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Classical Pathogen Biocontrol Research in Asia 1994-1995: Surveys for Pathogen Agents of *Hydrilla verticillata* (L.f.) Royle and *Myriophyllum spicatum* L.

by Judy F. Shearer

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

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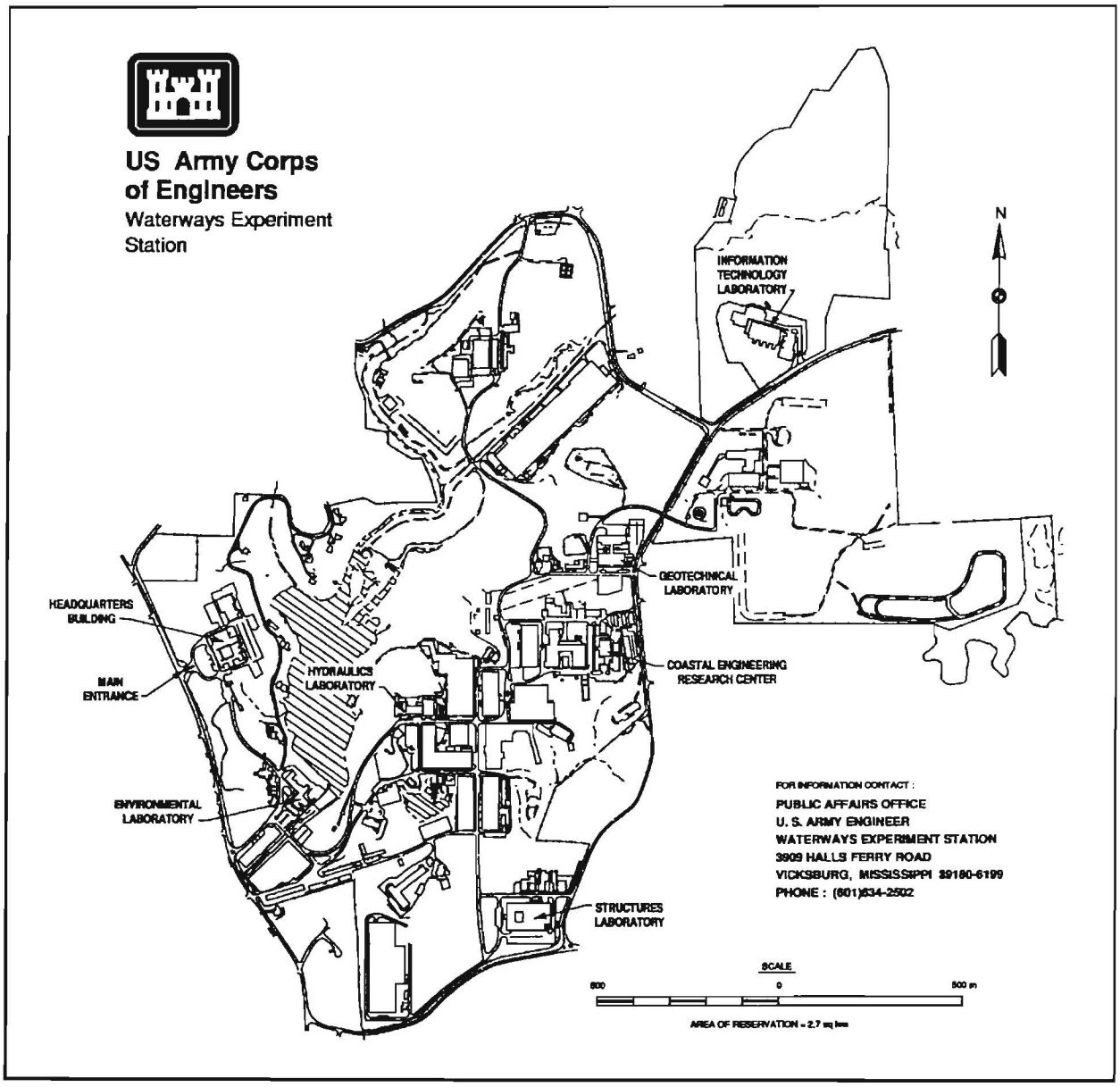

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Preface

The work reported herein was conducted as part of the Aquatic Plant Control Research Program (APCRP), Work Unit 32200 and Work Unit 32202. The APCRP is sponsored by the Headquarters, U.S. Army Corps of Engineers (HQUSACE), and is assigned to the U.S. Army Engineer Waterways Experiment Station (WES) under the purview of the Environmental Laboratory (EL). Funding was provided under Department of the Army Appropriation No. 96X3122, Construction General. The APCRP is managed under the Center for Aquatic Plant Research and Technology (CAPRT), Dr. John W Barko, Director. Mr. Robert Gunkel was Assistant Director for the CARPT. Program Monitor during this study was Ms. Denise White, HQUSACE.

This report was prepared by Dr. Judy F. Shearer, Aquatic Ecology Branch (AEB), Ecological Research Division (ERD), EL, WES. Technical assistance at WES was provided by Ms. Janis Lanier and Mr. Brian Durham and at the Sino American Biological Control Laboratory (SABCL) in Beijing by Mr. Zhiqun Chen. Dr. William Bruckart and the staff at the U.S. Department of Agriculture Agricultural Research Service Foreign Disease-Weed Science Laboratory, Fort Detrick, Frederick, MD, assisted in preservation and maintenance of the China cultures.

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At the time of publication of this report, Director of WES was Dr. Robert W. Whalin. Commander was COL Bruce K. Howard, EN.

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1 Introduction

In the search for pathogens as potential biocontrol agents for nuisance plants, two strategies have been employed: (a) an inundative approach using native organisms occurring on the nuisance plant in the infested region; and (b) a classical approach that involves searches for and importation of natural enemies of a nuisance plant from the native range (Wapshire, Delfosse, and Cullen 1989). Until recently, pathogen biocontrol work on *Hydrilla verticillata* (L.f.) Royle (hydrilla) and *Myriophyllum spicatum* L. (Eurasian watermilfoil) focused entirely on searching for and developing endemic microorganisms as biocontrol agents. Implementation of a classical approach began in 1994 with the initiation of survey work in Europe for pathogens of Eurasian watermilfoil (Harvey and Varley 1996) and in China for pathogens of both milfoil and hydrilla. Broadening the scope of the work by including a classical approach not only will strengthen the overall pathogen biocontrol effort but has the potential to enhance the existing insect biocontrol program by increasing the number and variety of agents available for management of problematic submersed macrophytes.

The principle of classical biocontrol is to search for natural enemies (i.e., potential biocontrol agents) of a target plant in regions of the world where the plant and its enemies coevolved. Ideally, searches for biocontrol agents should be concentrated near the centers of origin or diversification of the host species (Harley and Forno 1992). Because hydrilla and Eurasian watermilfoil are widely distributed throughout the Old World, and their exact center of origin remains uncertain (Cook and Luond 1982; Couch and Nelson 1985), the search strategy has been to examine specific areas in the reported range of the two species. With the easing of relations between the United States and China in the late 1980s, a previously untapped region became accessible for exploratory research. As both hydrilla and milfoil occur in China, it also became possible to broaden the search to include agents from temperate areas where the climatic conditions more closely match those in the United States where the plants have recently invaded.

