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Is Classical Biocontrol Using Fungi a Viable Option for Submersed Aquatic Plant Management?

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INTRODUCTION

Three different pathogen biological control strategies, classical, inundative, and augmentative have practical use on invasive weeds in the United States (Charudattan 2001a). Classical biological control involves the introduction of agents into a region that is not part of their natural range to permanently suppress populations of target weeds (Harley and Forno 1992). Inundative biological control involves the development of a bioherbicide delivered at inoculum levels sufficient to bring about control of a target weed (Harley and Forno 1992). Augmentation involves supplementing numbers of a native or naturalized pathogen into a weed population, timed to bring on an epidemic (Charudattan 2001a).

Historically, classical biological weed control has focused on the use of exotic insects as agents (Julien and Griffiths 1998); however, in the past 35 years the use of pathogens has been steadily increasing worldwide (Morin et al. 2006). In 2001, Charudattan (2001a) compiled a list of classical plant pathogenic agents that had been intentionally or accidentally released in various countries resulting in documented and verifiable success, promising results, or uncertain impacts. Of the 29 classical biocontrol agents listed in these three categories, six have been released in the United States: *Entyloma ageratinae* R. W. Barreto & H. C. Evans on mistflower (*Ageratina riparia* [Regel] King & H. E. Robins); *Puccinia chondrillina* Bubák on skeleton weed (*Chondrilla juncea* L.). *Puccinia carduorum* Jacky on nodding plumeless thistle (*Carduus nutans* L.); *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. f. sp. *clidemiae* E. E. Trujillo, Latterell & A. E. Rossi on Koster's curse (*Clidemia hirta* [L.] D. Don); *Septoria passiflorae* Syd. on banana poka (*Passiflora tripartita* [Juss.] Poir. var. *mollissima* [Kunth] Holm-Niesen & P. M. Jorg.); and *Puccinia punctiformis* (Str.) Röhl. on Canada thistle (*Cirsium arvense* [L.] Scop.). Since 2001, one additional agent, *Puccinia jaceae* Otth. var. *solstitialis* Savile, was recommended for release by the Technical Advisory Group (TAG) and approved for release by the Animal Plant Health Inspection Service (APHIS) for biological control of yellow starthistle (*Centaurea solstitialis* L.). The first releases were made in California in 2003 (Fisher et al. 2007). The adventive rust, *P. psidii* G. Wint., thought to have been accidently introduced into Florida during the 1970s, has been found to be a natural enemy of punk tree (*Melaleuca quinquenervia* [Cav.] S. T. Blake) (Rayamajhi et al. 2006). Had it not been accidently introduced it is highly unlikely that this rust would be approved for release in the United States because it infects more than 12 genera and 34 species in the family Myrtaceae (Rayachhetry et al. 2001).

All these fungi have been officially approved for release and have certain characteristics that make them excellent candidates for classical biological control. They are foliar pathogens that are readily dispersed by wind or rain-splash, they are relatively host-specific to the target plants in the release region, and they are capable of parasitizing and severely damaging vigorously growing plants. Included in this group are species that are generally highly specific and cause rust, smut, or mildew symptoms. Referred to as biotrophic (obligate) fungi, they coevolved with their host, are difficult to culture on standard laboratory media, and require the host to complete its life cycle. Included here from the organisms cited above would be *Puccinia* and *Entyloma* species. *Septoria* and *Colletotrichum* would be classified as facultative parasites because they can be readily cultured on artificial media and are capable of feeding on dead or decaying organic matter as well as parasitizing plants (Morin et al. 2006). Following the success of the agent *Septoria passiflorae* in managing banana poka in Hawaii, researchers seriously considered use of the pathogen in New Zealand. Prior to the release of the pathogen, host range testing was expanded, and the pathogen was found to have a much broader host range than anticipated. It was capable of infecting and causing disease on other species in the genus *Passiflora* including, *P. edulis*, of commercial value in New Zealand (Barton 2005). Consequently, the pathogen was deemed not suitable for use in New Zealand and a potentially catastrophic mistake was avoided. This research accentuated the importance of additional host testing prior to release of an agent into a new region.

