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A SURVEY OF THE CONTINENTAL UNITED STATES FOR PATHOGENS OF EURASIAN WATERMILFOIL

by

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A survey of the continental United States for pathogens of *Myriophyllum spicatum* (Eurasian watermilfoil) was conducted. More than 50 water bodies, located in 10 states, were sampled for diseased plants. Sample sites represented a geographic and climatic cross section of aquatic systems in the continental United States, including ponds, lakes, reservoirs, rivers, and canals.

At the conclusion of the survey, 792 isolates had been collected from tissue samples and maintained in pure culture. Of these, 462 were bacteria and 330 were fungi; many isolates were duplicates but were retained because they possibly represented different strains of the same species.

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Lytic enzyme assays indicated that 14 bacterial and 22 fungal isolates produced pectinase or cellulose. Enzyme assays were used to screen for the more promising isolates for additional study as potential biocontrol agents for the management of Eurasian watermilfoil. The 36 isolates were then assayed against healthy sprigs of the target species in test tubes. Results indicated that five fungal isolates should be considered for additional study.

PREFACE

This report describes a field survey and laboratory study designed to isolate and characterize potential microbiological agents for the control of Eurasian watermilfoil.

Funding for this study was provided by the Office, Chief of Engineers (OCE), US Army, under Appropriation No. 96X3122, Construction General, to the Aquatic Plant Control Research Program (APCRP), US Army Engineer Waterways Experiment Station (WES), Vicksburg, Miss. The OCE Technical Monitor of the APCRP was Mr. E. Carl Brown.

The principal investigator was Dr. William C. Zattau of the Wetlands and Terrestrial Habitat Group (WTHG), Environmental Resources Division (ERD), Environmental Laboratory (EL), WES. This report was reviewed by Drs. Kurt D. Getsinger, Douglas Gunnison, and Charles V. Klimas. Mr. Harvey L. Jones and Mses. Cindy L. Crist, Susan M. Hennington, Pat A. Miller, and Ramona H. Warren assisted in the study at WES. The report was edited by Ms. Jessica S. Ruff of the WES Information Technology Laboratory.

The field portion of this study could not have been conducted without the cooperation of many people. The author acknowledges the following individuals for their assistance regarding the states indicated: Alabama - Mr. Joe Zolczynski, Alabama Department of Conservation and Natural Resources, and Mr. Leon Bates, Tennessee Valley Authority; California - Mr. Edward Fonseca, San Francisco Water Department, and Dr. Randall Stocker, Imperial Valley Irrigation District; Florida - Mr. Jesse Van Dyke, Florida Department of Natural Resources; Louisiana - Mr. Doug Adams, Louisiana Department of Wildlife and Fisheries; New York - Mr. Robert Johnson, Cornell University; North Carolina - Dr. Graham Davis, East Carolina University; Texas - Mr. Paul Gray, Pat Mayse Lake Project Director, and Dr. John Rodgers, North Texas State University; Vermont - Ms. Virginia Garrison, Vermont Department of Water Resources; Washington - Mr. Robert Rawson, US Army Engineer District, Seattle; Wisconsin - Drs. John Andrews and Stan Nichols, University of Wisconsin.

Team leaders for the Biomanagement Team during the study were Mr. Edwin A. Theriot and Dr. Dana R. Sanders, Sr. The study was conducted under the direct supervision of Dr. Hanley K. Smith, Chief, WTHG, and under the general supervision of Dr. Conrad J. Kirby, Jr., Chief, ERD, and Dr. John Harrison, Chief, EL. Manager of the APCRP was Mr. J. Lewis Decell.

Commander and Director of WES was COL Dwayne G. Lee, CE. Technical Director was Dr. Robert W. Whalin.

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PART I: INTRODUCTION

Background

1. Eurasian watermilfoil (*Myriophyllum spicatum L.*) is a submersed perennial aquatic plant that grows in fresh to brackish waters. This aggressive rooted aquatic plant, native to Europe, Asia, and northern Africa, grows to lengths of approximately 10 to 13 ft (3 to 4 m) in depths of up to almost 23 ft (7 m) (Stockerl and Kent 1984). The plant, with long flexible stems and finely dissected leaves arranged in whorls of four, is anchored in the bottom sediment by a branched, fibrous root system. The species is monecious with emergent floral spikes. The primary means of propagation is through asexual reproduction by auto fragmentation, rhizome production, and axillary buds.

2. Eurasian watermilfoil is thought to have been introduced into North America in the latter part of the 19th century (Bayley, Rabin, and Southwick 1968; Reed 1977) and has since spread from the east to west coast of the United States (Reed 1977; Aiken, Newroth, and Wile 1979). Couch and Nelson (1986), studying herbaria records, have documented past and present populations in 33 states and the District of Columbia. Eurasian watermilfoil has developed into one of the most troublesome aquatic weed species in waters for which the US Army Corps of Engineers has primary management responsibility.

3. Problems associated with the plant include displacement of native vegetation, interference with navigational and recreational activities, impedance of water flow in natural drainage systems and man-made irrigation systems, decline of aesthetic and real estate values due to accumulation and decay of plant material, and associated depression of dissolved oxygen levels. Fragmentation, caused by water movement or man's activities (e.g., boat propellers), is a major mode of dispersal. Reed (1977) noted that some long-distance dispersal has been related to the aquarium and aquatic nursery trade.

Conventional Control

4. Conventional methods for control and management of Eurasian watermilfoil populations include mechanical harvesting, herbicide treatment, and drawdown. Mechanical harvesting, an expensive, labor-intensive method, generally provides only temporary relief. A common control technique in the Northeast, upper Midwest, and West Coast regions, harvesting must be repeated during the growing season in most water bodies. Nichols and Shaw (1986) noted that positive environmental benefits of harvesting include the removal of plant material prior to decay, thereby lessening oxygen depletion and aesthetic problems. Negative impacts include a temporary increase in turbidity, spread of potentially colonizing fragments, and stimulation of post-harvest growth by the remnant plants.

5. Chemical control is often convenient, quick, and effective, although herbicide use is limited by label restrictions. Current formulations provide a variety of treatment regimes, although problems associated with herbicide use include occasional unpredictable treatment results and upset of aquatic oxygen-carbon dioxide balance due to decaying vegetation (Nichols and Shaw 1986).

6. Winter drawdown is an effective management strategy for Eurasian watermilfoil in some areas. Potential problems with this method include conflicts with recreational use, unknown impacts on benthic organisms, and invasion by other undesirable plant species in dewatered areas.

Biological Control Agents of Eurasian Watermilfoil

7. Being an introduced species, Eurasian watermilfoil has no natural enemies in the United States. Several studies have been undertaken to find suitable biological agents for management purposes.

Insects

8. Buckingham, Bennett, and Ross (1981) investigated two insect species for control of Eurasian watermilfoil, and neither was recommended as a suitable biological control agent. Balciunas (1982) conducted a survey for insects and other macroinvertebrates associated with Eurasian watermilfoil in the United States. He made 71 collections in 11 states and determined that none of the insects identified in the study was a promising candidate for use

as a biological agent. Habeck (1983) investigated the use of aquatic larvae of the European moth, *Parapoynx* sp., as a biological agent for Eurasian watermilfoil and found the insect unsuitable due to its polyphagous nature.

Grass carp

9. Studies utilizing the latest strain of *Ctenopharyngodon idella*, the triploid grass carp, indicate that, although the fish can be used to control Eurasian watermilfoil, it is considered to be undesirable for this use because of its lack of specificity and possible indirect impact on sport fisheries.

Pathogens

10. Interest in pathogens of Eurasian watermilfoil was stimulated by two events in the mid- to late-1960's in the Chesapeake Bay area. Bayley (1971) described extensive mortality of Eurasian watermilfoil in two areas--Lake Venice, a 22-acre (9-ha) pond located in Anne Arundel County, Maryland, and an area near the Northeast River in Cecil County, Maryland--and suggested the declines were the result of diseases. At that time she suspected the causative agent of the Northeast River disease was a virus, although this was never proven. Hayslip and Zettler (1973) later reported a failure to introduce the Northeast River disease in Florida, and Bean, Fusco, and Klarman (1973) concluded that the Lake Venice and Northeast River events were not the result of phytopathogen activity. Later, Bayley, Rabin, and Southwick (1978) concluded that adverse environmental conditions were responsible for the observed declines since populations of other native species concurrently decreased.

11. Although these events were never shown to be the result of plant pathogen activity, the occurrences stimulated research into Eurasian watermilfoil population declines and interest in the use of pathogens for biological control. Elser (1967) cited a number of partial or complete disappearances of milfoil that occurred without apparent cause. Carpenter (1980) documented a sustained decline in Eurasian watermilfoil in Lake Wingra, Wisconsin. Davis and Brinson (1983) noted declines in Eurasian watermilfoil communities in Currituck Sound, North Carolina. Nichols and Shaw (1986) cited declines in New York, Washington, Wisconsin, Ontario, and British Columbia.

12. Several studies have been conducted for the purpose of locating and isolating pathogenic organisms for use as biological control agents for Eurasian watermilfoil. Hayslip and Zettler (1973) tested a bacterium isolated from Eurasian watermilfoil and a number of fungi obtained from other plant

species. Results indicated limited infection of the target species. Joyner and Freeman (1973) tested the pathogenicity of *Rhizoctonia solani* to Eurasian watermilfoil and found the fungus mildly pathogenic. Andrews and Hecht (1981) tested the pathogenicity of *Fusarium sporotrichioides* to Eurasian watermilfoil. The fungus that was isolated from Eurasian watermilfoil caused a localized necrosis, and the Andrews and Hecht data indicated that it existed as an epiphyte. Andrews, Hecht, and Bashirian (1982) tested another fungal isolate from Eurasian watermilfoil, *Acremonium curvulum*, and determined that the isolate grew epiphytically and endophytically without serious damage to the host plant. Gunner (1983) isolated celluloytic and pectinolytic microorganisms from the phyllosphere of Eurasian watermilfoil and enhanced the production of appropriate enzymes by repetitive culture in media rich in cellulose and pectin. Gunner (1985) has since reported results that demonstrated the ability of these microorganisms to control Eurasian watermilfoil under simulated field conditions.