In 1989, exploration began in China for biocontrol insects of Eurasian watermilfoil and hydrilla as a cooperative effort between the Corps of Engineers and the United States Department of Agriculture Agricultural Research Service (USDA/ARS). The research effort operated out of the Sino-American Biological Control Laboratory (SABCL) housed in the Biological Control Institute on the campus of the Chinese Academy of Agricultural Sciences, Beijing. Since that time, extensive surveys for insect

agents were accomplished in and around Beijing and in Liaoning, Inner Mongolia, Guangdong, and Hunan provinces (Balciunas 1990). The initial survey work was confined to searches for insect agents, because the importation of plant pathogenic organisms into the United States at that time was prohibitive. As pathogen importation restrictions eased and with the development of the USDA/ARS Foreign Disease-Weed Science Laboratory at Fort Detrick, Frederick, MD, a search for pathogens was added to the biocontrol survey efforts in China in 1994.

The purpose of the overseas research was to conduct surveys for disease-causing organisms of hydrilla and milfoil in China. Pure culture isolates of microorganisms isolated from hydrilla and milfoil tissue were shipped to the USDA quarantine facility at Fort Detrick, MD, for accession into their permanent collection of pathogenic organisms on nuisance plants. The first phase of the work at the quarantine center focused on isolate identification to pinpoint the organisms to be screened and tested for host specificity.

2 Materials and Methods

Water agar (H_2O, 1 L; agar 20 g) and Martin's agar (H_2O, 1 L; agar, 17 g; KH_2PO_4, 0.5 g; K_2HPO_4, 0.5 g; $MgSO_4$ 7 H_2O, 0.5 g; peptone, 0.5 g; dextrose, 10 g; yeast extract, 0.5 g; rose bengal, 0.05 g; streptomycin sulfate 0.03 g) were prepared for the initial isolations from plant tissue. Dilute corn meal agar (Difco Laboratories, Detroit, MI) (H_2O, 1 L; corn meal agar 8.5 g; agar 2 g) was used for axenic culturing of isolates for shipment to the quarantine center. For space-saving purposes, the media were prepoured into small plastic snap-cap petri dishes (50- by 9-mm) (Falcon Brand, Becton Dickinson Laboratories, Lincoln Park, NJ) or small plastic snap-cap test tubes (12- by 75-mm) (Falcon Brand). All media, solutions, and equipment necessary for microbial isolations from plant tissue were packaged in coolers or lockable cases at the biocontrol laboratory at the U.S. Army Engineer Waterways Experiment Station (WES), Vicksburg, MS, and shipped to China.

During summer 1994 surveys were conducted in and around the Beijing area and in 1995 were expanded to include sites in Liaoning, Hubei, and Jiangsu provinces (Figure 1). At each field site, the aquatic vegetation was examined and recorded, and, when present, samples of hydrilla and milfoil were collected (Table 1). When time allowed, plant material was examined in situ with 10X magnifying headsets. Sections of leaves, stems, or roots that appeared damaged were placed in plastic bags and transported in an ice chest back to the SABCL in Beijing or the hotel room when working in outlying provinces. Collected plant material was reexamined with a stereoscopic microscope at 30X magnification. Diseased pieces of leaves, stems, and/or roots were placed in small sieves with a site label and washed under running water for 1 hr.

After washing, the plant pieces (i.e., leaves, stems, and/or roots) were surface sterilized in separate batches in 10-percent chlorox for 1 min, rinsed in sterile water, blotted dry, and plated onto water agar and Martin's agar. The plates were incubated at room temperature and examined every few days for microbial growth. As colonies began growing from the plant pieces, small triangular sections (1- by 1- by 1-mm) were cut from the leading edge of a colony and placed on a corn meal agar slant. Each isolate placed on an agar slant was given a collection number, and notations were made on the collection site and plant material (Appendix A). The agar slants were incubated at room temperature and observed periodically to ensure that each tube contained a single pure culture. Any contaminated tubes were discarded. Tentative identifications were attempted

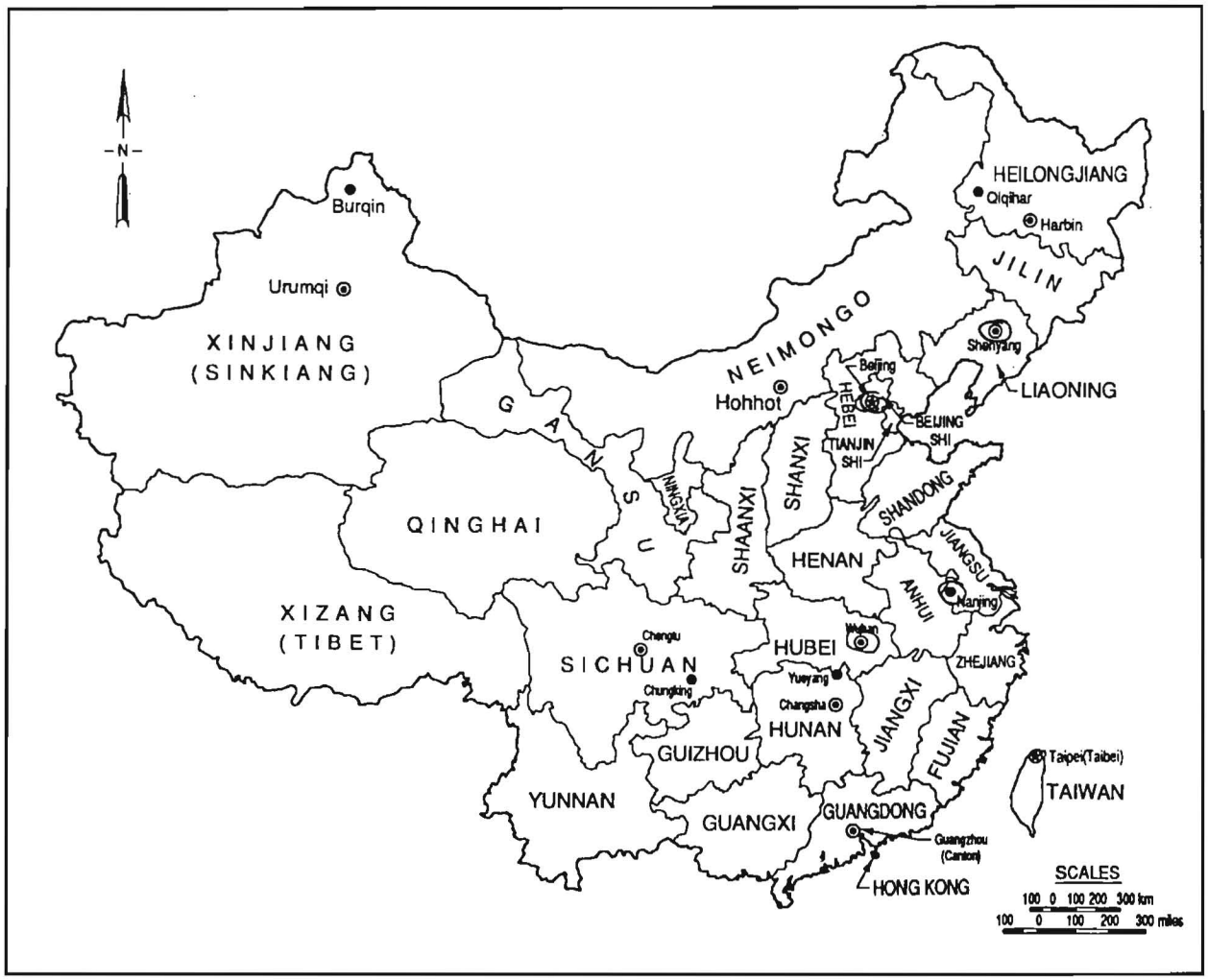

Figure 1. Map of China highlighting major cities in provinces visited during survey work for pathogens of Eurasian watermilfoil and hydrilla during 1994 and 1995

for all isolates based on morphology of the colonies on the agar slants. The tubes were individually wrapped in paper towels to lessen the chance of breakage, sealed in a box, hand-carried into the United States, and shipped directly to the USDA quarantine center.