Floating Plant Control

Note that only one obligate aquatic species, waterhyacinth (*Eichhornia crassipes* L.,), was on the aforementioned lists. It is parasitized by *Cercospora piaropi* Tharp. (synonym *C. rodmanii* Conway; Tessmann et al. 2001), a fungal species that has been extensively studied (Freeman and Charudattan 1984, Charudattan et al. 1985), patented by the University of Florida, and developed as a bioherbicide by Abbott Laboratories (Pennington and Theriot 1983). Field testing of the bioherbicide failed to produce desired results, and additional development of the product never ensued. Interest in the fungus

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has continued, however, and recent laboratory research has paired it with insects for use in an integrated approach for waterhyacinth management (Moran 2005, 2006, Martinez and Gomez 2007). A rust fungus, *Uredo eichhorniae* Gonz. Frag. and Cif., was discovered on waterhyacinth in South America in 1974 and studied in containment at the University of Florida (Freeman et al. 1976, 1981). Petition for its release into the United States was not approved because all life stages of the rust had not been documented (Charudattan 2001b). Species placed in the form-genus *Uredo* (i.e., an artificial taxonomic category for organisms with unknown relationships) could have an alternate host in an unrelated genus, making it almost impossible to get permission for release. Additional research has been undertaken overseas, and a petition has been requested to research the fungus in quarantine in the United States (Charudattan 2001b).

Submersed Plant Control

One of the challenges for aquatic biological control research is identifying and developing agents for submersed aquatic plants. Most research to date has focused on two species, hydrilla (*Hydrilla verticillata* [L.f.] Royle) and Eurasian watermilfoil (*Myriophyllum spicatum* L.). In general, classical pathogen research on submersed aquatic plants has lagged behind that of insect biocontrol research. Surveys of insect agents have been conducted in North America, Africa, Asia, and Australia (Balciunas et al. 2004, Johnson and Blossey 2004). Although more than 20 insect agents were identified as feeding on Eurasian watermilfoil overseas, none had the required host specificity to be considered for introduction into the United States (Johnson and Blossey 2004). Of 24 candidate insect agents documented on hydrilla overseas, four (*Bagous affinis* Hustache, *Bagous hydrillae* OBrien, *Hydrellia balciunasi* Bock, *Hydrellia pakistanae* Deonier) were approved for release, and of these, only the two *Hydrellia* species have become established.

One of the earliest attempts at overseas surveys for pathogens of aquatic plants was in 1971-1972, when Charudattan (1973) surveyed for pathogens of hydrilla in India. Out of 55 isolates collected on hydrilla, only two, a *Pythium* sp. and a *Sclerotium* sp., were damaging. Unfortunately members of the genus *Pythium* are generally recognized as saprotrophic soil-inhabiting species, which are opportunistic pathogens (Webster and Weber 2007) and unlikely to make good classical agents. Members of the genus *Sclerotium* often have broad host ranges including vegetables, flowers, legumes, cereals, and forage plants (Agrios 2005), also making them unlikely candidates for classical biocontrol.

Although not obtained through an intentional plant pathogen survey, in 1974 a fungal isolate, *Fusarium roseum* "Culmorum" (Lk. & Fr.) Snyd. & Hans., was obtained from diseased water soldier (*Stratiotes aloides* L.) plants obtained from the Netherlands and taken to Gainesville, Florida. The *Fusarium* isolate was capable of killing hydrilla (Charudattan and McKinney 1977, 1978); however, it was found to be non-host-specific in a seedling bioassay (Charudattan et al. 1980) and thus not suitable for classical biological control.

