insect biocontrol agents (Haag and Habeck 1991, Julien and Storrs 1996). Plant growth retardants also may increase the effectiveness of some insect biocontrol agents (Van and Center 1994).

Other studies have shown that fungal pathogens can enhance the effectiveness of herbicides applied at lower than the recommended rates. For instance, the microbial pathogen *M. terrestris* enhanced the performance of two herbicides commonly used to control hydrilla. In both laboratory tests and small-scale field trials, integrating low doses of fluridone or endothall with the pathogen increased the susceptibility of hydrilla to otherwise sublethal doses of these herbicides (Netherland and Shearer 1996, Nelson et al. 1998, Shearer and Nelson 2002). Similar results were obtained when the fungus was combined with 2, 4-D for the control of Eurasian watermilfoil (Nelson and Shearer 2005).

Shabana et al. (1998, 2003) explored the effects of combined attacks by fungal pathogens and an insect biocontrol agent on hydrilla. They found that isolates of *Botrytis* sp., *Cephalosporium* sp., *Fusarium culmorum*, and an unidentified fungus collected from hydrilla shoots or from soil and water surrounding hydrilla in ponds and lakes in Florida, were capable of killing hydrilla in a test-tube bioassay. *Fusarium culmorum*, the most effective isolate, was examined further in an aquarium test. The interaction of *F. culmorum* and *H. pakistanae* resulted in a higher level of damage on hydrilla shoots than either organism alone (Shabana et al. 2003). Maximum shoot kill was achieved at 20 to 30 C compared to 15 or 35 C. Thus, it may be possible to integrate fungal and insect natural enemies to control hydrilla.

In terrestrial environments, the ability of a weed to recover from the effects of herbivory diminishes as competition from neighboring plants increases (Van Driesche and Bellows 1996, Bellows and Headrick 1999). The interaction between plant competition and a weed's natural enemies often underlies successful weed biological control (Sheppard 1996). Recognition of the importance of plant competition to biological control success is creating a paradigm shift in the design of terrestrial biological weed control programs (McEvoy and Coombs 1999). By placing greater emphasis on manipulating bottom-up effects such as interspecific plant competition, minimizing disturbance, and introducing fewer but more effective natural enemies, the potential for nontarget damage can be minimized (McEvoy and Coombs 1999). This conservative approach has the added benefit of ensuring that introductions of safe natural enemies will continue in the future.

In the aquatic environment, there is evidence that interspecific competition from native plants may be equally important to the successful biological control of rooted submersed weeds. For example, the results of outdoor tank studies conducted in Florida indicate that selective herbivory by the ephydrid fly *H. pakistanae* and the stem-mining weevil *Bagous hydrillae* O'Brien, two introduced natural enemies of hydrilla, shifts the competitive balance in favor of eelgrass (*Vallisneria americana* [Michx.]), a commonly occurring native species frequently associated with hydrilla (Van et al. 1998). More recent mescosm experiments in Mississippi and Texas using typical densities of *H. pakistanae* show a reduction in both biomass and tuber production when herbivory is combined with competition from eelgrass (Doyle et al. 2007). In another study in Minnesota, competition from native plant species appears to be an important factor contributing to the sustained biological control of Eurasian watermilfoil by the weevil *E. lecontei* (Newman et al. 1998).

MANAGEMENT PRIORITIES

The submersed aquatic plants hydrilla, Eurasian watermilfoil and Brazilian egeria are widely recognized as three of the worst invasive aquatic weeds in the U.S., with millions of dollars spent annually to control large infestations of these plants in all types of waterbodies. Since 2000, several hydrilla biotypes in Florida have developed resistance to the herbicide fluridone (Michel et al. 2004). As a result, interest in the submersed aquatic weed problem has increased dramatically because of the herbicide resistance issue (Hoyer et al. 2005, Netherland et al. 2005).

The discovery of fluridone resistance is cause for concern for several reasons. First, the resistance problem will make it difficult for aquatic plant managers in Florida to control hydrilla in a cost-effective and selective manner. This can lead to the eventual spread and establishment of resistant biotypes throughout hydrilla's introduced range. Secondly, fluridone is the only aquatic herbicide approved by the U.S. EPA for managing large infestations of the aforementioned submersed aquatic weed species. However, the overuse of fluridone eventually could lead to similar resistance problems in Eurasian watermilfoil and Brazilian egeria. Finally, no other registered herbicides are available with comparable environmental, cost, and application characteristics to replace fluridone (Hoyer et al. 2005). Therefore, biological control is a viable alternative because it is one of the few tactics currently available that is not only economical but can provide the kind of selective control needed, without damaging nontarget species or the environment. Furthermore, the public is becoming increasingly more receptive to the development of effective nonchemical alternatives such as biological control for managing aquatic weeds.