For identification of the isolates at the quarantine center, each isolate was plated onto potato carrot agar (PCA) (carrots, 10 g; potatoes, 10 g; agar, 20 g: H_2O, 1 L) and potato dextrose agar (PDA) (Difco Laboratories, Detroit, MI). The cultures were incubated in the dark at 25 °C for 2 weeks prior to observation. Most initial identifications were made from colony characteristics of the isolates on the culture media followed by microscopic observation of spores and spore-producing bodies on slide mounts made from the cultures.

Long-term preservation of the China isolates was provided by the National Cancer Institute located at Fort Detrick, Frederick, MD. The isolates will be maintained by their technicians and made available as needed for additional studies.

Table 1
Sites Visited in Search of Pathogen Biocontrol Agents of Hydrilla And Eurasian Watermilfoil During Survey Work in China, 1994 and 1995

Year	Site Name	Location	Collection
7/18/94	August 1st Lake	Beijing	Hydrilla and milfoil
7/19/94	Qing Hua University Canal	Beijing	Hydrilla
7/19/94	Qing Hua University Lake	Beijing	Hydrilla
7/20/94	Lan-dian-chang	Beijing	Hydrilla and milfoil
7/21/94	Qiao zhuang	Outskirts Beijing	Hydrilla
7/21/94	Han-jia chuan	Outskirts Beijing	Hydrilla
7/21/94	San-jia-dian	West of Beijing	Hydrilla and milfoil
7/22/94	Huai-roi Reservoir	Huai-roi	Hydrilla and milfoil
7/25/94	Yu River	North of Beijing	Hydrilla and milfoil
7/19/95	Ma san jia Canal	South of Shenyang, Liaoning Province	Hydrilla
7/20/95	Shi Fu	North of Shenyang, Liaoning Province	None
7/21/95	Yuhong	South of Shenyang, Liaoning Province	Hydrilla
7/21/95	Hunhe River	Shenyang	None
7/25/95	East Lake	Wuhan	Hydrilla and milfoil
7/26/95	Liangzi Lake	Ganghou	Hydrilla and milfoil
7/28/95	Pearl Spring	North of Nanjing	Milfoil
7/29/95	Tianhe River	Southeast of Nanjing	Hydrilla and milfoil
7/29/95	Jing chun dahe	Southeast of Nanjing	Hydrilla and milfoil
7/29/95	Canal east of Quinghui River	Southeast of Nanjing	None
7/29/95	Xuanwuhu Lake	Nanjing	None
7/30/95	Baohua san	Jurong County west of Nanjing	None
8/1/95	Huai-roi Reservoir	Huai-roi	Hydrilla and milfoil
8/3/95	Qing Hua University Lake	Beijing	None
8/7/95	San jia-dian Reservoir	West of Beijing	Hydrilla and milfoil
8/8/95	Old Summer Palace	Beijing	Hydrilla and milfoil

3 Results and Discussion

Twenty-five sites were visited in China in July 1994 and July and August 1995 as part of a joint effort to survey for insects and pathogens as potential biocontrol agents of hydrilla and Eurasian watermilfoil (Table 1). While most sites had populations of aquatic plants, the canal east of the Quinghui River near Nanjing was void of vegetation. Shi Fu (Liaoning Province), Xuanwuhu Lake (Nanjing), Hunhe River (Liaoning Province), and Baohua san (west of Nanjing) lacked hydrilla and Eurasian watermilfoil. A large population of milfoil had been present in the Hunhe River,[1] but extensive dredging at the site has resulted in a loss of vegetation in the river and adjacent backwater areas. At Qing Hua University Lake, Beijing, a small population of hydrilla had evidence of insect and/or pathogen damage in 1994, but in 1995, only two small undamaged strands of hydrilla were found.

A diverse community of aquatic plants was generally found growing in association with hydrilla and/or milfoil at most visited sites and included one or more species in the genera *Ceratophyllum, Myriophyllum, Vallisneria, Potamogeton, Utricularia, Najas, Chara, Lemna, Hydrocharis, Salvinia, Euryale, Nymphoides, Trapa, Trapella, Sagittaria, Typha, Monochoria, Alisma*, and *Murdannia*. In sites that had evidence of disturbance, species richness was lacking, but vast monocultures of hydrilla and milfoil were not in evidence.

Disease epidemics directly attributable to pathogens were not observed on hydrilla or milfoil during summer surveys in China 1994 and 1995. Damaged meristematic tips, stem lesions, leaf spots, striping of leaves, and chlorosis provided evidence that disease was impacting these submersed aquatic plants. A complete list of fungal species isolated from hydrilla and milfoil can be found in Appendix A. Not all of the isolates obtained from the plant tissues are considered to be potential agents for biocontrol. Some including species of *Trichoderma, Penicillium, Aspergillus, Cladosporium, Alternaria*, and *Epicoccum* are ubiquitous and can be found associated with both aquatic and terrestrial plant material under a variety of conditions.

[1] Personal Communication, 1995, Professor Gang Guang Qing, Scientist, Liaoning Province, China.

Of particular interest in the group of isolates obtained from China are potential pathogens in the genera *Pythium, Phytophthora, Fusarium, Cylindrocladium, Glomerella*, and *Mycoleptodiscus*. One or more species in each of these genera have been reported to be disease-producing organisms on a variety of plants (Domsch, Gams, and Anderson 1980; Farr et al. 1989).

Species of *Pythium* and *Phytophthora*, two zoosporic Phycomycetes in the family Pythiaceae, together make up one of the most destructive groups of plant pathogens (Walker 1969). They cause a variety of diseases including root rots, stem cankers, fruit rots, and damping off. Many are associated with moist habitats but are not strictly confined to them. Asexual reproduction and dissemination of zoospores require the presence of water. While many species have a broad host range occurring on over 100 genera of plants, others are more restricted in their host range, e.g., *Phytophthora infestans*, which causes late blight of potatoes.

Species in the genus *Fusarium* range from mild facultative parasites to highly developed pathogens that invade the vascular system of their hosts (Walker 1969). In addition to vascular wilts, plant parasitic *Fusariums* cause root and stem rot, fruit rot, or ear diseases. Host-specific strains are known as *formae speciales,* and many of these have been found to consist of two or more races (Booth 1971). Many of the highly pathogenic vascular strains are included within the complex of *Fusarium oxysporum* section Elegans (Booth 1971). Farr et al. (1989) list over 40 *formae speciale*s of *F. oxysporum* in the compiled list of fungi on plant and plant products in the United States. Host plants range from herbaceous to woody and include members of the Magnoliopsida and the Liliopsida. Although not commonly isolated from hydrilla and milfoil in China, a *Fusarium* sp. was found on milfoil at San-jia-dian, and an *F. oxysporum* was found on hydrilla leaves collected at the same location in 1994 and 1995.