In 1992-1993, researchers from the former Soviet Union, surveyed for pathogens of Eurasian watermilfoil in Russia, Kazakhstan, Uzbekistan, and Tadzhikistan. They isolated 680 fungal strains, 290 of which were screened for efficacy (Dolgovskaya et al. 1994). Five strains were found that killed Eurasian watermilfoil plants. The fungi were reisolated from the treated plants, thus satisfying Koch's postulates, and retested, giving the same results. These fungi were never identified, and no further reports resulted from this work. Unfortunately, with the collapse of the Soviet Union in the early 1990s research funding suffered and work on the isolates was in all likelihood discontinued. If the isolates were adequately preserved and if funding once again became available, testing could resume; otherwise, the original collection sites would have to be revisited. According to Johnson and Blossey (2004) areas comprising the former Soviet Union were never surveyed for Eurasian watermilfoil insect agents; therefore, a combined effort of searching for both pathogens and insects would likely be required.

United States Army Corps of Engineers overseas searches for pathogens were not undertaken until the 1990s when parts of the People's Republic of China (PRC) were surveyed in 1994-1995 for pathogens of Eurasian watermilfoil and hydrilla (Shearer 1997), and 12 European countries were surveyed in 1993-1995 for pathogens of Eurasian watermilfoil (Harvey and Evans 1997). Most of the more than 400 fungal pathogens isolated from Eurasian watermilfoil collected in Europe and Asia were from genera known to be common colonizers of plant tissues. Testing of the European isolates revealed that 11 of the 291 that were initially screened (Table 1) provided good control of Eurasian watermilfoil in small-scale testing (Harvey and Evans 1997). Three of the isolates did not sporulate on standard media and could not be identified to species. The remaining eight have broad host ranges or are described in the literature as weakly pathogenic at best and would not be considered as candidates for classical biological control. As a result, the 1993-1995 European collections warrant no further evaluation.

A total of 97 accessions of PRC isolates were screened for pathogenicity on hydrilla and Eurasian watermilfoil at the USDA/ARS/Foreign Disease-Weed Science Research Laboratory (FDWSRU) located at Fort Detrick, Frederick, Maryland. A *Phoma* sp. and three *Mycoleptodiscus terrestris* isolates were equally efficacious on both hydrilla and Eurasian watermilfoil in preliminary tests, while a *Cylindrocladium* isolate was efficacious only on Eurasian watermilfoil (Table 1). Foreign isolates of *M. terrestris* are unlikely to receive release approval for classical biological control use in the United States because the species is reported to parasitize other plant species (Verma and Charudattan 1993). Additionally, an indigenous isolate of *M. terrestris* from Texas is currently undergoing bioherbicide development in the United States (Shearer and Jackson 2006), lessening the need to use foreign isolates for this purpose. Of the two remaining isolates, the *Phoma* sp. proved ineffective in further tests and the *Cylindrocladium* was not further evaluated because it failed to grow following retrieval from storage. Classical pathogen biological control work is currently on hold due to lack of funding for additional foreign surveys and evaluations.

Unfortunately, none of the aforementioned foreign isolates collected fall in the rust, smut, or powdery mildew categories (i.e., those pathogens preferred as classical biological

TABLE 1. ISOLATES FROM OVERSEAS COLLECTIONS OF EURASIAN WATERMILFOIL AND HYDRILLA THAT PROVIDED GOOD CONTROL AGAINST THE HOST PLANT IN SMALL SCALE SCREENING IN THE LABORATORY.

Isolate	Location	Collection date
European Collections		
Indeterminate Coelomycete	Bedfordshire, England	6/20/94
Indeterminate Hyphomycete 1	Hampshire, England	7/6/94
Coniothyrium fuckelii Sacc.	Powys, Wales	8/4/94
Gliocladium roseum Banier.	Bedfordshire, England	6/20/94
Acremonium sp.	Suffolk, England	8/18/94
Cylindrocarpon destructans (Zinssm.) Scholten.	Cumbria, England	9/7/94
Indeterminate Hyphomycete 2	Cumbria, England	9/7/94
Fusarium solani (Martius) Sacc.	Cambridgeshire, England	11/3/93
Gliocladium roseum Banier.	Gerardmer, France	4/5/94
Cryptosporiopsis sp.	Hereford, England	6/13/95
Embellisia cf. *telluster* E. G. Simmons	Devon, England	9/4/93
Peoples Republic of China Collections		
Mycoleptodiscus terrestris (Gerd.) Ostazeski 1	Huai-roi Reservoir	7/22/94
Mycoleptodiscus terrestris (Gerd.) Ostazeski 2	Huai-roi Reservoir	7/22/94
Mycoleptodiscus terrestris (Gerd.) Ostazeski 3	Huai-roi Reservoir	7/22/94
Phoma sp.	Qiao Zhuang	7/21/94
Cylindrocladium sp.	Huai-roi Reservoir	7/22/94