Approach

13. Plant pathogens possess characteristics that make them candidates as biocontrol agents. Often host-specific, self-perpetuating, and with rapid reproduction rates, pathogens are capable of quickly infecting and damaging target species. The use of such organisms as control agents is based on the tendency for pathogens to be common regulating influences on population levels of aquatic plant species in natural systems. No such natural population control occurs when an exotic species such as Eurasian watermilfoil is introduced into susceptible aquatic systems where natural enemies are nonexistent, resulting in unchecked growth. This problem might be alleviated if a host-specific pathogen were introduced into the system, thereby suppressing the Eurasian watermilfoil populations. To date no host-specific pathogen of Eurasian watermilfoil has been found.

Rationale

14. The cost and long-term ineffectiveness of conventional control methods support the need to find effective biological control agents for the management of Eurasian watermilfoil. Although it has become obvious that

there are no widespread native or indigenous pathogens currently acting to halt the spread of the species in the continental United States, a virulent bacterial or fungal pathogen of the plant may exist. Such a localized pathogen population could provide a source for biological control agents. Therefore, a thorough survey for pathogens was conducted.

Purpose and Objectives

15. The purpose of this study was to isolate bacterial and fungal pathogens from Eurasian watermilfoil for their development as candidate biological agents for the control of Eurasian watermilfoil. Specific objectives were as follows:

a. Examine populations of Eurasian watermilfoil in the continental United States for evidence of phytopathogen activity.

b. Isolate microorganisms from diseased tissue.

c. Select candidate microorganisms by assaying isolates for production of cellulase and pectinase, enzymes lytic to selected plant tissue.

d. Test selected candidate microorganisms for their ability to infect and damage healthy Eurasian watermilfoil plants.

PART II: METHODS AND MATERIALS

Site Selection

16. Survey sites represented a geographic and climatic cross section of aquatic systems in the continental United States, including ponds, lakes, reservoirs, rivers, and canals. Unexplained diebacks of Eurasian watermilfoil had been reported from many of these areas.

Site Descriptions

17. Appendix A provides specific locations and dates of all collections; Figure 1 shows general site locations. General information concerning the collection areas appears below, with the plant acreages as estimated by local authorities.

Alabama

18. Guntersville Reservoir, a Tennessee Valley Authority (TVA) lake heavily infested with Eurasian watermilfoil, is located in north-central Alabama. This plant has been the dominant submersed aquatic plant in TVA mainstream reservoirs for the last 25 years (Bates, Burns, and Webb 1986). Plant samples from these sites were provided by TVA personnel.

19. Numerous sites were sampled in Mobile Bay. At the beginning of this survey (spring 1984), the Eurasian watermilfoil in the bay covered an estimated 3,000 to 4,000 acres (1,200 to 1,600 ha).

California

20. Lower Crystal Springs Reservoir, Pilarcitos Lake, and San Andreas Lake, located in northern California, serve as potable water reservoirs for the city of San Francisco. These water bodies, managed by the San Francisco Water Department, have minor infestations of Eurasian watermilfoil.

21. The Imperial Valley Irrigation District, in southern California, consists of 507,000 acres (205,000 ha) irrigated by approximately 1,700 miles (2,700 km) of canal, 600 miles (965 km) of which contain Eurasian watermilfoil. Several canals were sampled.

Florida

22. Sample sites were located on the Apalachicola River, Deer Point Lake, Waukulla River, and Lake Seminole. The Apalachicola River estuarine

Figure 1. Sampling sites for pathogens of Eurasian watermilfoil

system contained an estimated 320 acres (130 ha) of milfoil in 1985, compared to an estimated 800 acres (325 ha) in 1984. Sample sites were located near Apalachicola, at the mouth of Apalachicola Bay.

23. Deer Point Lake, a potable water source for Panama City, contained an estimated 500 acres (200 ha) of Eurasian watermilfoil in 1984 (Schardt 1985). The main body of the lake and several feeder creeks were sampled.

24. Eurasian watermilfoil was first detected in the Waukulla River in 1983, and a total of 8 acres (3 ha) was reported as of 1985 (Schardt 1985). Sampling sites were located near St. Marks. Sampling sites at Lake Seminole were near Three Rivers State Park on the Florida side of the lake.

Louisiana

25. The eastern and western shorelines of the southern portion of Toledo Bend Reservoir were sampled. Milfoil is a nuisance plant in many of the bays in the reservoir.

New York

26. Three areas in New York were sampled. Cayuga Lake, one of the Finger Lakes, has several isolated populations of the aquatic weed. Several sites in and around the Sodus Bay area of Lake Ontario were sampled, as were

several small research ponds, near Ithaca, on land owned by Cornell University.

North Carolina

27. Collections were made at several locations in the Outer Banks area of coastal North Carolina. Sites were located in Kitty Hawk Bay and Coinjock Bay. According to Davis and Brinson (1983), populations of Eurasian watermilfoil have dramatically decreased in these areas in recent years.

Texas

28. Pat Mayse Lake was impounded in 1968, and Eurasian watermilfoil was first documented in 1976. The population of the weed peaked in 1981, covering approximately 100 surface acres (40 ha) of the 6,000-acre (2,400-ha) lake. Since then, due to a period of drastic changes in lake elevation accompanied by increased turbidity, as well as a herbicide treatment program, the population of milfoil has declined.

Vermont

29. Seven bodies of water were sampled in Vermont, including Lakes Bomoseen, Carmi, Hortonia, and St. Catherine, Glen Lake, Metcalf Pond, and the St. Albans Bay area of Lake Champlain.

30. Eurasian watermilfoil has been in Lake Champlain since the early 1960's. The milfoil populations in Lakes Carmi and St. Catherine most likely began in the mid-1970's, whereas introduction into Lake Bomoseen, Glen Lake, Metcalf Pond, and Lake Hortonia was in the early 1980's. The milfoil population appears to be rapidly increasing in the state.

Washington

31. Union, Juanita, Yarrow, and Fairweather Bays and Cozy Cove, all located on Lake Washington near Seattle, were sampled. Eurasian watermilfoil is the dominant aquatic plant in Lake Washington. Surface coverage has increased dramatically in the sample areas in recent years (Zisette 1985).

32. Sample sites were located on four reservoirs of the Columbia River system. In Banks Lake, sparse populations of Eurasian watermilfoil were sampled. Wells Reservoir had a low population of aquatic plants due to recent drawdowns conducted for archaeological salvage operations. Rocky Reach Reservoir and Rock Island Reservoir both have large aquatic plant populations, with Eurasian watermilfoil being a dominant species.

33. The majority of Lake Osoyoos, formed by the Okanogan River, is in Canada. The US portion was sampled, as was a short stretch of the Okanogan River south of Lake Osoyoos.

Wisconsin

34. A number of Wisconsin Lakes were sampled for pathogens of Eurasian watermilfoil. Lakes Kegonsa, Mendota, Waubesa, and Wingra and the Yahara River, all in the vicinity of Madison, had large populations of the weed. Seven lakes east of Madison (Lac La Belle, Lakes Fowler and Pewaukee, and Oconomowoc, Lower Phantom, Pine, and Whitewater Lakes) were also sampled.

Collection of Samples

35. Tissue samples from Eurasian watermilfoil plants were collected either from shore or by boat. Sites were initially scanned for diseased vegetation, and portions of diseased and nearby nondiseased vegetation were collected. In areas of visually healthy vegetation, samples were collected on a random basis. Samples with some accompanying water were placed in sterile Whirl-Paks, marked with an identifying number, and placed in a cooler. Upon return to the laboratory, samples were refrigerated prior to examination.

Processing of Samples

36. Plant material was washed with sterile distilled water to remove debris and was placed in a translucent plastic container on a light box. Transmitted light passing through the plant tissue highlighted diseased areas. Closer observation was done with a stereo dissecting microscope.

37. Diseased plant tissue (Figures 2-5) was surface sterilized in a dilute (5-percent) solution of sodium hypochloride for 60 sec. Small pieces of tissue were aseptically cut from stem or leaf sections with a sterile scalpel and plated onto petri plates containing either potato dextrose agar (PDA) or nutrient agar (NA). PDA is a selective medium for fungi whereas NA is selective for bacteria. The petri plates were incubated at 28° C for 3 to 5 days. Fungal or bacterial colonies were subcultured onto fresh plates of the appropriate medium until pure cultures were obtained. Isolates were maintained in PDA or NA test tubes under constant refrigeration and transferred to fresh tubes as necessary.

