US Army Corps of Engineers

AQUATIC PLANT CONTROL
RESEARCH PROGRAM

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PROCEEDINGS, 19TH ANNUAL MEETING, AQUATIC PLANT CONTROL RESEARCH PROGRAM

26-29 NOVEMBER 1984
GALVESTON, TEXAS

June 1985
Final Report

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The 19th Annual Meeting of the US Army Corps of Engineers Aquatic Plant Control Research Program was held in Galveston, Texas, on 26-29 November 1984, to review current research activities and to afford an opportunity for presentation of operational needs. Papers presented at the meeting are included in this report. These papers include reports of research being conducted in the areas of biological, chemical, and mechanical control methods, and the ecology of problem plants. In addition, papers are included that present the operational problems in various Corps of Engineer Districts. A specific section on aquatic plant problems in the Potomac River is also included.

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PREFACE

The 19th Annual Meeting of the US Army Corps of Engineers Aquatic Plant Control Research Program was held in Galveston, Texas, on 26-29 November 1984. The meeting is required by Engineer Regulation (ER) 1130-2-412 paragraph 4c and was organized by personnel of the Aquatic Plant Control Research Program (APCRP), Environmental Laboratory (EL), US Army Engineer Waterways Experiment Station (WES), Vicksburg, Miss.

The organizational activities were carried out and presentations by WES personnel were prepared under the general supervision of Dr. John Harrison, Chief, EL. Mr. J. Lewis Decell was Program Manager, APCRP. Mr. W. N. Rushing, APCRP, was responsible for planning and chairing the meeting. Mr. E. Carl Brown was Technical Monitor for the Office, Chief of Engineers, US Army.

This report was edited by Ms. Jamie W. Leach of the WES Publications and Graphic Arts Division (P&GAD). All copy layout was accomplished by Ms. Gracie B. Park, P&GAD.

COL Robert C. Lee, CE, was Commander and Director of the WES at the time of the meeting. COL Allen F. Grum, USA, was Director of WES during the publication of this report. Mr. Fred R. Brown and Dr. Robert W. Whalin were Technical Directors.

CONTENTS

Page

AGENDA

19th Annual Meeting
U.S. Army Corps of Engineers
AQUATIC PLANT CONTROL RESEARCH PROGRAM

Galveston, Texas
26-29 November 1984

MONDAY, 26 NOVEMBER 1984

10:00 a.m. -5:30 p.m.	Registration—West Promenade
6:30 p.m.	Reception—Music Hall Terrace

TUESDAY, 27 NOVEMBER 1984
General Session, Music Hall

8:00 a.m.	Registration Continues
8:30 a.m.	Call to Order and Announcements —W. N. Rushing, Waterways Experiment Station (WES), Vicksburg, Mississippi
8:40 a.m.	Welcome to the Galveston District —COL Alan L. Laubscher, Commander, USAE District, Galveston, Texas
8:50 a.m.	General Comments —F. R. Brown, Technical Director, WES, Vicksburg, Mississippi
9:00 a.m.	Comments by the Technical Monitor —E. Carl Brown, Natural Resources Management Branch, Office, Chief of Engineers (OCE), Washington, DC
9:10 a.m.	Comments by the Program Manager —J. L. Decell, Aquatic Plant Control Research Program (APCRP), WES, Vicksburg, Mississippi
9:25 a.m.	BREAK
9:40 a.m.	USAE Division/District Presentations Aquatic Plant Problems—Operations Activities Lower Mississippi Valley Division, New Orleans District —Frank J. Cali North Atlantic Division, New York District, Lake Champlain, Vermont —David J. Papp North Atlantic Division, New York District —Simeon M. Hook North Central Division, St. Paul District —Wayne Koerner North Pacific Division, Seattle District —Robert M. Rawson South Atlantic Division, Charleston District —John L. Carothers South Atlantic, Jacksonville District —Edward Knight

South Atlantic Division, Jacksonville District, Aquatic Plant Control Operations Support Center
—James T. McGehee