control agents), and pathogens in these categories have not been reported as disease-causing agents on the submersed aquatic plants, Eurasian watermilfoil, or hydrilla. These primarily foliar pathogens are more adapted to wind rather than water dispersal, although rain splash may at times be involved in spore movement. In the case of powdery mildews, free water is inhibitory to spore germination (Webster and Weber 2007), making it highly unlikely that rust, smut, or powdery mildew fungi would be found on the foliage of submersed aquatic plants. A rust or smut might possibly attack the floral inflorescence of Eurasian watermilfoil when it blooms and extends above the water surface; however, such an agent would be a poor choice for classical biological control because Eurasian watermilfoil does not consistently flower, and sexual reproduction appears unimportant in many populations (Smith and Barko 1990, Johnson and Blossey 2004). The investment of valuable resources required to bring such an agent to the point of release would not be warranted, especially because seeds do not seem to be a major factor in reproduction and spread.

Reports have documented classical pathogen biological control agents in groups of fungi other than rusts, smuts, or powdery mildews, although to date the number has been minimal (Charudattan 2001a, Morin et al. 2006). Of the 29 classical fungal pathogens cited by Charudattan (2001a) as in use or under development worldwide, only seven species in six genera were not rusts, smuts, or powdery mildews. In all cases, the anamorphic (asexual) stage of the fungus induced disease in the host. Only one was identified for potential use on an aquatic plant: *Cercospora piaropi* for waterhyacinth management in South Africa. It seems an unlikely candidate for use in the classical sense, however, because research in the United States suggested it would be most effective as an inundative agent, where large inoculum levels would be required to achieve waterhyacinth control (Pennington and Theriot 1983). The other six species were in the genera *Phaeoramularia*, *Phloeospora*, *Phoma*, *Septoria*, and *Cercospora*, all of which contain species reported to be confined to a single host genus (Farr et al. 1989). From these particular genera, several *Phoma* species were found in surveys in both Europe and the PRC (Harvey and Evans 1997, Shearer 1997). Efficacy testing revealed them to be weakly pathogenic on Eurasian watermilfoil and/or hydrilla and not good classical biocontrol candidates.

Although overseas surveys of hydrilla and Eurasian watermilfoil are unlikely to yield classical biological control agents among the rusts, smuts, or powdery mildews, future endeavors to search for pathogens should not be curtailed. To date, searches have covered only a small area of the native range of the two plants. In previous surveys for pathogens and insects for Eurasian watermilfoil biocontrol, most sites were visited only once during a short window in the growing season, and thus the full spectrum of possible natural enemies has not been fully explored. A logical direction for future work would be to combine insect and pathogen surveys. This approach would have the advantage of being cost-effective and simultaneously providing biocontrol researchers a better understanding of the interactions of fungi, insects, and the damage they inflict on the host (Cock et al. 2008).

A far greater range of hydrilla populations were surveyed overseas for insect biocontrol agents than for pathogens and over a longer time period. Future overseas work should target searches for pathogens in regions not previously surveyed. Surveys for both pathogens and additional insects that damage stems, roots, apical tips, turions, and/or tubers should target areas of the world that have received limited attention, such as Southeast Asia (Balciunas et al. 2004). Results of DNA work suggest that United States dioecious hydrilla populations align closely with accessions from India, Nepal, and Vietnam, whereas monoecious hydrilla populations cluster closely with an accession from Seoul, Korea (Madeira et al. 1997, 2007), suggesting hydrilla populations in these regions should be examined with great intensity for insect and pathogen agents.

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