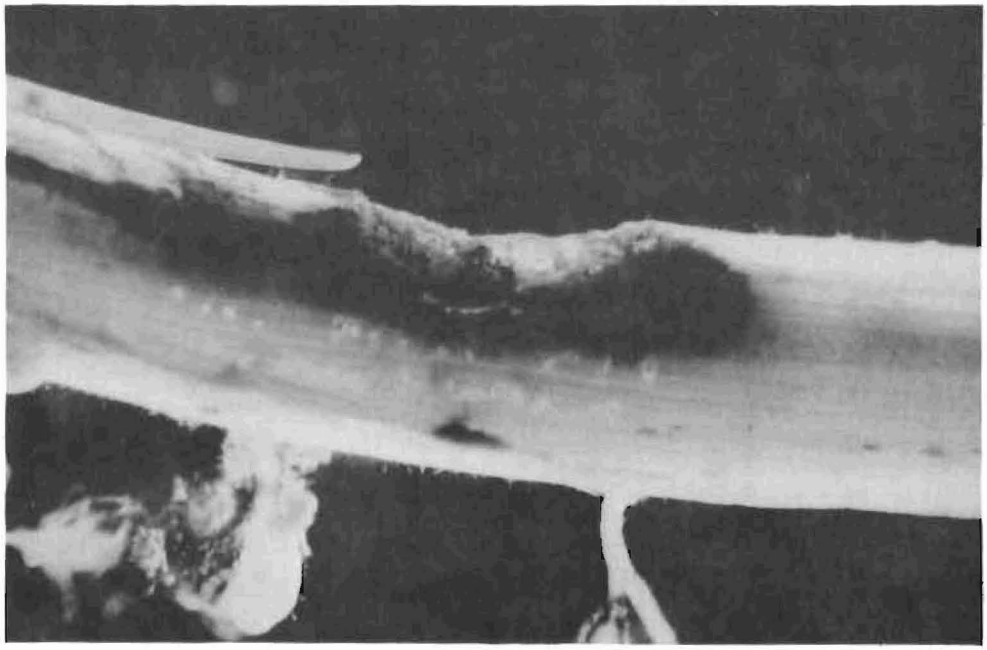

Figure 2. Infected area on Eurasian watermilfoil stem

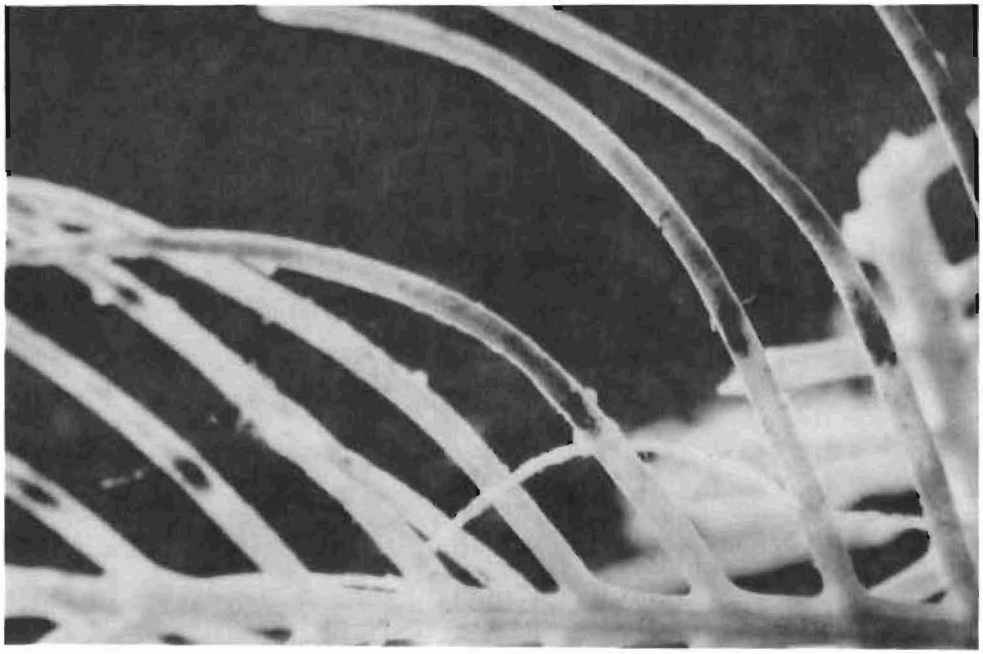

Figure 3. Eurasian watermilfoil leaf divisions showing disease symptoms

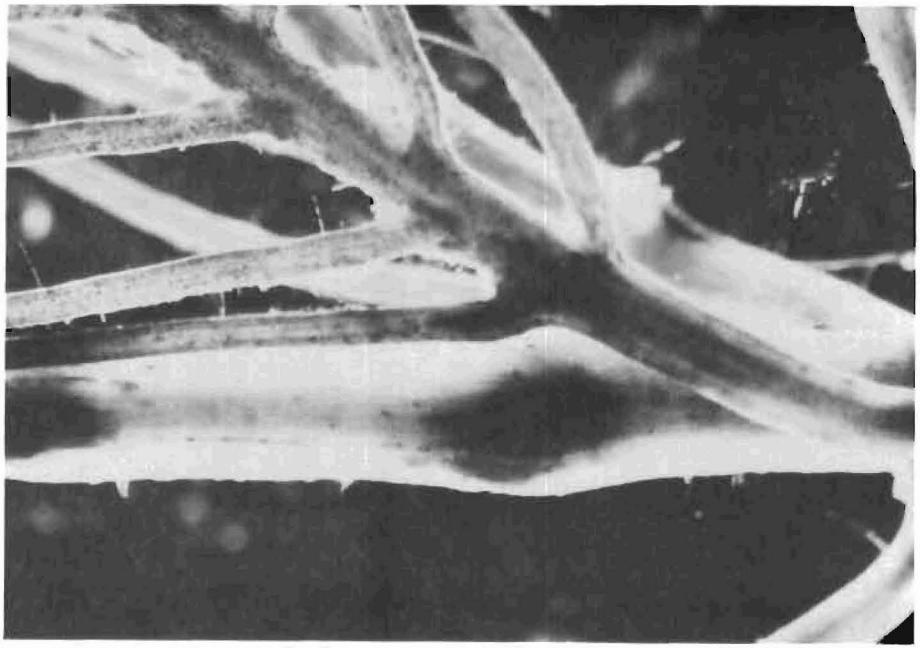

Figure 4. Internal diseased area causing swelling of Eurasian watermilfoil stem

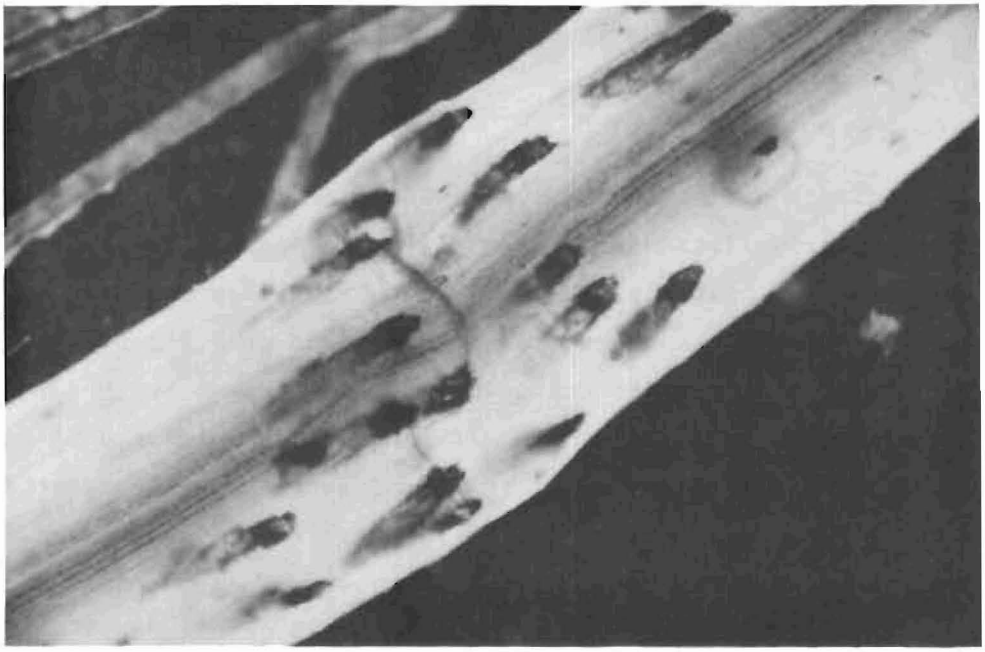

Figure 5. Insect feeding scars provide entry points for invasive microbes into Eurasian watermilfoil stem

Lytic Enzyme Screening

38. All isolates were screened for lytic enzyme production by challenging the organism with an appropriate growth medium. Cellulase production was determined by inoculating the isolate onto agar petri plates incorporating cellulose as the sole carbohydrate source (Skerman 1967). Cellulase production was indicated by a clearing of the cloudy growth medium. Pectinase production was determined by growing the organism on a pectate agar that consisted of nutrient agar amended with 0.05-percent sodium chloride overlaid with a thin layer of sodium polypectate gel (Paton 1959). Production of pectinase was indicated by depressions of the assay medium (Figure 6).

Inoculum Viability

39. At the time of assay plant inoculation, serial dilutions were conducted to determine the concentration and viability of all isolates. One millitre of each prepared inoculant was serially diluted in sterile distilled water and pipetted onto appropriate NA or PDA petri plates. Counts of colony forming units (CFU's) were made after 1 to 2 days incubation at 25° C.

Test Tube Assay

40. Candidate bacterial and fungal isolates for test tube assay were selected by a positive lytic enzyme assay. These organisms were then tested for ability to infect, parasitize, and damage healthy sprigs of Eurasian watermilfoil in test tubes. Bacterial inoculum was produced by incubating the isolate in screw-top test tubes containing 10 ml nutrient broth (NB) for 24 hr at 25° C with frequent agitation. Fungal inoculum was produced by growing the isolate in 50 ml V-8 broth contained in 125-ml screw-top Erlenmeyer flasks on a reciprocal shaker for 72 hr at 25° C. The resultant mycelium and broth were then blended for 2 to 5 sec in a sterile stainless steel blender. Inoculum consisted of 1.0 ml of the incubated, inoculated NB or blended V-8 broth. Serial dilutions were conducted to determine the inoculum concentrations. Controls consisted of 1.0 ml of sterile NB, sterile V-8 broth, or distilled water (no-treatment control).