Cylindrocladium species have a worldwide distribution and have been reported as pathogens of angiosperm and gymnosperm hosts (Crous, Phillips, and Wingfield 1991). Isolation of a *Cylindrocladium* from submersed aquatic vegetation appears rather unusual in that most of the known hosts are woody terrestrial plants. Disease symptoms in woody hosts include damping off, seedling blight, leaf spots, stem cankers, shoot blight, and root disease. As with other pathogens, some species of *Cylindrocladium* have a broad host range, and others are limited to one or two hosts. If the China isolates prove to be highly pathogenic on hydrilla or milfoil in initial screening, further tests will have to be undertaken to ensure that they are not also pathogenic on woody species.

Glomerella cingulata and its more frequently reported anamorph, *Colletotrichum gloeosporioides,* induce a variety of disease symptoms on their hosts including fruit rots, leaf spots, and stem anthracnoses (Domsch, Gams, and Anderson 1980; Sutton 1980). A *formae speciales* of this group, *Colletotrichum gloeosporioides* (Penz.) Sacc. f.sp. *aeschynomene,* an anthracnose pathogen of northern jointvetch (*Aeschenomene virginica*) (Daniel et al. 1973), was registered as the mycoherbicide COLLEGO in 1982 (Bowers 1986; TeBeest 1993). Another *formae speciales*, *Colletotrichum gloeosporioides* (Penz.) Sacc. f.sp. *malvae*, was studied for biocontrol of round-leaved mallow and velvetleaf (Mortensen 1988) and

was later developed into the mycoherbicide Biomal (Harris 1993). Another species, *Colletotrichum truncatum*, has shown promise as a biocontrol agent for control of hemp sesbania (Boyette 1991) and is currently undergoing fermentation and formulation development as a mycoherbicide (Egley, Hanks, and Boyette 1993). A strain of *Glomerella cingulata* isolated from milfoil in Europe has induced disease development on milfoil in small-scale laboratory screening (Varley and Harvey 1996). Several isolates of *G. cingulata* have been obtained from milfoil in the United States and are currently undergoing screening in the biocontrol laboratory at WES. It appears that this group of pathogens may hold promise as a good source of biocontrol agents for hydrilla or milfoil.

Strains of *Mycoleptodiscus terrestris* have been isolated from hydrilla and Eurasian watermilfoil from a number of locations in the United States. Originally described from collections in the United States (Gerdemann 1953), but not isolated from milfoil during survey work in Europe,[1] the distribution of the fungus was thought to be North American. The distribution now appears to be far more cosmopolitan, as several isolates were obtained from plant tissue of hydrilla and milfoil in China. A total of six isolates of *M. terrestris* were obtained from milfoil and hydrilla stem tissue collected at Huai-Roi Reservoir in China in 1994. On a return trip to the site in 1995, both hydrilla and milfoil were collected and several fungal isolates were obtained from the plant material, but *M. terrestris* was not among them. Of interesting note was that total biomass of hydrilla and milfoil was much reduced from the previous year. Eighteen isolates of *M. terrestris* were obtained from milfoil stem and leaf tissue collected at East Lake and Liangze Lake in central China, Hubei Province. Upon examination of cultures at the USDA quarantine center, 3 of the 18 isolates were found to be sporulating abundantly in pure culture. This phenomenon is unusual in that *M. terrestris* isolates may be induced to sporulate on excised plant tissue, but they rarely sporulate on standard culture media (PCA and PDA). Morphologically, several of the isolates exhibited cultural characteristics that were quite different from isolates obtained from plant material in the United States.

Mycoleptodiscus terrestris has been considered as a biocontrol candidate for milfoil and hydrilla in the United States. A strain isolated from milfoil in Massachusettes was extensively studied for biocontrol purposes by EcoScience, Inc., Worchester, MA (Gunner 1983, 1985, 1987; Gunner et al. 1990; Stack 1990). Following promising results using a mycelial matrix of the fungus on milfoil in laboratory and field studies, formulation efforts were begun whereby the fungus was incorporated into calcium alginate pellets. Approval for field testing Aqua-Fyte in selected States was granted in 1993. Because of poor results in the field using the formulated fungus and faced with a reformulation task, the company discontinued research efforts on mycoherbicide product development with *M. terrestris* (Shearer 1996b). Another strain of *M. terrestris* isolated from hydrilla in Texas has proved to be an effective pathogen on hydrilla in laboratory and field studies (Joye 1990; Joye and Cofrancesco 1991; Shearer 1996a) and is being considered for product development. Strains of *M. terrestris*

[1] Personal Communication, 1996, Harry Evans, Plant Pathologist, International Institute of Biological Control, Ascot, Berkshire, UK.

isolated from the native range of hydrilla and milfoil may prove to be more effective as biocontrol agents than those currently in use in the United States. If screening and host-specificity tests are promising, the application for release of the organism may be given increased consideration because the fungus already occurs as an endemic.

4 Conclusions and Recommendations

Overseas survey work presents a wide array of challenges to biocontrol researchers. Working in aquatic ecosystems presents a range of problems not faced by scientists surveying for biocontrol agents on terrestrial plants. Boats may not be readily available, and efforts to find biocontrol agents of the target aquatic plants are often confined to easily accessible shoreline areas. Rarely can one observe the plants in situ in their entirety. Once a collector enters the water, the sediment is disturbed and turbidity exacerbates the ability to easily observe the submersed macrophytes and symptoms of disease.

Within the host country, finding individuals with expertise in the distribution and ecology of aquatic macrophytes is often difficult. Background information on diseases of plants that are not economically important may be lacking because they are less frequently studied from a pathology point of view than are crop plants. For example, as of 1989, Farr et al. (1989) listed almost 400 reported pathogenic organisms on corn (*Zea maize*), whereas there was only a single organism reported on hydrilla.

Worldwide, water bodies have been greatly impacted by agriculture, industry, and burgeoning populations. They may be destroyed as the need for agricultural land increases, and they are drained and put into cultivation. Left in place, they are often impacted by disturbance and pollutants, which affects the aquatic habitat not only in terms of plant diversity and richness but in the complex of organisms closely associated with the plants.

Using a team of research scientists experienced in plant pathology and entomology has great merit when undertaking foreign exploration for biocontrol agents. Natural enemies of a targeted plant usually include a complex of organisms both insects and pathogens. Training in a particular discipline often biases the scope of observation (i.e., entomologists look for insects, and plant pathologists look for pathogens). Two or more people carefully scrutinizing plant material provide increased chances that more organisms can be found. The team approach also means that foreign cooperators assist several researchers in a combined effort rather than individually, meaning a savings in cost and time.