Identification and Screening of New Classical Biocontrol Agents

Adequate long-term funding and agency commitment are needed to continue overseas surveys and screening of new natural enemies of hydrilla (e.g., Overholt and Cuda 2005), Eurasian watermilfoil, Brazilian egeria, and other widespread invasive submersed plants such as parrotfeather (Cilliers 1999) and curlyleaf pondweed (*Potamogeton crispus* L.). Programs targeting species of regional importance like water chestnut (*Trapa natans* L.; Pemberton 1999, 2002, Ding et al. 2006a, b) and Indian swampweed (*Hygrophila polysperma*; Cuda and Sutton 2000) also will benefit by increased financial as well as agency support. Until recently, surveys for new biological control agents of the aforementioned aquatic weeds have been lacking, largely due to inadequate funding for foreign exploration and screening. Overseas surveys should adopt the scoring system recently proposed by Forno and Julien (2000) for selecting and prioritizing new arthropods as candidates for biological control of submersed aquatic weeds. This system can readily identify potentially effective natural enemies in the native range because greater importance is placed on objective criteria, such as the type of damage caused by both adults and immatures, the duration of the attack, and number of generations annually. Candidate arthropods incapable of completing their development en-

tirely on the submersed weed should be identified early on in a project and dropped from further consideration.

Resources also are needed to screen plant pathogens collected during the 1990s in Asia and Europe from both hydrilla and Eurasian watermilfoil (Shearer 1997, Harvey and Varley 1996, Harvey and Evans 1997, Balciunas et al. 2002). These disease organisms, which are currently stored in a high security quarantine laboratory in Ft. Dietrick, Maryland, may have potential to be used in inoculative releases or for development of new bioherbicides if they are found to be sufficiently viable and host specific. Screening of the pathogen *F. graminearum*, recently discovered impacting Brazilian egeria in South America, should also be investigated and developed as soon as possible (Nachtigal and Pitelli 2000). Finally, additional overseas surveys are needed to identify new, currently undiscovered pathogens for all invasive submersed aquatic plants.

Taxonomic Expertise

The loss of taxonomic expertise for natural enemies as well as their host plants must be addressed. Systematists highly trained in traditional and molecular methods are needed to better understand the genetic diversity of the weed targets and their natural enemies. The need for continued advancement in taxonomic understanding is underscored by the success of *Cyrtobagous salviniae* (originally misidentified) in controlling *Salvina molesta* (also originally misidentified; Buckingham 1994, Forno and Julien 2000), the recent discovery of hybrid watermilfoils (Moody and Les 2002), and the fact that many potential agents discovered in previous exploratory surveys remain unidentified. Advances in taxonomic expertise will facilitate the process of "biotype matching" by increasing the likelihood of selecting biological control agents highly adapted to a particular weed biotype, or possibly a new hybrid.

Post-release Monitoring and Assessment

Effective monitoring is needed to evaluate new biological control projects to determine which agents are effective and what factors limit or enhance their success (Blossey and Skinner 2000, Forno and Julien 2000, Blossey 2004). Funding often has been limited to screening, introduction, establishment, and spread of agents, with little attention given to quantifying their effectiveness or potential unanticipated effects (McClay 1995, McEvoy and Coombs 1999, Kaslichuk et al. 2004). Monitoring programs are often underfunded or inadequate in scope and do not identify where and why control is or is not successful (Blossey 2004). For example, debates over the degree of success of biological control on water hyacinth and hydrilla in North America are largely due to poor monitoring after release. Development of objective evaluations of success also is needed (Forno and Julien 2000, Delfosse 2004). Toward this goal, monitoring should include historical or untreated reference sites for comparisons, be long-term (>2 years), and evaluate the direct and indirect effects on target and nontarget plants as well as agent performance (Syrett et al. 2000, Blossey 2004). Monitoring also should take place at a regional scale to determine generality

of success and to further identify climatic and environmental factors that impact success. In addition to providing information on the success or failure of specific projects or agents, successful evaluation programs should identify the causes of success or failure and development of better selection, release and management strategies (Syrett et al. 2000, Blossey 2004).

Standardized procedures/techniques for post-release monitoring of biological control agents are essential to confirm establishment and assess the effects on the target weed and the associated plant community (Forno and Julien 2000, Blossey 2004). However, the growth habit of submersed aquatic weeds presents a challenge because it does not readily lend itself to sampling procedures normally used by terrestrial researchers to quantify biological control agent impacts. Biological control researchers need to develop collaborative projects with aquatic plant ecologists and watershed managers who have the appropriate expertise to address this problem. Development of precise but efficient methods to assess agent densities and their impacts on target plants and aquatic plant community response, for both degree of control and for nontarget impacts, will increase the likelihood of accurate assessment. Finally, to maintain the high standards of the discipline, practitioners of classical biological control of aquatic weeds should adhere to the guidelines in the International Code of Best Practices for Classical Biological Control of Weeds (Balciunas 2000, Balciunas and Coombs 2004).

Further Assessment of Non-classical Biological Control

Continued work on the use of native and naturalized agents is needed. For example, the native milfoil weevil *E. lecontei* successfully controls Eurasian watermilfoil in some lakes, but is limited by fish predation in others (Newman 2004, Ward and Newman 2006). The extent and degree of this limitation, along with other limiting factors, is unknown even though the weevil is being stocked in more than 80 lakes (Maple 2006). Rigorous evaluation of these projects should result in more effective selection of lakes and more efficient use of resources. Testing of established hydrilla biological control agents, specifically the tip-mining midge *Criocotpus lebetis* Sublette (Cuda et al. 2002) and the two *Hydrellia* flies (Center et al. 1997b, Grodowitz et al. 1997) should be completed as soon as possible to assess their developmental and reproductive performance on the fluridone-resistant hydrilla biotypes.