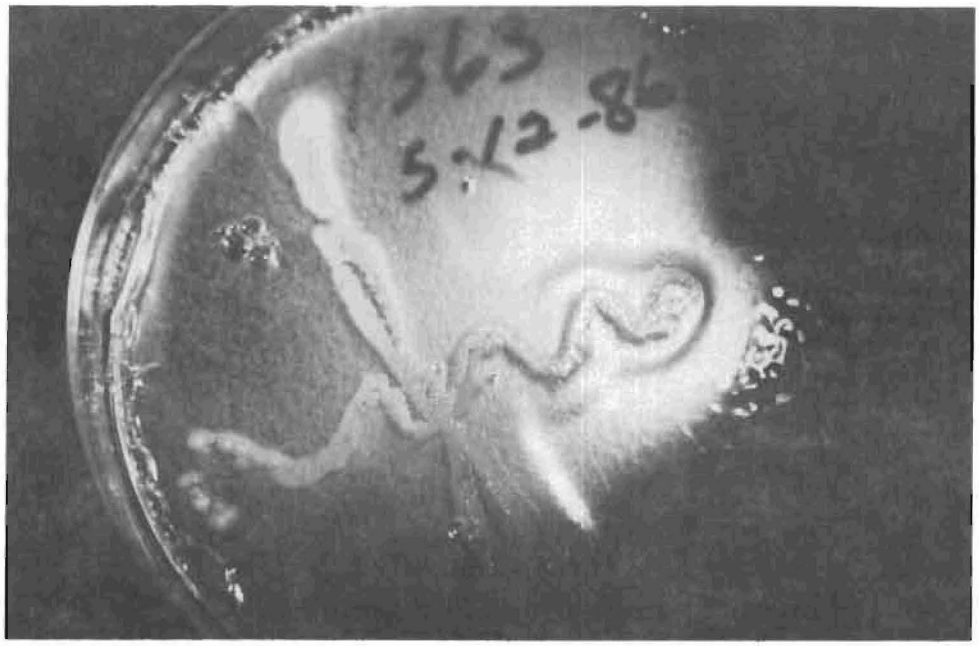

Figure 6. Pectinase assay medium with depression resulting from use of pectin by bacterial isolate

41. Assay plants were grown in monoculture in greenhouse tanks containing modified Hoagland's plant growth solution (Table 1). The plants were rooted in sediment obtained from a local lake. Healthy, nondiseased stem apices, 11 cm in length, were cut from the greenhouse plants, washed in sterile distilled water, and placed in capped 200-ml test tubes containing 150 ml of the sterile modified Hoagland's solution. Assay plants were kept in a growth chamber at 25° C under a 12-hr day/night cycle (Figure 7).

42. The inoculum was pipetted into the test tubes containing the assay plants. There were five replicates of each isolate and control.

43. A damage index value (Table 2) between 1 and 5 was used to rate the assay, which ran 6 weeks. A value was assigned to each sprig prior to inoculation and each week after inoculation.

Reisolation

44. At the conclusion of the assay, attempts were made to reisolate the assay inoculum. Sections of diseased Eurasian watermilfoil tissue were removed from the test tube, washed in sterile distilled water, and surface sterilized in a dilute (5-percent) solution of sodium hypochloride for 60 sec.

Table 1

Greenhouse and Test Tube Plant Culture Solution

Substance	Value
Compound	
$CaCl_2 \cdot 2H_2O$	0.0917 g/ℓ
$KHCO_3$	0.0154 g/ℓ
$MgSO_4$ (anhydrous)	0.0337 g/ℓ
$NaHCO_3$	0.0584 g/ℓ
Elemental composition	
Na^+	16.0 mg/ℓ
K^+	6.0 mg/ℓ
Ca^{++}	25.0 mg/ℓ
Mg^{++}	6.8 mg/ℓ
HCO_3^-	51.8 mg/ℓ
Cl^-	44.2 mg/ℓ
SO_4^{--}	26.9 mg/ℓ

Notes: Ionic strength, 3.8 mM. Measured parameters: pH, 7.9; conductivity, 280 µS/cm, 25° C; total inorganic carbon, 10.2 mg/ℓ.

Sections of this tissue were made with a sterile scalpel and plated on either NA or PDA. Colonies were subcultured until pure and then compared to the original inoculant using cultural and taxonomical characteristics.

Identification of Isolates

45. Fungal isolates selected for test tube assay were sent to Dr. Tim Schubert, Florida Department of Agricultural and Consumer Services, University of Florida, for taxonomic characterization.

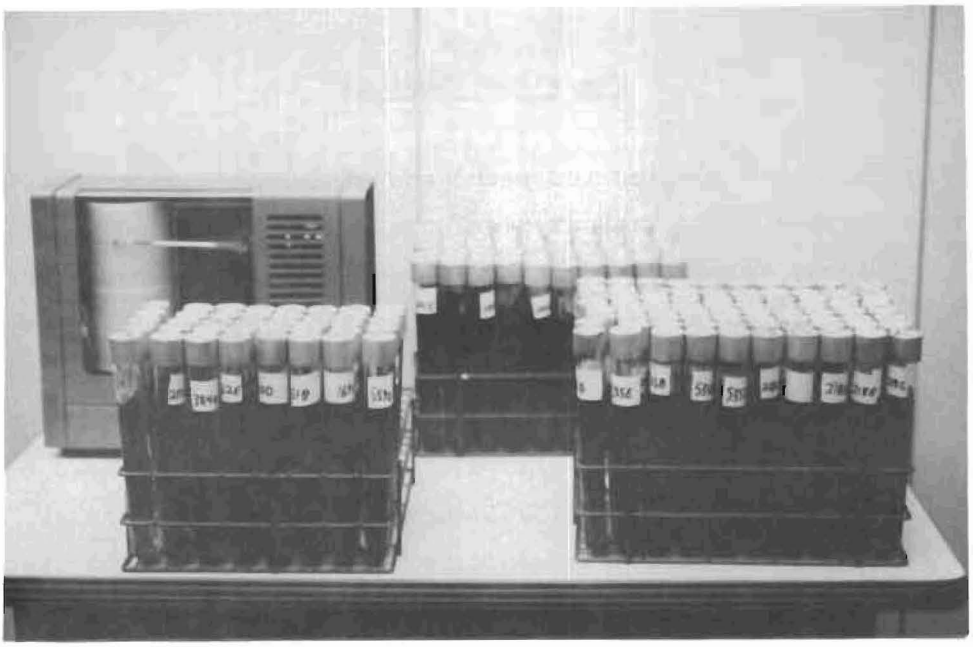

Figure 7. Test tube assay in environmental growth chamber

Table 2

Index of Plant Damage Values

Index Value	Description
1	Vigorous, healthy plants. No evidence of chlorosis, disease, or damage.
2	Faintly chlorotic plants only slightly paler than 1, exhibiting few or no damaged areas.
3	Chlorotic plants, or plants exhibiting less than 50 percent disease or damage.
4	Markedly chlorotic plants, or plants exhibiting pronounced disease or damage exceeding 50 percent of sprig.
5	100 percent chlorotic to brown plants, plants with broken stems and most leaves transparent and disintegrating, or obviously dead plants.

PART III: RESULTS

Observed Pathology

46. Of the areas sampled for pathogens of Eurasian watermilfoil during the survey, three had sites with atypical plants: Coinjock Bay, North Carolina; Lake Bomoseen, Vermont; and Lake Osoyoos, Washington. Numerous plants in a population of Eurasian watermilfoil in Coinjock Bay appeared to be diseased. These plants, collected in June 1984, were decomposing or in a state similar to advanced senescence. Plants in surrounding populations were healthy. Two isolates from these plants (Nos. 212 and 217) caused heavy damage to healthy Eurasian watermilfoil during the test tube assay.

47. Plants at the Lake Bomoseen site, sampled August 1985, were covered with a white flocculant that, under microscopic examination, appeared to consist of a collection of epiphytes. Under the epiphytic covering, the stems and leaves were chlorotic. Over an acre of milfoil was affected, and gaps existed in the mat of this local population. Isolates obtained from this site were found to be nonpathogenic to Eurasian watermilfoil.

48. At several Lake Osoyoos sites, milfoil plants were prostrate, and some exhibited limited chlorosis during sampling in August 1985. Low turbidity in this lake permitted visual observation of the prostrate milfoil, a condition that would have gone unnoticed in many survey areas. Microscopic examination indicated no overt pathological conditions. Several isolates were obtained from the plant tissue and were determined to be nonpathogenic.

49. Although no widespread disease outbreaks were observed during this survey, many sampled Eurasian watermilfoil plants exhibited symptoms of phytopathogen activity, such as leaf spots, stem spots, and chlorosis. These plants, which were returned to the laboratory for microscopic examination and phytopathogen isolation, yielded the majority of the isolates collected during the survey.

Isolate Collection

50. At the conclusion of the 2-year survey, 792 isolates were maintained in pure culture; of these, 462 were bacteria and 330 were fungi.

Several isolates appeared to be duplicates but were maintained because they may have represented different strains of the same species.

Lytic Enzyme Assay

51. Lytic enzyme assays performed on the isolates indicated that 14 bacterial isolates and 22 fungal isolates produced lytic enzymes (Table 3). These 36 isolates were considered candidates for further testing.

Isolate Identification

52. Reliable identification of fungal isolates depends on their production of characteristic reproductive structures. A number of these isolates could not to be identified due to their inability to produce such structures (Table 4). Bacterial isolates were not identified since none of them produced significant damage in the test tube assay.

53. Many microorganisms isolated during this study did not exhibit lytic enzyme production. These organisms were not candidates for further assay and probably represented epiphytic microflora that survived the surface sterilization process.

Inoculum Viability

54. At the time of assay plant inoculation, serial dilutions were conducted to determine the concentration and viability of all isolates. These results are presented in Table 5.