South Atlantic Division, Wilmington District
—Charles R. Wilson

South Atlantic Division, Mobile District
—Michael J. Eubanks

South Atlantic Division, Lake Seminole
—Joe Kight*

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—Joyce Johnson

Southwestern Division, Tulsa District
—Loren M. Mason

Pat Mayse Lake, Texas
—John H. Rodgers, Jr., North Texas State University, Denton, Texas

Southwestern Division, Little Rock District
—Clyde Gates*

12:00 noon	LUNCH
1:30 p.m.	Ecology of Aquatic Plant Species—Overview: Effects of Sediment Composition—Final Report on Work Unit Environmental Interactions—Initiation of Work Unit —J. W. Barko, WES
1:50 p.m.	Effects of Water Chemistry on Aquatic Plant Species —R. M. Smart, WES
2:05 p.m.	The Development of Computer-Aided Evaluation Systems —B. M. Sabol, WES
2:25 p.m.	BREAK
2:40 p.m.	Biological Control Technology Development—Overview —E. A. Theriot, WES
2:50 p.m.	The Control of Hydrilla with Plant Pathogens —E. A. Theriot, WES
3:05 p.m.	Overseas Searches and Evaluation of Insects for Control of Hydrilla —J. K. Balciunas, University of Florida, Aquatic Plant Management Laboratory, Ft. Lauderdale
3:20 p.m.	Update on Control of Eurasian Watermilfoil with Microorganisms —H. B. Gunner, University of Massachusetts, Amherst
3:35 p.m.	Domestic Survey of Pathogenic Microorganisms on Eurasian Watermilfoil —W. C. Zattau, WES
3:50 p.m.	The Control of Waterhyacinth with Neochetina —R. M. Stewart, WES
4:05 p.m.	Biological Control of Waterhyacinth and Alligatorweed in Galveston District and at Jean Lafitte Park, Louisiana —A. F. Cofrancesco, WES
4:25 p.m.	Biological Control of Waterhyacinth in the California Delta —R. M. Stewart, WES

* Presentation not submitted for inclusion in proceedings.

WEDNESDAY, 28 NOVEMBER 1984
General Session — Music Hall

8:30 a.m.	Chemical Control Technology Development—An Overview and Description of New Work Units Controlled-Release Poly GMA 2,4-D Development and Evaluation Herbicide Application Technique Development for Flowing Water Herbicide Concentration Effects on Stressed Plants —H. W. Westerdahl, WES
8:50 a.m.	Evaluation of Herbicide/Adjuvant Mixtures in Flowing Water —K. D. Getsinger, WES
9:05 a.m.	Herbicide Threshold Concentrations and Exposure Time Requirements —J. F. Hall, WES
9:20 a.m.	Herbicide Evaluation Program —T. K. Van, USDA, APML, Ft. Lauderdale, Florida
9:35 a.m.	Development and Evaluation of Controlled-Release Fluridone Pellets —R. L. Dunn, Southern Research Institute, Birmingham, Alabama
9:50 a.m.	BREAK
10:05 a.m.	A New Aquatic Plant Infestation in Florida —J. C. Joyce, Director, Center for Aquatic Weeds, University of Florida, Gainesville
10:15 a.m.	Latest Developments of the Triploid White Amur —J. M. Malone, J. M. Malone and Son Enterprises, Lonoke, Arkansas
10:35 a.m.	Triploid White Amur Research in Washington State —G. Pauley and Gary Thomas, University of Washington, Seattle
11:05 a.m.	Induced Triploidy in the White Amur —J. Cassani, Lee County Hyacinth Control District, Fort Myers, Florida
11:20 a.m.	The Lake Conroe Texas White Amur Project —R. Betsill, Texas A&M University, College Station
11:35 a.m.	Discussion, Questions and Answers
12:00 noon	LUNCH
1:30 p.m.	The Potomac River Hydrilla Project—Introduction —J. L. Decell, WES
1:45 p.m.	Toward Development of an Operational Program for the Potomac —R. Pace, USAE District, Baltimore, Maryland*
2:00 p.m.	Survey of the Submersed Aquatic Vegetation on the Potomac in the Immediate Washington Area —N. Rybicki, U.S. Geological Survey, Reston, Virginia
2:15 p.m.	Survey of the Submersed Aquatic Vegetation on the Potomac from Quantico, Virginia, to the Highway 301 Bridge —R. A. Allaire, Northern Virginia Community College, Woodbridge, Virginia
2:30 p.m.	Preliminary Laboratory Research Results on Monoecious Hydrilla —T. K. Van, USDA, Ft. Lauderdale, Florida
2:45 p.m.	Preliminary Laboratory Research Results on Monoecious Hydrilla —L. W. J. Anderson, USDA, Davis, California
3:00 p.m.	Demonstration Trials on the Potomac—Fall 1984 —W. N. Rushing, WES