Classical biocontrol using pathogens is still a relatively new and little used weed management technology in the United States compared with insect biocontrol that dates back to 1902 when insects were introduced into Hawaii for the control of lantana (Julien 1992). That pathogens can be effective biocontrol agents has been documented following successes with the release of *Entyloma compositarum* Farlow to control hamakua pamakani in Hawaii and *Puccinia chondrillina* Bubak. & Syd to control skeleton weed in California (TeBeest, Yang, and Cisar 1992). Despite successes with release of these classical agents in the 1970s, progress has been slow; only two additional pathogens, one a nematode, have been intentionally released in the United States, and nine more were under investigation as of 1991 (Watson 1991). The lack of progress has been attributed to a variety of factors including the inherent nature of classical biocontrol (Evans 1995) and the lack of funding, personnel, quarantine facilities, and foreign collaborators (Watson 1991). The addition of the quarantine facility at Fort Detrick and increased availability of foreign cooperators may now aid the development of classical pathogen biocontrol agents including those of hydrilla and milfoil.

With the acquisition and identification of a number of potential pathogens among the isolates obtained from hydrilla and milfoil in China, screening of the isolates on the target plants will begin spring 1996. A continuation of the survey work needs to be expanded into other countries where hydrilla and milfoil are native. The recent possibility of being able to use cooperators in Russia and eastern Europe holds great promise for expanding the scope of the work into additional unsurveyed regions.

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Appendix A
Isolates Collected in China, Summer 1994 and 1995

Isolate number, collection site, province, date, substrate, identification, and status of fungal isolates collected in China summer 1994 and 1995. Status of isolates: Active = undergoing testing at the U.S. Department of Agriculture Agricultural Research Service Foreign Disease-Weed Science Laboratory, Fort Detrick, Frederick, MD; No growth = fungus was not viable when plated at the quarantine center; Discarded = bacteria, double picks, and contaminated cultures were eliminated from the test list; =(xxx) a morphologically similar isolate based on plate culture.

Isolate No.	Location	Province	Date	Substrate	Identification	Status
C194	August 1st Lake	Beijing	7/18/94	Hydrilla stem	*Pythium* sp.	Active
C294	August 1st Lake	Beijing	7/18/94	Hydrilla stem	Phycomycete	No growth
C394	August 1st Lake	Beijing	7/18/94	Hydrilla stem	*Trichoderma harzianum*	Active
C494	August 1st Lake	Beijing	7/18/94	Milfoil stem	*Tritirachium* sp.	Active
C594	August 1st Lake	Beijing	7/18/94	Water	*Trichoderma harzianum*	=394
C694	August 1st Lake	Beijing	7/18/94	Water	Bacterium	Discarded
C794	Qing Hua University Canal	Beijing	7/19/94	Hydrilla stem	Bacterium	Discarded
C894	Qing Hua University Canal	Beijing	7/19/94	Hydrilla stem	Bacterium	Discarded
C994	Qing Hua University Canal	Beijing	7/19/94	Hydrilla stem	Bacterium	Discarded
C1094	Qing Hua University Canal	Beijing	7/19/94	Hydrilla stem	No growth	Discarded
C1194	Qing Hua University Canal	Beijing	7/19/94	Hydrilla stem	Moniliaceous hyphomycete	Active

C1294	Qing Hua University Canal	Beijing	7/19/94	Hydrilla stem	*Trichoderma harzianum*	=594
C1394	Qing Hua University Canal	Beijing	7/19/94	Hydrilla stem	*Glomerella cingulata*	Active
C1494	Qing Hua University Lake	Beijing	7/19/94	Hydrilla stem	Moniliaceous hyphomycete	Active
C1594	Qing Hua University Lake	Beijing	7/19/94	Hydrilla leaves	*Chaetomium* sp.	Active
C1694	Qing Hua University Lake	Beijing	7/19/94	Hydrilla leaves	Moniliaceous hyphomycete	Active
C1794	Qing Hua University Lake	Beijing	7/19/94	Hydrilla leaves	*Chaetomium* sp.	Active
C1894	Qing Hua University Canal	Beijing	7/19/94	Hydrilla leaves	*Glomerella cingulata*	=1394
C1994	Lan-dian-chang	Beijing	720/94	Milfoil stem	Contaminated	Discarded
C2094	Lan-dian chang	Beijing	7/20/94	Milfoil stem	*Botryoderma* sp.	Active
C2194	Lan-dian-chang	Beijing	7/20/94	Milfoil stem	No growth	Discarded
C2294	Lan-dian-chang	Beijing	7/20/94	Milfoil stem	*Pythium* sp.	Active
C2394	Lan-dian-chang	Beijing	7/20/94	Milfoil stem	No growth	Discarded
C2494	Lan-dian-chang	Beijing	7/20/94	Milfoil leaves	*Fusarium* sp.	Active
C2594	Lan-dian-chang	Beijing	7/20/94	Milfoil leaves	*Chaetomium funicola*	Active
C2694	Lan-dian-chang	Beijing	7/20/94	Milfoil stem	*Sclerotium rolfsii*	Active
C2794	Lan-dian-chang	Beijing	7/20/94	Hydrilla stem	Bacterium	Discarded
C2894	Qing Hua University Canal	Beijing	7/19/94	Hydrilla leaves	Coelomycete	Active
C2994	Lan-dian-chang	Beijing	7/20/94	Hydrilla leaves	*Geotrichum* sp.	Active
C3094	Lan-dian-chang	Beijing	7/20/94	Hydrilla leaves	No growth	Discarded
C3194	Lan-dian-chang	Beijing	7/20/94	Hydrilla leaves	*Botryomonilia* sp.	Active
C3294	Lan-dian-chang	Beijing	7/20/94	Hydrilla stem	*Botryomonilia* sp.	=3194
C3394	San-jia-dian	Beijing	7/21/94	Hydrilla leaves	*Rhizosphaera* sp.	Active
C3494	San-jia-dian	Beijing	7/21/94	Hydrilla leaves	*Rhizosphaera* sp.	=3394
C3594	Qiao zhuang	Beijing	7/21/94	Hydrilla leaves	Moniliaceous hyphomycete	Active
C3694	Qiao zhuang	Beijing	7/21/94	Hydrilla stem	*Phoma* sp.	Active
C3794	Qiao zhuang	Beijing	7/21/94	Hydrilla stem	Moniliaceous hyphomycete	Active
C3894	San-jia-dian	Beijing	7/21/94	Milfoil stem	*Cylindrocladium* sp.	Active
C3994	San-jia-dian	Beijing	7/21/94	Milfoil stem	No growth	Discarded
C4094	San-jia-dian	Beijing	7/21/94	Milfoil stem	Phycomycete	Active