Test Tube Assay

55. Sprigs of healthy Eurasian watermilfoil were challenged by the candidate isolates that tested positive for lytic enzyme production. The isolate and control (sterile uninoculated NB, V-8 broth, and sterile distilled water) assays were assigned a damage index value (Table 2). These results are presented in Table 6 and Appendixes B, C, and D.

Table 3

Isolates Positive for Lytic Enzyme Production

Isolate No.	Site Source	Bacteria/ Fungi	Enzyme Produced*
114	Apalachicola Bay	Bacteria	C, P
115	Apalachicola Bay	Bacteria	C, P
116	Apalachicola Bay	Bacteria	C, P
156	Deer Point Lake	Bacteria	C, P
162	Apalachicola Bay	Fungus	C, P
169	Apalachicola Bay	Fungus	C
170	Apalachicola Bay	Bacteria	P
172	Apalachicola Bay	Bacteria	P
189	Toledo Bend Reservoir	Fungus	C, P
192	Toledo Bend Reservoir	Fungus	C, P
201	Coinjock Bay	Bacteria	C
212	Coinjock Bay	Fungus	C, P
217	Coinjock Bay	Fungus	C, P
218	Guntersville Reservoir	Bacteria	C, P
308	Lake Wingra	Bacteria	P
327	Yahara River	Fungus	P
328	Yahara River	Fungus	P
329	Yahara River	Fungus	P
332	Yahara River	Fungus	P
351	Lac La Belle	Fungus	P
378	Pine Lake	Fungus	P
384	Lake Waubesa	Fungus	P
418	Mobile Bay	Bacteria	P
424	Mobile Bay	Fungus	P
429	Mobile Bay	Fungus	P
440	Wakulla River	Fungus	P
464	Deer Point Lake	Fungus	P
508	Apalachicola Bay	Fungus	P
509	Apalachicola Bay	Bacteria	P
511	Apalachicola Bay	Bacteria	P
520	Metcalf Pond	Fungus	P
535	Lake Bomoseen	Fungus	P
551	St. Albans Bay	Bacteria	P
559	Kitty Hawk Bay	Fungus	P
561	Kitty Hawk Bay	Fungus	P
565	Kitty Hawk Bay	Bacteria	P

* C = cellulose; P = pectinase.

Table 4

Fungal Isolates Examined in Test Tube Assay

Isolate No.	Scientific Name
162	Actinomycete
169	Rhizoctonia
189	Nonsporulating isolate*
192	*Aspergillus* sp.
212	Nonsporulating isolate
217	*Trichoderma* sp.
327	*Aspergillus* sp.
328	*Aspergillus* sp.
329	Aspergillus niger group
332	*Aspergillus* sp.
351	Nonsporulating isolate
378	*Penicillium* sp.
384	*Phoma* sp.
424	Nonsporulating isolate
429	*Gleocladium* sp.
440	*Penicillium* sp.
464	Nonsporulating isolate
508	*Penicillium* sp.
520	*Penicillium* sp.
535	*Curvularia lunata*
559	Nonsporulating isolate
561	*Penicillium* sp.

* Nonsporulating isolates could not be reliably identified.

Fungal isolates

56. Mean damage index (MDI) values of the fungal isolates, when compared to the no-treatment control, indicate that 19 fungal isolates had a significantly greater ($p = 0.05$) value after 1 week, 21 after 2 weeks, 2 after 3 weeks, 3 after 4 weeks, 9 after 5 weeks, and 5 at the conclusion of the assay.

57. Differences are less dramatic when the fungal isolates' MDI values are compared to that of the V-8 broth control, although there are short-term similarities. After 1 week, 19 fungal isolates had significantly greater MDI values than did the V-8 control; this number decreased to two from weeks 2 through 5, with none at week 6.

58. Isolate No. 212 had a 3.6 MDI at week 1, increasing to a 4.0 MDI at week 2. Isolate No. 464 was the next most damaging isolate with a 3.2 MDI at

Table 5

Inoculum Viability for Test Tube Assay (n = 2)

Isolate No.	Bacteria/ Fungi	CFU/ml
114	Bacteria	5×10^7
115	Bacteria	2×10^7
116	Bacteria	2×10^8
156	Bacteria	7×10^6
162	Fungus	3×10^4
169	Fungus	8×10^3
170	Bacteria	5×10^6
172	Bacteria	3×10^7
189	Fungus	8×10^5
192	Fungus	4×10^4
201	Bacteria	1×10^7
212	Fungus	4×10^4
217	Fungus	9×10^3
218	Bacteria	3×10^8
308	Bacteria	7×10^7
327	Fungus	6×10^3
328	Fungus	2×10^4
329	Fungus	5×10^4
332	Fungus	9×10^3
351	Fungus	4×10^5
378	Fungus	1×10^4
384	Fungus	8×10^3
418	Bacteria	3×10^8
424	Fungus	7×10^3
429	Fungus	6×10^4
440	Fungus	6×10^3
464	Fungus	1×10^5
508	Fungus	3×10^4
509	Bacteria	4×10^7
511	Bacteria	2×10^7
520	Fungus	2×10^8
535	Fungus	7×10^4
551	Bacteria	8×10^8
559	Fungus	5×10^3
561	Fungus	1×10^4
565	Bacteria	3×10^8

week 1, increasing to a 3.8 MDI at week 2. (These isolates' MDI values are not significantly different.) The isolate with the highest MDI at the completion of the assay was No. 429 (value = 4.6).

59. Of the fungal assay test tube replicates, 21 contained Eurasian watermilfoil plants that had MDI values ranging to 5 at the conclusion of the assay. These replicates were spread out among 14 isolates, with five having two replicates rated 5 and one isolate having three replicates rated 5. For reference, none of the no-treatment and NB control replicates rated 5, whereas one replicate of the V-8 broth control rated 5 at the conclusion of the assay.

Bacterial isolates

60. The results of the bacterial assay indicated that eight isolates had a significantly greater MDI than the NB control after 1 week, four after 2 weeks, two after 3 weeks, and none for weeks 4, 5, and 6. When compared to the no-treatment control, eight bacterial isolates showed significantly greater damage after 1 week, eight after 2 weeks, and none for weeks 3 through 6. None of the bacterial replicates rated 5 on the damage index.

Table 6

Weekly Mean Damage Index Values for Each Isolate (n = 5)

No.	Microorganism	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
114	Bacterial	2.2	2.4	2.4	2.4	3.0	3.8
115	Bacterial	2.0	2.0	2.0	2.0	2.6	3.2
116	Bacterial	2.2	2.2	2.2	2.2	3.0	3.4
156	Bacterial	2.2	2.4	2.8	2.8	3.2	3.8
162	Fungal	2.0	2.2	2.2	2.2	3.4	4.0
169	Fungal	1.8	2.0	2.4	2.4	3.4	4.0
170	Bacterial	1.2	1.2	1.2	2.4	3.0	3.4
172	Bacterial	2.0	2.0	2.0	2.0	3.0	3.6
189	Fungal	2.0	2.6	2.6	2.6	3.6	4.0
192	Fungal	3.0	3.0	3.0	3.2	3.4	4.2
201	Bacterial	2.0	2.0	2.0	2.0	3.2	3.4
202	Control	1.2	1.4	1.8	2.4	3.4	3.6
212	Fungal	3.6	4.0	4.2	4.2	4.4	4.4
217	Fungal	2.4	2.4	2.4	2.8	3.4	4.4
218	Bacterial	2.0	2.0	2.0	2.2	2.6	3.0
308	Bacterial	2.4	2.4	2.4	2.4	2.6	2.8
327	Fungal	2.4	2.6	3.2	3.2	3.8	3.8
328	Fungal	3.0	3.0	3.0	3.0	3.2	3.2
329	Fungal	2.0	2.0	2.8	3.0	3.0	3.8
332	Fungal	2.0	3.0	3.0	3.0	3.2	3.4
351	Fungal	1.0	1.0	2.4	2.4	3.0	3.2
378	Fungal	1.0	2.6	2.8	2.8	3.4	4.2
384	Fungal	2.0	2.4	2.4	2.4	3.2	4.0
418	Bacterial	1.2	1.2	1.2	2.0	2.6	2.8
424	Fungal	2.2	3.0	3.0	3.0	3.8	4.0
427	Control	1.2	3.0	3.0	3.0	3.2	4.0
429	Fungal	2.2	2.4	2.6	3.0	3.8	4.6
440	Fungal	2.4	3.0	3.0	3.2	3.4	3.8
464	Fungal	3.2	3.8	3.8	3.8	4.0	4.4
508	Fungal	2.2	3.0	3.0	3.2	3.2	4.0
509	Bacterial	1.4	1.4	1.4	2.0	2.6	3.2
511	Bacterial	1.2	1.2	1.2	1.2	2.0	2.6
520	Fungal	2.2	2.4	2.4	2.6	3.2	3.8
535	Fungal	1.4	2.2	3.2	3.4	3.6	4.0
551	Bacterial	1.0	1.0	1.0	2.0	2.0	3.0
559	Fungal	2.4	2.4	2.6	2.8	3.6	4.4
561	Fungal	2.2	2.8	2.8	2.8	3.8	3.8
564	Control	1.0	1.0	2.8	2.8	2.8	3.6
565	Bacterial	1.4	1.4	1.8	2.0	2.8	3.2

PART IV: DISCUSSION

Field Observations

61. The fact that atypical plants were found at only three sites during the 2-year survey was somewhat surprising. The survey encompassed a representative cross section of aquatic habitats with a variety of Eurasian watermilfoil populations, diversity of climate, water use regimes, and water qualities. These factors exposed resident Eurasian watermilfoil populations to varying stresses that could predispose the plants to phytopathogen activity if appropriate microorganisms had been present. The wide geographical range covered would have likely allowed contact between milfoil and commonly occurring pathogens. Additionally, many aquatic plant management professionals were aware of this project and would have brought any disease outbreaks or unusual population declines to the attention of the investigator. These factors suggest that during the time frame of this survey, there was no significant disease activity in regard to Eurasian watermilfoil in the survey areas.