* Presentation not submitted for inclusion in proceedings.

3:15 p.m.	Results of Mechanical Harvesting Demonstration Trial, Fall 1984 —B. M. Sabol, WES
3:30 p.m.	Future Work on the Potomac River Hydrilla Project —J. L. Decell, WES
3:45 p.m.	Questions and Answers
4:00 p.m.	Closing Remarks —J. A. Pfeiffer, Jr., R&D Directorate, OCE, Washington, DC
4:15 p.m.	Adjourn 19th Annual Meeting

THURSDAY, 29 NOVEMBER 1984
West Parlour

8:00 a.m. -11:00 a.m.	FY 86 Civil Works R&D Program Review, Directorate of Research and Development, OCE (Corps of Engineers Representatives ONLY)

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19th Annual Meeting
U.S. Army Corps of Engineers
AQUATIC PLANT CONTROL RESEARCH PROGRAM

Galveston, Texas
26-29 November 1984

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CONVERSION FACTORS, NON-SI TO SI (METRIC) UNITS OF MEASUREMENT

Non-SI units of measurement used in this report can be converted to SI (metric) units as follows:

Multiply	By	To Obtain
acres	4046.873	square metres
acre-feet	1233.489	cubic metres
cubic yards	0.7645549	cubic metres
feet	0.3048	metres
gallons per acre	0.00093	cubic decimetres per square metre
gallons (U.S. liquid)	3.785412	cubic decimetres
horsepower (550 foot-pounds per second)	745.6999	watts
inches	25.4	millimetres
miles per hour	1.609347	kilometres per hour
miles (U.S. statute)	1.609347	kilometres
pounds (force) per square inch	6894.757	pascals
pounds (mass)	0.000112	kilograms
pounds (mass) per gallon	0.12	kilograms per cubic decimetre
square miles	2.589998	square kilometres
tons (mass) per acre	0.22	kilograms per square metre
tons (2000 lb mass)	907.1847	kilograms
yards	0.9144	metres

19th Annual Meeting
US Army Corps of Engineers

AQUATIC PLANT CONTROL RESEARCH PROGRAM

INTRODUCTION

As part of the Corps of Engineers (CE) Aquatic Plant Control Research Program (APCRP) it is required that a meeting be held each year to provide for professional presentation of current research projects and review current operations activities and problems. Subsequent to these presentations, the Civil Works Research and Development Program Review is held. This program review is attended by representatives of the Civil Works and Research Development Directorates of the Office of the Chief of Engineers; the Program Manager, APCRP; and representatives of the operations elements of various Division and District Engineer Offices.

The overall objective of this annual meeting is to thoroughly review Corps aquatic plant control needs and establish priorities for future research, such that identified needs are satisfied in a timely manner.

The technical findings of each research effort conducted under the APCRP are reported to the Manager, APCRP, US Army Engineer Waterways Experiment Station (WES), each year in the form of quarterly progress reports and a final technical report. Each technical report is given wide distribution as a means of transferring technology to the technical community. Technology transfer to the field operations elements is effected through the conduct of demonstration projects in various District Office problem areas and through publication of Instruction Reports (IR), Engineering Circulars (EC), and Engineering Manuals (EM). Periodically, results are presented through publication of an APCRP Information Exchange Bulletin which is distributed to both the field units and the general community. Public-oriented brochures, movies, and speaking engagements are used to keep the general public informed.

The printed proceedings of the annual meetings and program reviews are intended to provide Corps management with an annual summary to ensure that the research is being focused on the current operational needs on a nationwide scale.