C4194	San-jia-dian	Beijing	7/21/94	Milfoil stem	*Plectosphaerella cucumeris*	Active
C4294	San-jia-dian	Beijing	7/21/94	Milfoil stem	*Alternaria alternata*	Active
C4394	San-jia-dian	Beijing	7/21/94	Milfoil stem	Phycomycete - *Pythium*	=4094
C4494	Han-jia-chuan	Beijing	7/21/94	Hydrilla leaves	*Trichoderma* sp.	Active
C4594	Han-jia-chuan	Beijing	7/21/94	Hydrilla leaves	*Trichoderma* sp.	Active
C4694	Han-jia-chuan	Beijing	7/21/94	Hydrilla leaves	*Colletotrichum gloeosporioides*	Active
C4794	Han-jia-chuan	Beijing	7/21/94	Hydrilla leaves	*Microsphaeropsis olivacea*	Active
C4894	Han-jia-chuan	Beijing	7/21/94	Hydrilla stem	*Trichoderma* sp.	Active
C4994	San-jia-dian	Beijing	7/21/94	Hydrilla leaves	*Fusarium oxysporum*	Active
C5094	San-jia-dian	Beijing	7/21/94	Milfoil stem	Bacterium	Active
C5194	San-jia-dian	Beijing	7/21/94	Milfoil stem	Phycomycete - *Pythium*	=4094
C5294	San-jia-dian	Beijing	7/21/94	Milfoil stem	Phycomycete	Active
C5394	San-jia-dian	Beijing	7/21/94	Milfoil stem	Phycomycete - *Pythium*	=4094
C5494	San-jia-dian	Beijing	7/21/94	Milfoil stem	Phycomycete	Active
C5594	Huai-Roi Reservoir	Beijing	7/22/94	Milfoil leaves	*Mycoleptodiscus terrestris*	Active
C5694	Huai-Roi Reservoir	Beijing	7/22/94	Milfoil stem	*Mycoleptodiscus terrestris*	Active
C5794	Huai-Roi Reservoir	Beijing	7/22/94	Milfoil stem	*Mycoleptodiscus terrestris*	=5694
C5894	Huai-Roi Reservoir	Beijing	7/22/94	Milfoil stem	*Mycoleptodiscus terrestris*	=5594
C5994	Huai-Roi Reservoir	Beijing	7/22/94	Milfoil stem	*Cylindrocladium* sp.	=3894
C6094	Huai-Roi Reservoir	Beijing	7/22/94	Hydrilla leaves	*Pythium* sp.	Active
C6194	Huai-Roi Reservoir	Beijing	7/22/94	Hydrilla leaves	*Trichoderma* sp.	Active
C6294	Huai-Roi Reservoir	Beijing	7/22/94	Hydrilla leaves	*Cylindrocladium* sp.	=3894
C6394	Huai-Roi Reservoir	Beijing	7/22/94	Hydrilla leaves	Moniliaceous hyphomycete	Active
C6494	Huai-Roi Reservoir	Beijing	7/22/94	Hydrilla stem	*Trichoderma aureoviride*	Active
C6594	Huai-Roi Reservoir	Beijing	7/22/94	Hydrilla stem	*Mycoleptodiscus terrestris*	Active
C6694	Huai Roi Reservoir	Beijing	7/22/94	Hydrilla stem	*Mycoleptodiscus terrestris*	=6594
C6794	Huai-Roi Reservoir	Beijing	7/22/94	Hydrilla stem	Moniliaceous hyphomycete	Active
C6894	Huai-Roi Reservoir	Beijing	7/22/94	Hydrilla leaves	*Plecotsphaerella cucumeris*	=4194
C6994	Huai-Roi Reservoir	Beijing	7/22/94	Hydrilla leaves	*Trichoderma* sp.	Active
C7094	Huai-Roi Reservoir	Beijing	7/22/94	Hydrilla leaves	*Trichoderma* sp.	=6994
C7194	Huai-Roi Reservoir	Beijing	7/22/94	Hydrilla leaves	*Trichoderma* sp.	=6994

C7294	Huai-Roi Reservoir	Beijing	7/22/94	Hydrilla leaves	No growth	Discarded
C7394	Huai-Roi Reservoir	Beijing	7/22/94	Hydrilla leaves	*Plectosphaerella cucumeris*	=4194
C7494	Huai-Roi Reservoir	Beijing	7/22/94	Hydrilla stem	*Cylindrocladium* sp.	=3894
C7594	Huai-Roi Reservoir	Beijing	7/22/94	Milfoil stem	*Pythium* sp.	Active
C7694	Huai-Roi Reservoir	Beijing	7/22/94	Milfoil stem	*Pythium* sp.	=7594
C7794	Huai-Roi Reservoir	Beijing	7/22/94	Milfoil stem	*Pythium* sp.	=7594
C7894	Huai-Roi Reservoir	Beijing	7/22/94	Milfoil leaves	*Cylindrocladium* sp.	=3894
C7994	Huai-Roi Reservoir	Beijing	7/22/94	Milfoil leaves	Phycomycete	Active
C8094	Huai-Roi Reservoir	Beijing	7/22/94	Milfoil leaves	*Cylindrocladium* sp.	=3894
C8194	Lan-dian-chang	Beijing	7/20/94	Milfoil stem	*Colletotrichum truncatum*	Active
C8294	Huai-Roi Reservoir	Beijing	7/22/94	Milfoil leaves	*Cylindrocladium* sp.	=3894
C8394	Yu River	Beijing	7/25/94	Milfoil root	*Cylindrocladium* sp.	=3894
C8494	Yu River	Beijing	7/25/94	Milfoil leaves	*Cylindrocladium* sp.	=3894
C8594	Yu River	Beijing	7/25/94	Milfoil stem	*Plectosphaerella cucumeris*	=4194
C8694	Yu River	Beijing	7/25/94	Milfoil leaves	*Plectosphaerella cucumeris*	=4194
C8794	Yu River	Beijing	7/25/94	Milfoil leaves	*Cylindrocladium* sp.	=3894
C8894	Yu River	Beijing	7/25/94	Milfoil stem	*Cylindrocladium* sp.	=3894
C195	Ma san jis Canal	Liaoning	7/19/95	Hydrilla stem	*Cladosporium* sp.	Active
C295	Ma san jia Canal	Liaoning	7/19/95	Hydrilla stem	*Acremonium charticola*	Active
C395	Ma san jia Canal	Liaoning	7/19/95	Hydrilla stem	*Drecslera fugax*	Active
C495	Ma san jia Canal	Liaoning	7/19/95	Trapa leaves	Phycomycete	Active
C595	Ma san jia Canal	Liaoning	7/19/95	Trapa leaves	*Sclerotium rolfsii*	Active
C695	Ma san jia Canal	Liaoning	7/19/95	Trapa leaves	*Pythium* sp.	Active
C795	Ma san jia Canal	Liaoning	7/19/95	Trapa leaves	*Trichoderma* sp.	Active
C895	Ma san jia Canal	Liaoning	7/19/95	Trapa leaves	*Pythium* sp.	Active
C995	Ma san jia Canal	Liaoning	7/19/95	Trapa leaves	*Sclerotium* sp.	Active
C1095	Liangze Lake	Hubei	7/26/95	Milfoil stem	*Cylindrocladium* sp.	Active
C1195	East Lake	Hubei	7/25/95	Milfoil stem	*Mycoleptodiscus terrestris*	Active
C1295	East Lake	Hubei	7/25/95	Milfoil stem	*Pythium* sp.	Active
C1395	Liangze Lake	Hubei	7/26/95	Milfoil stem	*Mycoleptodiscus terrestris*	=1195
C1495	Liangze Lake	Hubei	7/26/95	Milfoil stem	*Mycoleptodiscus terrestris*	=1195
C1595	East Lake	Hubei	7/25/95	Milfoil roots	Double pick	Active