Isolate Pathology

62. Assay results indicated that the lytic enzyme-producing fungal isolates produced measurable Eurasian watermilfoil decline significantly faster than did the no-treatment control, especially in the short term. One week after the inoculation of Eurasian watermilfoil sprigs, 19 of 22 fungal isolates produced a significantly greater ($p = 0.05$) MDI than did the no-treatment control. After 6 weeks, only five isolates maintained a significantly greater MDI than did the no-treatment control. These five isolates should be assayed against intact, rooted Eurasian watermilfoil in aquaria to further examine their suitability as biocontrol agents.

63. The question arises as to why there was such a difference between the MDI of the no-treatment control and that of the V-8 broth control. The sterile V-8 broth possibly promoted a population explosion of the microorganisms resident on the test plants, leading to plant decline or early senescence. This decline did not occur in the no-treatment control because growth-promoting media constituents (i.e., V-8 juice) were not added. Data

from weeks 5 and 6 demonstrated that in excess of 80 percent of the assay plants (control and test) that received inoculum containing V-8 juice experienced similar declines as measured by MDI values. Less than 10 percent of the fungal replicates had MDI values significantly greater or smaller than that of the V-8 control at weeks 5 and 6. This indicates that most damage not related to the V-8 juice occurred in the first weeks of the assay before the V-8 juice had time to stimulate the resident microflora, perhaps indicating a growth lag phase response. The V-8, although dilute, did increase the nutritional value of the solution. Pennington (1985) noted this phenomenon and suggested nutritional enrichment of the resident microflora's habitat to control Eurasian watermilfoil.

64. The results indicate that the assayed bacterial isolates are not strong candidates for future testing as biological control agents. However, they may be candidates for testing in combination studies with other microorganisms that provide entry points into the target plant. Bacterial pathogens often require prior damage to infect plants.

65. The bacterial isolates likely represent endemic microflora, epiphytes, or weak pathogens that survived the surface sterilization procedure. This observation is supported by the fact that the NB control plants, populated only with the resident microflora, had the highest MDI at week 5 (although not significantly higher than others).

66. These tests suggest several mechanisms that may have contributed to the observed decline of Eurasian watermilfoil assay plants. The fungal or bacterial inoculant may have caused the damage by infecting the previously healthy sprig. The V-8 broth or nutrient broth may have stimulated growth of the resident microflora causing general plant decline irrespective of the inoculant. A third possibility is that enzymes or toxins, produced by the fungal or bacterial isolate prior to plant inoculation and pipetted into the test tube as part of the inoculant, promoted breakdown of the plant tissue. All of these explanations are possible, and further study is needed for a definitive answer.

PART V: CONCLUSIONS AND RECOMMENDATIONS

67. Specific conclusions of this study are as follows:

a. A cross section of populations of Eurasian watermilfoil in the continental United States was surveyed for phytopathogen activity. No widespread disease outbreaks were detected during the 2-year survey (1984-1985). Numerous plants that were sampled showed limited pathogen activity.

b. Laboratory isolation procedures yielded 792 pure culture isolates from the plants; 330 were fungi, and 462 were bacteria.

c. Lytic enzyme assays indicated that 36 of the isolates were candidates for assay against healthy Eurasian watermilfoil plants.

d. Several fungal isolates were determined by test tube assay using healthy Eurasian watermilfoil to be candidates for future, larger scale assay. No bacterial isolates are candidates for additional study as pathogens of Eurasian watermilfoil.

68. It is recommended that five candidate microorganisms (Table 7) be tested in aquarium studies to determine their efficacy as biological control agents of Eurasian watermilfoil.

Table 7

Isolates Recommended for Additional Study

No.	Collection Site	Reason for Selection
212	Kitty Hawk Bay	Highest MDI, weeks 1-5
217	Kitty Hawk Bay	MDI significantly > NTC* at week 6
429	Mobile Bay	Highest MDI at conclusion of assay (week 6)
464	Deer Point lake	Second highest MDI, weeks 1-5; tied for 2d, week 6
559	Kitty Hawk Bay	MDI significantly > NTC, weeks 5 and 6

* NTC = no-treatment control.

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APPENDIX A: DATES AND LOCATIONS OF COLLECTIONS OF EURASIAN WATERMILFOIL

Date	Site	Location	County/ Parish
		Alabama	
3 May 1984	Mobile Bay	Boat Ramp, Causeway	Baldwin
3 May 1984	Mobile Bay	Bay Minet Bay	Baldwin
3 May 1984	Mobile Bay	D'Olives Bay	Baldwin
3 May 1984	Mobile Bay	Big Batteau Bay	Baldwin
24 May 1984	Guntersville Res.	Crow Creek	Jackson
24 May 1984	Guntersville Res.	Grant 2,4,5	Jackson
8 Aug 1984	Mobile Bay	Boat Ramp, Causeway	Baldwin
8 Aug 1984	Mobile Bay	Big Batteau Bay	Baldwin
8 Aug 1984	Mobile Bay	D'Olives Bay	Baldwin
8 Aug 1984	Mobile Bay	Chocolatta Bay	Baldwin
8 Aug 1984	Mobile Bay	Bay Minet Bay	Baldwin
18 Jun 1985	Mobile Bay	Boat Ramp, Causeway	Baldwin
18 Jun 1985	Mobile Bay	Bay Minet Bay	Baldwin
18 Jun 1985	Mobile Bay	D'Olives Bay	Baldwin
18 Jun 1985	Mobile Bay	Big Batteau Bay	Baldwin
18 Jun 1985	Mobile Bay	Justin Bay	Baldwin
		California	
18 Jun 1984	All American Canal	Due S. Mount Signal	Imperial
18 Jun 1984	All American Canal	W. of Calexico Golf Course	Imperial
19 Jun 1984	East Highline Canal	At State Highway 78	Imperial
19 Jun 1984	East Highline Canal	At Orange Lateral Canal	Imperial
19 Jun 1984	East Highline Canal	1 mi. N. St. Hwy. 78	Imperial
19 Jun 1984	East Highline Canal	1 mi. S. St. Hwy. 78	Imperial
21 Jun 1984	Lower Crystal Spgs	Northwest corner	San Mateo
21 Jun 1984	Lower Crystal Spgs	North end	San Mateo
21 Jun 1984	Lower Crystal Spgs	East shore 0.5 mi N. dam	San Mateo
21 Jun 1984	Pilarcitos Lake	Dam area	San Mateo
21 Jun 1984	Pilarcitos Lake	Western shoreline	San Mateo
21 Jun 1984	San Andreas Lake	Dam area	San Mateo
21 Jun 1984	San Andreas Lake	North end	San Mateo
19 Sep 1984	Lower Crystal Spgs	Northwest corner	San Mateo
19 Sep 1984	Lower Crystal Spgs	North end	San Mateo
19 Sep 1984	Lower Crystal Spgs	East shore 0.5 mi N. dam	San Mateo
19 Sep 1984	Pilarcitos Lake	Dam area	San Mateo
19 Sep 1984	Pilarcitos Lake	Western shoreline	San Mateo
19 Sep 1984	San Andreas Lake	Dam area	San Mateo
19 Sep 1984	San Andreas Lake	North end	San Mateo

Date	Site	Location	County/ Parish
		California (Concluded)	
21 Sep 1984	East Highline Canal	At State Highway 78	Imperial
21 Sep 1984	East Highline Canal	At Orange Lateral Canal	Imperial
21 Sep 1984	East Highline Canal	1 mi. N. State Hwy. 78	Imperial
21 Sep 1984	East Highline Canal	1 mi. S. State Hwy. 78	Imperial
25 Jul 1985	Lower Crystal Spgs	North end	San Mateo
25 Jul 1985	Lower Crystal Spgs	Northwest corner	San Mateo
25 Jul 1985	Lower Crystal Spgs	East shore 0.5 mi N. dam	San Mateo
25 Jul 1985	Pilarcitos Lake	Dam area	San Mateo
25 Jul 1985	Pilarcitos Lake	West shoreline	San Mateo
25 Jul 1985	San Andreas Lake	Dam area	San Mateo
25 Jul 1985	San Andreas Lake	North end	San Mateo
27 Jul 1985	East Highline Canal	At State Highway 78	Imperial
27 Jul 1985	East Highline Canal	At Orchard Canal	Imperial
27 Jul 1985	East Highline Canal	At Orange Lateral Canal	Imperial
27 Jul 1985	East Highline Canal	1 mi. N. State Hwy 78	Imperial
27 Jul 1985	East Highline Canal	1 mi. S. State Hwy 78	Imperial
		Florida	
9 May 1984	Deer Point Lake	Bear Creek	Bay
9 May 1984	Deer Point Lake	North Bay	Bay
9 May 1984	Deer Point Lake	Bay George	Bay
9 May 1984	Deer Point Lake	Powerline	Bay
9 May 1984	Apalachicola Bay	Turtle Bay	Franklin
9 May 1984	Apalachicola Bay	Harbor Bay	Franklin
9 May 1984	Apalachicola Bay	Scipio Creek	Franklin
10 May 1984	Lake Seminole	State Highway 271 Bridge	Jackson
14 Aug 1984	Lake Seminole	Three Rivers St. Pk.	Jackson
14 Aug 1984	Lake Seminole	Bay N. 271 Bridge	Jackson
14 Aug 1984	Waukulla River	Mouth of Boggy Creek	Waukulla
14 Aug 1984	Waukulla River	1 mi. S. 98 bridge	Waukulla
14 Aug 1984	Waukulla River	Shell Isl. Marina area	Waukulla
15 Aug 1984	Deer Point Lake	Bear Creek	Bay
15 Aug 1984	Deer Point Lake	North Bay	Bay
15 Aug 1984	Deer Point Lake	Ecofina Creek	Bay
15 Aug 1984	Deer Point Lake	Cedar Creek	Bay
15 Aug 1984	Apalachicola Bay	Turtle Bay	Franklin
15 Aug 1984	Apalachicola Bay	Scipio Creek	Franklin
15 Aug 1984	Apalachicola Bay	Harbor Bay	Franklin
15 Aug 1984	Apalachicola Bay	Apalachicola River	Franklin
11 Jun 1985	Apalachicola Bay	Turtle Bay	Franklin
11 Jun 1985	Apalachicola Bay	Scipio Creek	Franklin