Trained personnel and funding are needed to conduct statewide surveys to confirm the presence/absence of established biological control agents of hydrilla, Eurasian watermilfoil, and other aquatic weeds outside their currently known range. Such surveys are essential because if a biological agent is not present in a particular state, then additional host range testing of at-risk native plant species may be required before the organism can be imported into that state.

Mass rearing and release of large numbers of high quality introduced and native biological control agents can lead to better control of the weed target in a shorter period of time, but can be an expensive enterprise (Grodowitz et al. 2004). However, costs of rearing natural enemies can be reduced dramatically by using outdoor ponds (Grodowitz et al. 2004) or working with local correctional facilities and training inmates to recognize/handle the biological control agents (Osborne 2005). Additional research on mass rearing of different agents may result in effective inundative control or better establishment of new populations.

Integration of Tactics

To gain greater acceptance by stakeholders and the general public, more emphasis should be placed on integrating biological control with other tactics (e.g., herbicides, revegetation). Research should be targeted at directly assessing integrative approaches rather than *ad hoc* evaluation. For instance, addressing the fluridone resistance problem in hydrilla will require additional resources for new research on removal techniques for grass carp (Netherland et al. 2005), and combining lower stocking rates with a revegetation program may minimize adverse effects to native plant species. This approach was proposed for Eurasian watermilfoil control at Houghton Lake, Michigan (Getsinger et al. 2002), but was not adequately implemented.

More research is needed on revegetation as well as promoting a positive native plant response in combination with compatible invasive plant control methods (e.g., biological control) (Van et al. 1998, Doyle et al. 2007). Failure to incorporate this aspect into a management program may result in reduced habitat, poor weed control, and/or replacement of one invasive with another (Newman et al. 1998, McEvoy and Coombs 1999). Finally, funding should be made available so that industry can mass produce and market effective bioherbicide products (e.g., *M. terrestris*). These commercially produced bioherbicides could be used alone or in combination with lower concentrations of fluridone for controlling susceptible hydrilla. The combination could preserve the selectivity and cost effectiveness characteristics of this herbicide.

Foundational Research

Greater emphasis should be placed on basic research in support of biological control. For instance, studies on plant/biological control agent physiology, the influence of larval and adult nutrition on reproduction, mechanisms of host location (Marko et al. 2005), and the effects of deleterious microorganisms (e.g., *Wolbachia*) on survival and reproduction are needed to gain insight into the factors impacting the effectiveness of biological control agents. Foundational research on waterhyacinth, hydrilla and Eurasian watermilfoil has advanced our understanding of factors regulating success in different systems and our ability to integrate biological control with other management practices. Adoption and testing of new ecological niche models such as the Genetic Algorithm for Rule Set Production (GARP) can help to predict where biological control agents and their target weeds are likely to establish (Stockwell and Peters 1999). The GARP model also could be used for early detection and rapid response to new weed problems before they reach the U.S. Increasing the adoption of GARP would facilitate more rapid biological control response by fostering collaboration with overseas researchers much earlier in those countries where the plant is considered native.

Outreach

Public education about the safety of biological control needs improvement (e.g., Scoles et al. 2005). At the same time, scientists need to continue developing innovative state-of-the-art tools for biological control technology transfer (Grodowitz et al. 1996). For example, two computer-based information/expert systems have been developed and recently updated that contain information on biological control and other methods available for aquatic plant management (Whitaker et al. 2004). These two training tools, including the Noxious and Nuisance Plant Management Information System (PMIS) and the Aquatic Plant Information System (APIS), are easy to use, readily available, and will enable the general public to gain a greater appreciation for and acceptance of biological control technology.

Other examples of successful outreach programs that provide training in biological control are the annual short courses held in California and Florida and teaching modules for primary and secondary schools developed by the University of Florida's, Institute of Food and Agricultural Sciences, Center for Aquatic and Invasive Plants (CAIPS 2005). Established in 1978, CAIPS is a multi-disciplinary research, teaching and extension unit devoted to the study and management of freshwater aquatic and invasive plants. The Aquatic, Wetland and Invasive Plant Information Retrieval System (APIRS) is the information office for the Center. APIRS maintains the world's largest on-line aquatic and wetland plant research database, and produces a variety of educational materials relating to aquatic ecosystems. One of these is a companion website for the general public, which was developed in collaboration with the Florida Department of Environmental Protection, Bureau of Invasive Plant Management. This website not only addresses biological control but also other aspects of aquatic plant management. Finally, professional organizations such as the Aquatic Plant Management Society (APMS 2006) and the Weed Science Society of America (WSSA 2005) should continue producing educational materials to extend and develop public interest in biological control as the basis for integrated management of invasive aquatic plants.

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