C1695	Liangze Lake	Hubei	7/26/95	Milfoil stem	*Cylindrocladium* sp.	=1095
C1795	East Lake	Hubei	7/25/95	Milfoil leaves	*Khuskia oryzae*	Active
C1895	East Lake	Hubei	7/25/95	Milfoil leaves	*Khuskia oryzae*	=1795
C1995	East Lake	Hubei	7/25/95	Milfoil leaves	*Khuskia oryzae*	=1795
C2095	East Lake	Hubei	7/25/95	Milfoil leaves	*Pythium* sp.	Active
C2195	East Lake	Hubei	7/25/95	Milfoil stem	Coelomycete	Active
C2295	East Lake	Hubei	7/25/95	Milfoil stem	*Cylindrocladium* sp.	=1095
C2395	East Lake	Hubei	7/25/95	Milfoil stem	*Pythium* sp.	Active
C2495	East Lake	Hubei	7/25/95	Milfoil stem	*Pythium* sp.	Active
C2595	East Lake	Hubei	7/25/95	Milfoil stem	*Mycoleptodiscus terrestris*	=1195
C2695	East Lake	Hubei	7/25/95	Milfoil stem	*Pythium* sp.	Active
C2795	East Lake	Hubei	7/25/95	Milfoil stem	*Cylindrocladium* sp.	=1095
C2895	East Lake	Hubei	7/25/95	Milfoil stem	*Pythium* sp.	Active
C2995	East Lake	Hubei	7/25/95	Milfoil stem	*Mycoleptodiscus terrestris*	=1195
C3095	East Lake	Hubei	7/25/95	Milfoil stem	*Pythium* sp.	Active
C3195	East Lake	Hubei	7/25/95	Milfoil stem	*Mycoleptodiscus terrestris*	=1195
C3295	East Lake	Hubei	7/25/95	Milfoil stem	No growth	Discarded
C3395	East Lake	Hubei	7/25/95	Milfoil roots	Double pick	Discarded
C3495	East Lake	Hubei	7/25/95	Milfoil stem	*Mycoleptodiscus terrestris*	=1195
C3595	East Lake	Hubei	7/25/95	Milfoil stem	Double pick	Active
C3695	Liangze Lake	Hubei	7/26/95	Milfoil stem	*Mycoleptodiscus terrestris*	=1195
C3795	Liangze Lake	Hubei	7/26/95	Milfoil stem	No growth	Discarded
C3895	Liangze Lake	Hubei	7/26/95	Milfoil stem	*Pythium* sp.	Active
C3995	Liangze Lake	Hubei	7/26/95	Milfoil stem	*Mycoleptodiscus terrestris*	=1195
C4095	Liangze Lake	Hubei	7/26/95	Milfoil stem	*Mycoleptodiscus terrestris*	=1195
C4195	Liangze Lake	Hubei	7/26/95	Milfoil stem	*Fusarium* sp.	Active
C4295	Liangze Lake	Hubei	7/26/95	Milfoil stem	Double pick	Active
C4395	Liangze Lake	Hubei	7/26/95	Milfoil leaves	*Mycoleptodiscus terrestris*	=1195
C4495	Pearl Spring	Jiangsu	7/28/95	Milfoil stem	*Epicoccum purpurascens*	Active
C4595	Pearl Spring	Jiangsu	7/28/95	Milfoil stem	*Trichoderma hamatum*	Active
C4695	Pearl Spring	Jiangsu	7/28/95	Milfoil stem	*Cylindrocladium* sp.	=1095
C4795	Pearl Spring	Jiangsu	7/28/95	Milfoil stem	*Cylindrocladium* sp.	=1095
C4895	Pearl Spring	Jiangsu	7/28/95	Milfoil stem	*Cylindrocladium* sp.	=1095
C4995	Pearl Spring	Jiangsu	7/28/95	Milfoil stem	*Cylindrocladium* sp.	=1095

C5095	Pearl Spring	Jiangsu	7/28/95	Milfoil stem	*Cylindrocladium* sp.	=1095
C5195	Pearl Spring	Jiangsu	7/28/95	Milfoil stem	*Penicillium* sp.	Active
C5295	Pearl Spring	Jiangsu	7/28/95	Milfoil stem	*Cylindrocladium* sp.	=1095
C5395	Pearl Spring	Jiangsu	7/28/95	Milfoil stem	*Cylindrocladium* sp.	=1095
C5495	Pearl Spring	Jiangsu	7/28/95	Milfoil stem	*Cylindrocladium* sp.	=1095
C5595	Pearl Spring	Jiangsu	7/28/95	Milfoil stem	Phycomycete	Active
C5695	Pearl Spring	Jiangsu	7/28/95	Milfoil stem	*Pythium* sp.	Active
C5795	Pearl Spring	Jiangsu	7/28/95	Milfoil stem	*Cylindrocladium* sp.	=1095
C5895	Pearl Spring	Jiangsu	7/28/95	Milfoil stem	*Cylindrocladium* sp.	=1095
C5995	Pearl Spring	Jiangsu	7/28/95	Milfoil stem	*Cylindrocladium* sp.	=1095
C6095	Pearl Spring	Jiangsu	7/28/95	Milfoil roots	Phycomycete	Active
C6195	Pearl Spring	Jiangsu	7/28/95	Milfoil roots	Phycomycete	Active
C6295	Pearl Spring	Jiangsu	7/28/95	Milfoil leaves	Phycomycete	Active
C6395	Pearl Spring	Jiangsu	7/28/95	Milfoil leaves	Double pick	Discarded
C6495	Pearl Spring	Jiangsu	7/28/95	Milfoil leaves	*Cylindrocladium* sp.	=1095
C6595	Pearl Spring	Jiangsu	7/28/95	Milfoil leaves	*Cylindrocladium* sp.	=1095
C6695	Tianhe River	Jiangsu	7/29/95	Milfoil stem	*Cylindrocladium* sp.	=1095
C6795	Tianhe River	Jiangsu	7/29/95	Milfoil stem	*Pythium* sp.	Active
C6895	Tianhe River	Jiangsu	7/29/95	Milfoil stem	*Sclrotium rolfsii*	Active
C6995	Tianhe River	Jiangsu	7/29/95	Milfoil stem	*Thielavia terricola*	Active
C7095	Tianhe River	Jiangsu	7/29/95	Milfoil stem	*Thielavia terricola*	=6995
C7195	Tianhe River	Jiangsu	7/29/95	Hydrilla leaves	Double pick	Discarded
C7295	Jing chun dahe River	Jiangsu	7/29/95	Milfoil stem	Moniliaceous hyphomycete	Active
C7395	Jing chun dahe River	Jiangsu	7/29/95	Milfoil stem	*Pythium* sp.	Active
C7495	Jing chun dahe River	Jiangsu	7/29/95	Milfoil stem	Phycomycete	Active
C7595	Tianhe River	Jiangsu	7/29/95	Trapa leaf	Phycomycete	Active
C7695	East Lake	Hubei	7/25/95	Milfoil leaves	*Mycoleptodiscus terrestris*	=1195
C7795	East Lake	Hubei	7/25/95	Milfoil stem	*Mycoleptodiscus terrestris*	=1195
C7895	East Lake	Hubei	7/25/95	Milfoil stem	*Pythium* sp.	Active
C7995	Liangze Lake	Hubei	7/26/95	Milfoil stem	*Mycoleptodiscus terrestris*	=1195
C8095	East Lake	Hubei	7/25/95	Milfoil stem	*Mycoleptodiscus terrestris*	=1195
C8195	East Lake	Hubei	7/25/95	Milfoil stem	*Mycoleptodiscus terrestris*	=1195
C8295	East Lake	Hubei	7/25/95	Milfoil stem	*Mycoleptodiscus terrestris*	=1195