Date	Site	Location	County/ Parish
		Florida (Concluded)	
11 Jun 1985	Deer Point Lake	Bear Creek	Bay
11 Jun 1985	Deer Point Lake	North Bay	Bay
11 Jun 1985	Deer Point Lake	Cedar Creek	Bay
12 Jun 1985	Lake Seminole	Three Rivers St. Pk.	Jackson
12 Jun 1985	Lake Seminole	Bay N. 271 Bridge	Jackson
12 Jun 1985	Waukulla River	Shell Isl. Marina Area	Waukulla
12 Jun 1985	Waukulla River	1 mi. S. 98 Bridge	Waukulla
		Louisiana	
15 May 1984	Toledo Bend Res.	Louisiana Island	Sabine
15 May 1984	Toledo Bend Res.	Pirates Cove	Sabine
15 May 1984	Toledo Bend Res	J and L Cove	Sabine
15 May 1984	Toledo Bend Res.	Quiet Cove	Sabine
15 May 1984	Toledo Bend Res.	Lab Cove	Sabine
		New York	
6 Jun 1984	Cornell Ponds	Research ponds 1,2,34,35	Tompkins
6 Jun 1984	Cayuga Lake	Northeast corner	Cayuga
6 Jun 1984	Cayuga Lake	State Park	Seneca
6 Jun 1984	Lake Ontario	E. Sodus Bay	Wayne
6 Jun 1984	Lake Ontario	S. Sodus Bay	Wayne
6 Jun 1984	Lake Ontario	Otabs Marina	Wayne
15 Aug 1985	Cornell Ponds	Research ponds 1,2,34,35	Tompkins
15 Aug 1985	Cayuga Lake	State Park	Seneca
15 Aug 1985	Cayuga Lake	Marina	Seneca
15 Aug 1985	Lake Ontario	S. Sodus Bay	Wayne
		North Carolina	
1 Jun 1984	Kitty Hawk Bay	State Wildlife boat ramp	Dare
1 Jun 1984	Currituck Sound	NW. Coinjock Bay	Currituck
27 Aug 1984	Kitty Hawk Bay	State Wildlife boat ramp	Dare
27 Aug 1984	Currituck Sound	Canal Parallel CJB	Currituck
27 Aug 1984	Currituck Sound	Bell Island	Currituck
28 Aug 1984	Pamlico River	Alligator Cut	Beaufort
28 Aug 1984	Pamlico River	South Creek	Beaufort
8 Aug 1985	Kitty Hawk Bay	Boat ramp	Dare
8 Aug 1985	Currituck Sound	NW. Coinjock Bay	Currituck

Date	Site	Location	County/ Parish
		Texas	
15 Jul 1984	Pat Mayse Lake	Sanders Cove beach	Lamar
15 Jul 1984	Pat Mayse Lake	Sanders Cove dock	Lamar
15 Jul 1984	Pat Mayse Lake	South bank	Lamar
15 Jul 1984	Pat Mayse Lake	East bank	Lamar
10 Sep 1985	Pat Mayse Lake	South bank	Lamar
10 Sep 1985	Pat Mayse Lake	East bank	Lamar
10 Sep 1985	Pat Mayse Lake	South bank	Lamar
10 Sep 1985	Pat Mayse Lake	Sanders Cove beach	Lamar
10 Sep 1985	Pat Mayse Lake	Sanders Cove dock	Lamar
		Vermont	
3 Jun 1984	Lake Champlain	St. Albans Bay, St. pier	Franklin
3 Jun 1984	Lake Champlain	St. Albans Bay, St. beach	Franklin
3 Jun 1984	Lake Champlain	St. Albans Bay, west shore	Franklin
3 Jun 1984	Lake Carmi	Southwestern Corner	Franklin
4 Jun 1984	Lake St. Catherine	State Park area	Rutland
4 Jun 1984	Lake St. Catherine	Boat ramp	Rutland
4 Jun 1984	Lake St. Catherine	Lake St. Catherine Inn	Rutland
4 Jun 1984	Lake St. Catherine	Bay E. of Cone's Point	Rutland
4 Jun 1984	Lake Bomoseen	State Route 4 Bridge	Rutland
4 Jun 1984	Lake Bomoseen	State Park area	Rutland
4 Jun 1984	Lake Bomoseen	Floating bridge	Rutland
4 Jun 1984	Lake Bomoseen	N. of Captain Johns	Rutland
4 Jun 1984	Lake Bomoseen	Cove behind Rabbit Island	Rutland
4 Jun 1984	Glen Lake	Outlet 1	Rutland
4 Jun 1984	Glen Lake	Outlet 2	Rutland
4 Jun 1984	Lake Hortonia	Outlet access	Rutland
30 Aug 1984	Lake St. Catherine	State Park area	Rutland
30 Aug 1984	Lake St. Catherine	Boat ramp	Rutland
30 Aug 1984	Lake St. Catherine	Lake St. Catherine Inn	Rutland
30 Aug 1984	Lake St. Catherine	West shore	Rutland
30 Aug 1984	Lake Bomoseen	State Route 4 bridge	Rutland
30 Aug 1984	Lake Bomoseen	State Park area	Rutland
30 Aug 1984	Lake Bomoseen	Floating bridge	Rutland
30 Aug 1984	Lake Bomoseen	West shore	Rutland
30 Aug 1984	Lake Hortonia	Outlet access	Rutland
30 Aug 1984	Glen Lake	Outlet 1	Rutland
30 Aug 1984	Glen Lake	Outlet 2	Rutland
31 Aug 1984	Lake Champlain	St. Albans Bay	Franklin
31 Aug 1984	Lake Carmi	Southwest corner	Franklin
31 Aug 1984	Metcalf Pond	Cottage area	Franklin

Date	Site	Location	County/ Parish
		Vermont (Concluded)	
12 Aug 1985	Lake Champlain	St. Albans Bay	Franklin
12 Aug 1985	Lake Carmi	Southwest corner	Franklin
12 Aug 1985	Metcalf Pond	Cottage area	Franklin
13 Aug 1985	Lake St. Catherine	State Park area	Rutland
13 Aug 1985	Lake St. Catherine	Boat ramp	Rutland
13 Aug 1985	Lake St. Catherine	Lake St. Catherine Inn	Rutland
13 Aug 1985	Lake St. Catherine	West shore	Rutland
13 Aug 1985	Lake Bomoseen	State Route 4 bridge	Rutland
13 Aug 1985	Lake Bomoseen	State Park area	Rutland
13 Aug 1985	Lake Bomoseen	Floating bridge	Rutland
13 Aug 1985	Lake Hortonia	Outlet access	Rutland
13 Aug 1985	Glen Lake	Outlet 1	Rutland
13 Aug 1985	Glen Lake	Outlet 2	Rutland
		Washington	
25 Jun 1984	Banks Lake	Steamboat Rock area	Grant
25 Jun 1984	Banks Lake	Eagles Cove	Grant
26 Jun 1984	Osoyoos Lake	Grubbs Cove	Okanogan
26 Jun 1984	Osoyoos Lake	Smith Point	Okanogan
26 Jun 1984	Osoyoos Lake	USA Border-east side	Okanogan
26 Jun 1984	Osoyoos Lake	Old Mobile Station	Okanogan
26 Jun 1984	Okanogan River	0.25 mi. S. Osoyoos L.	Okanogan
27 Jun 1984	Lake Washington	Union Bay	King
27 Jun 1984	Lake Washington	Fairweather Cove	King
27 Jun 1984	Lake Washington	Cozy Cove	King
27 Jun 1984	Lake Washington	Yarrow Bay	King
27 Jun 1984	Lake Washington	Jaunita Bay	King
13 Sep 1984	Rocky Reach Res.	At Entiat confluence	Chelan
13 Sep 1984	Rocky Reach Res.	Entiat City Park	Chelan
13 Sep 1984	Rocky Reach Res.	Earthquake Point	Chelan
13 Sep 1984	Rocky Reach Res.	Daroga Park	Chelan
13 Sep 1984	Rocky Reach Res.	Anvil Cove	Douglas
13 Sep 1984	Rock Island Res.	Old boat basin	Chelan
13 Sep 1984	Rock Island Res.	Mouth of Wenatchee River	Chelan
13 Sep 1984	Rock Island Res.	Hannah Mining Co.	Douglas
13 Sep 1984	Rock Island Res.	Wenatchee Railroad	Chelan
14 Sep 1984	Wells Reservoir	Pateros boat ramp	Okanogan
14 Sep 1984	Wells Reservoir	At Okanogan confluence	Okanogan
14 Sep 1984	Wells Reservoir	Brewster boat launch	Okanogan
14 Sep 1984	Wells Reservoir	Casmir	Okanogan
14 Sep 1984	Wells Reservoir	Kirk Island	Okanogan

Date	Site	Location	County/ Parish
		Washington (Concluded)	
17 Sep 1984	Lake Washington	Union Bay	King
17 Sep 1984	Lake Washington	Fairweather Cove	King
17 Sep 1984	Lake Washington	Cozy Cove	King
17 Sep 1984	Lake Washington	Yarrow Bay	King
17 Sep 1984	Lake Washington	Jaunita Bay	King
		Wisconsin	
18 Jul 1984	Pine Lake	North end	Waukesha
18 Jul 1984	Lake Fowler	City park	Waukesha
18 Jul 1984	Lac La Belle	North end	Waukesha
18 Jul 1984	Lake Pewaukee	City beach, N., E. side	Waukesha
18 Jul 1984	Lower Phantom Lake	Beach area	Waukesha
18 Jul 1984	Oconomowoc Lake	North end	Waukesha
18 Jul 1984	Whitewater Lake	State Recreation area	Walsworth
19 Jul 1984	Lake Mendota	University Bay	Dane
19 Jul 1984	Lake Wingra	Vilas Park area	Dane
19 Jul 1984	Lake Waubesa	Goodland Park	Dane
19 Jul 1984	Lake Kegonsa	West side	Dane
19 Jul 1984	Yahara River	Lottes Lane	Dane
12 Sep 1985	Lake Mendota	University Bay	Dane
12 Sep 1985	Lake Wingra	Vilas Park area	Dane
12 Sep 1985	Lake Waubesa	Goodland Park	Dane
12 Sep 1985	Lake Kegonsa	West side	Dane
13 Sep 1985	Pine Lake	North end	Waukesa
13 Sep 1985	Lake Fowler	Park	Waukesa
13 Sep 1985	Lac La Belle	North end	Waukesa
13 Sep 1985	Lake Pewaukee	City beach, N., E. side	Waukesa
13 Sep 1985	Lower Phantom Lake	Beach area	Waukesa
13 Sep 1985	Oconomowoc Lake	North end	Waukesa
13 Sep 1985	Whitewater Lake	State Recreation area	Walsworth
13 Sep 1985	Yahara River	Lottes Lane	Dane

APPENDIX B: RANKED WEEKLY MEAN DAMAGE INDEX VALUES FOR FUNGAL ISOLATES (n = 5)

Week 1	
Isolate	MDI
351	1.0
378	1.0
535	1.4
169	1.8
332	2.0
329	2.0
384	2.0
162	2.0
189	2.0
520	2.2
561	2.2
508	2.2
424	2.2
429	2.2
440	2.4
327	2.4
217	2.4
559	2.4
328	3.0
192	3.0
464	3.2
212	3.6

Week 2	
Isolate	MDI
351	1.0
169	2.0
329	2.0
535	2.2
162	2.2
384	2.4
520	2.4
429	2.4
217	2.4
559	2.4
378	2.6
189	2.6
327	2.6
561	2.8
332	3.0
508	3.0
424	3.0
440	3.0
328	3.0
192	3.0
464	3.8
212	4.0

Week 3	
Isolate	MDI
162	2.2
351	2.4
169	2.4
384	2.4
520	2.4
217	2.4
429	2.6
559	2.6
189	2.6
329	2.8
378	2.8
561	2.8
332	3.0
508	3.0
424	3.0
440	3.0
328	3.0
192	3.0
535	3.2
327	3.2
464	3.8
212	4.2

Week 4	
Isolate	MDI
162	2.2
351	2.4
169	2.4
384	2.4
520	2.6
189	2.6
217	2.8
559	2.8
378	2.8
561	2.8
429	3.0
329	3.0
332	3.0
424	3.0
328	3.0
508	3.2
440	3.2
192	3.2
327	3.2
535	3.4
464	3.8
212	4.2

Week 5	
Isolate	MDI
351	3.0
329	3.0
384	3.2
520	3.2
332	3.2
328	3.2
508	3.2
162	3.4
169	3.4
217	3.4
378	3.4
440	3.4
192	3.4
189	3.6
559	3.6
535	3.6
561	3.8
429	3.8
424	3.8
327	3.8
464	4.0
212	4.4

Week 6	
Isolate	MDI
351	3.2
328	3.2
332	3.4
329	3.8
520	3.8
440	3.8
561	3.8
327	3.8
384	4.0
508	4.0
162	4.0
169	4.0
189	4.0
535	4.0
424	4.0
378	4.2
192	4.2
217	4.4
559	4.4
464	4.4
212	4.4
429	4.6

APPENDIX C: RANKED WEEKLY MEAN DAMAGE INDEX VALUES FOR BACTERIAL ISOLATES (n = 5)

Week 1	
Isolate	MDI
551	1.0
170	1.2
418	1.2
511	1.2
509	1.4
565	1.4
115	2.0
172	2.0
201	2.0
218	2.0
114	2.2
116	2.2
156	2.2
308	2.4

Week 2	
Isolate	MDI
551	1.0
170	1.2
418	1.2
511	1.2
509	1.4
565	1.4
115	2.0
172	2.0
201	2.0
218	2.0
116	2.2
114	2.4
156	2.4
308	2.4

Week 3	
Isolate	MDI
551	1.0
170	1.2
418	1.2
511	1.2
509	1.4
565	1.8
115	2.0
172	2.0
201	2.0
218	2.0
116	2.2
114	2.4
308	2.4
156	2.8

Week 4	
Isolate	MDI
511	1.2
551	2.0
418	2.0
509	2.0
565	2.0
115	2.0
172	2.0
201	2.0
218	2.2
116	2.2
170	2.4
114	2.4
308	2.4
156	2.8

Week 5	
Isolate	MDI
511	2.0
551	2.0
418	2.6
509	2.6
115	2.6
218	2.6
308	2.6
565	2.8
172	3.0
116	3.0
170	3.0
114	3.0
308	3.2
156	3.2

Week 6	
Isolate	MDI
511	2.6
418	2.8
308	2.8
551	3.0
218	3.0
509	3.2
115	3.2
565	3.2
116	3.4
170	3.4
201	3.4
172	3.6
114	3.8
156	3.8

APPENDIX D: ANALYSIS OF VARIANCE OF LYTIC ENZYME POSITIVE FUNGAL ISOLATES

Week 1			Week 2			Week 3			Week 4			Week 5			Week 6		
Isolate	Mean	Grouping*	Isolate	Mean	Grouping*	Isolate	Mean	Grouping*	Isolate	Mean	Grouping*	Isolate	Mean	Grouping*	Isolate	Mean	Grouping*
212	3.6	A	212	4.0	A	212	4.2	A	212	4.2	A	212	4.4	A	429	4.6	A
464	3.2	B A	464	3.8	A	464	3.8	A	464	3.8	B A	464	4.0	B A	212	4.4	B A
328	3.0	B	328	3.0	B	327	3.2	B	535	3.4	B C	429	3.8	B A C	464	4.4	B A
192	3.0	B	192	3.0	B	535	3.2	B	192	3.2	D C	327	3.8	B A C	559	4.4	B A
440	2.4	C	440	3.0	B	328	3.0	C B	327	3.2	D C	561	3.8	B A C	217	4.4	B A
217	2.4	C	508	3.0	B	440	3.0	C B	440	3.2	D C	424	3.8	B A C	192	4.2	B A C
327	2.4	C	424	3.0	B	192	3.0	C B	508	3.2	D C	535	3.6	B D C	378	4.2	B A C
559	2.4	C	427	3.0	B	427	3.0	C B	429	3.0	D C E	189	3.6	B D C	169	4.0	B D A C
561	2.2	D C	332	3.0	B	508	3.0	C B	328	3.0	D C E	559	3.6	B D C	427	4.0	B D A C
520	2.2	D C	561	2.8	C B	332	3.0	C B	332	3.0	D C E	440	3.4	B E D C	384	4.0	B D A C
424	2.2	D C	189	2.6	C B D	424	3.0	C B	424	3.0	D C E	169	3.4	B E D C	189	4.0	B D A C
508	2.2	D C	378	2.6	C B D	329	2.8	C B D	329	3.0	D C E	162	3.4	B E D C	162	4.0	B D A C
429	2.2	D C	327	2.6	C B D	561	2.8	C B D	427	3.0	D C E	192	3.4	B E D C	508	4.0	B D A C
332	2.0	D C	217	2.4	C E D	564	2.8	C B D	561	2.8	D F E	378	3.4	B E D C	424	4.0	B D A C
189	2.0	D C	429	2.4	C E D	378	2.8	C B D	564	2.8	D F E	217	3.4	B E D C	535	4.0	B D A C
329	2.0	D C	384	2.4	C E D	429	2.6	C E D	378	2.8	D F E	384	3.2	E D C	440	3.8	B D E C
384	2.0	D C	520	2.4	C E D	189	2.6	C E D	217	2.8	D F E	328	3.2	E D C	520	3.8	B D E C
162	2.0	D C	559	2.4	C E D	559	2.6	C E D	559	2.8	D F E	332	3.2	E D C	561	3.8	B D E C
169	1.8	D E	535	2.2	E D	351	2.4	E D	189	2.6	G F E	508	3.2	E D C	329	3.8	B D E C
535	1.4	F E	162	2.2	E D	520	2.4	E D	520	2.6	G F E	520	3.2	E D C	327	3.8	B D E C
427	1.2	F	169	2.0	E	217	2.4	E D	384	2.4	G F	427	3.2	E D C	564	3.6	D E C
351	1.0	F	329	2.0	E	384	2.4	E D	351	2.4	G F	351	3.0	E D	332	3.4	D E
378	1.0	F	351	1.0	F	169	2.4	E D	169	2.4	G F	329	3.0	E D	328	3.2	E
564	1.0	F	564	1.0	F	162	2.2	E	162	2.2	G	564	2.8	E	351	3.2	E

Notes: n = 5. Includes no-treatment control (564) and V-8 control (427).
* Means with the same letter are not significantly different (Duncan's multiple range test).