C8395	Huai-Roi Reservoir	Beijing	8/1/95	Milfoil stem	*Cylindrocladium* sp.	=1095
C8495	Huai-Roi Reservoir	Beijing	8/1/95	Milfoil stem	*Cylindrocladium* sp.	=1095
C8595	Huai-Roi Reservoir	Beijng	8/1/95	Milfoil stem	*Plectosphaerella cucumeris*	Active
C8695	Huai-Roi Reservoir	Beijing	8/1/95	Milfoil stem	*Plectosphaerella cucumeris*	=8695
C8795	Huai-Roi Reservoir	Beijing	8/1/95	Milfoil stem	Phycomycete	Active
C8895	Huai-Roi Reservoir	Beijing	8/1/95	Milfoil stem	Phycomycete	=8795
C8995	Huai-Roi Reservoir	Beijing	8/1/95	Milfoil leaves	*Cylindrocladium* sp.	=1095
C9095	Huai-Roi Reservoir	Beijing	8/1/95	Milfoil leaves	*Drechslera* sp.	Active
C9195	Huai-Roi Reservoir	Beijing	8/1/95	Milfoil leaves	*Trichoderma harzianum*	Active
C9295	Huai-Roi Reservoir	Beijing	8/1/95	Hydrilla stem	*Pythium* sp.	Active
C9395	Huai-Roi Reservoir	Beijing	8/1/95	Hydrilla stem	*Cylindrocladium* sp.	=1095
C9495	Huai-Roi Reservoir	Beijing	8/1/95	Hydrilla stem	*Arthrinium* sp.	Active
C9595	Huai-Roi Reservoir	Beijing	8/1/95	Hydrilla leaves	*Pythium* sp.	Active
C9695	Huai-Roi Reservoir	Beijing	8/1/95	Hydrilla leaves	*Cylindrocladium* sp.	=1095
C9795	Huai-Roi Reservoir	Beijing	8/1/95	Hydrilla leaves	*Plectosphaerella cucumeris*	=1095
C9895	Huai-Roi Reservoir	Beijing	8/1/95	Hydrilla leaves	*Drechslera* sp.	=395
C9995	Huai-Roi Reservoir	Beijing	8/1/95	Hydrilla leaves	Double pick	Discarded
C10095	Liangze Lake	Hubei	7/26/95	Milfoil stem	*Mycoleptodiscus terrestris*	Active
C10195	Huai-Roi Reservoir	Beijing	8/1/95	Hydrilla stem tip	*Khuskia oryzae*	=1795
C10295	Huai-Roi Reservoir	Beijing	8/1/95	Hydrilla stem tip	*Pythium* sp.	Active
C10395	Huai-Roi Reservoir	Beijing	8/1/95	Milfoil leaves	*Drechslera* sp.	=395
C10495	San-jia-dian	Beijing	8/7/95	Milfoil stem	*Cylindrocladium* sp.	=1095
C10595	San-jia-dian	Beijing	8/7/95	Milfoil stem	Phycomycete	Active
C10695	San-jia-dian	Beijing	8/7/95	Milfoil stem	*Fusarium* sp.	Active
C10795	East Lake	Hubei	7/25/95	Soil	NA	Active
C10895	San-jia-dian	Beijing	8/7/95	Hydrilla leaves	No growth	Discarded
C10995	San-jia-dian	Beijing	8/7/95	Hydrilla leaves	No growth	Discarded
C11095	San-jia-dian	Beijing	8/7/95	Hydrilla leaves	Moniliaceous hyphomycete	Active
C11195	San-jia-dian	Beijing	8/7/95	Hydrilla leaves	Moniliaceous hyphomycete	No growth

C11295	San-jia-dian	Beijing	8/7/95	Hydrilla leaves	Moniliaceous hyphomycete	Active
C11395	San-jia-dian	Beijing	8/7/95	Hydrilla leaves	*Fusarium oxysporum*	Active
C11495	San-jia-dian	Beijing	8/7/95	Hydrilla stem	*Aspergillus* sp.	Active
C11595	San-jia-dian	Beijing	8/7/95	Hydrilla stem	Discarded	Active
C11695	San-jia-dian	Beijing	8/7/95	Hydrilla leaves	Moniliaceous hyphomycete	Active
C11795	San-jia-dian	Beijing	8/7/95	Hydrilla leaves	No growth	Discarded
C11895	San-jia-dian	Beijing	8/7/95	Hydrilla leaves	Moniliaceous hyphomycete	=11095
C11995	San-jia-dian	Beijing	8/7/95	Hydrilla leaves	No growth	Discarded
C12095	San-jia-dian	Beijing	8/7/95	Hydrilla leaves	No growth	Discarded
C12195	San-jia-dian	Beijing	8/7/95	Hydrilla leaves	No growth	Discarded
C12295	San-jia-dian	Beijing	8/7/95	Hydrilla leaves	Moniliaceous hyphomycete	=11095
C12395	San-jia-dian	Beijing	8/7/95	Hydrilla stem	Moniliaceous hyphomycete	Active
C12495	San-jia-dian	Beijing	8/7/95	Hydrilla stem	*Cladosporium* sp.	Active
C12595	San-jia-dian	Beijing	8/7/95	Hydrilla stem	Moniliaceous hyphomycete	=12395
C12695	San-jia-dian	Beijing	8/7/95	Hydrilla stem	No growth	Discarded
C12795	San-jia-dian	Beijing	8/7/95	Hydrilla stem	No growth	Discarded
C12895	Old Summer Palace	Beijing	8/8/95	Hydrilla stem	No growth	Discarded
C12995	Old Summer Palace	Beijing	8/8/95	Hydrilla stem	*Colletotrichum gloeosporioides*	Active
C13095	Old Summer Palace	Beijing	8/8/95	Hydrilla leaves	*Trichoderma* sp.	Active
C13195	Old Summer Palace	Beijing	8/8/95	Hydrilla leaves	*Trichoderma* sp.	Active
C13295	Old Summer Palace	Beijing	8/8/95	Hydrilla leaves	Dematiaceous hyphomycete	Active
C13395	Old Summer Palace	Beijing	8/8/95	Hydrilla leaves	No growth	Active
C13495	Old Summer Palace	Beijing	8/8/95	Hydrilla leaves	Dematiaceous hyphomycete	=13295

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Twenty-five sites were visited in China in 1994 and 1995 as part of a joint effort to survey for insects and pathogens as potential biological control agents of hydrilla and Eurasian watermilfoil. During the limited survey time, over 200 fungal isolates were obtained from plant tissues and water samples. Axenic cultures of the fungi were sent to the U.S. Department of Agriculture Agriculatural Research Service Foreign Disease-Weed Science Laboratory, Frederick, MD, where they were tentatively identified and accessed into a permanent collection. Not all of the isolates are considered to be potential agents for biological control. Of particular interest are pathogens in the genera *Pythium*, *Phytophthora*, *Fusarium*, *Cylindrocladium*, *Glomerella*, and *Mycoleptodiscus*. One or more species in each of these genera have been reported to be disease-producing organisms on a variety of plants.

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