The contents of this report include the presentations of the 19th Annual Meeting held in Galveston, Texas, 26-29 November 1984.

COMMENTS BY THE TECHNICAL MONITOR

by
E. Carl Brown*

INTRODUCTION

The Natural Resources Management Branch of the Office, Chief of Engineers, (OCE), US Army, has responsibility for the overall staff supervision of the Corps' Aquatic Plant Control Research Program (APCRP). As a member of that group, I was assigned the responsibility of Technical Monitor for the research efforts associated with the program just before our 1983 annual meeting in Raleigh, N.C., and have been favorably impressed with the dedication and professionalism of the people (public and private) I have met through the research and control program.

OBJECTIVES

Two simple objectives for this presentation are:

- Emphasize the importance of communication and cooperation between the various public agencies, industry, environmental organizations, and private citizens in accomplishing aquatic plant control in an efficient and environmentally acceptable manner.
- Present some views on the value and objectives of aquatic plant control research.

COMMUNICATION AND COOPERATION

Represented here today are (a) Corps employees, (b) representatives from other Federal agencies, (c) State employees, (d) industry representatives, (e) students, (f) academic institutions, (g) interested citizens, and (h) media representatives. Thank you all for showing an interest in the program. This mix of participants is germane to some thoughts I want to convey about cooperation and communication.

As most of you know, aquatic plants are somewhat of an enigma. In the broad picture, they present a "Doctor Jekyll and Mr. Hyde"situation. On one hand, aquatic plants can be highly beneficial in the nutrient cycle providing valuable food and cover for fish and wildlife. On the other hand, when overabundant, aquatic plants can interfere with drainage, irrigation, water supply, navigation, and recreation, as well as create aesthetic problems of unsightly and smelly masses of material.

People who have experienced or witnessed constraints in water use or management due to aquatic plants know the problem can be very real. Those who have not experienced such problems may wonder what all the concern is about. Further, because of the Doctor Jekyll and Mr. Hyde situation, aquatic plant

* US Army Corps of Engineers, Washington, D.C.

problems can be sensitive, emotional, and political issues. It is interesting to note, for example, the attention an infestation of hydrilla in the Potomac River received in this election year. You will hear more about that later in the meeting.

Some rather rigid lines can be drawn by various interest groups on how best to deal with aquatic plant problems. Our planners, control operations people, and researchers sometimes get caught in this tangled web, trying to determine the proper course of action.

To the point, to deal properly with aquatic plant problems, there must be thorough and timely communication and cooperation between many entities, including Federal and State agencies, environmental organizations, private citizens, and industry. We, in the Corps, are eager to understand the concerns of all parties interested in aquatic plants and to do what we can to properly deal with aquatic plant problems. We invite continued help, cooperation, and support in this effort.

RESEARCH

One of my objectives is to present some views on aquatic plant control research. Research can be necessary to the continued equilibrium of a program, and without it the program is not likely to function at its best. Further, I maintain that we should set, and always keep in sight, a lofty goal for our Aquatic Plant Control Research Program. Our goal should be to minimize the need for control operations — idealistic, but a good goal.

Our research program is clearly oriented to identification of new or more efficient tools for aquatic plant control. There is, of course, an additional and valuable benefit from the program. Because of the research activities, we have available to us a cadre of expertise that we can use whenever and wherever a special need arises. This cadre of experts is good backup for our Aquatic Plant Control Support Center in Jacksonville, Florida, and has been helpful with four special problems in the last year alone.

In our research efforts, we have a good mix of biological, environmental interaction, chemical, and mechanical system evaluation work units. Obviously, chemical and mechanical control methods are important to the overall approach to management of aquatic plants. Biological controls and better knowledge of ecosystem interactions hold promise for additional long-range, cost-effective, and evironmentally compatible solutions to aquatic plant problems.

SUMMARY

Problems caused by aquatic plants touch the lives of many Americans. While funding procedures sometimes constrain how fast we in the Corps can react to a problem, we do have strong interest in the program. To the extent that authorities and funding will allow, we will continue to do what we can to help with aquatic plant problems. But we must remember that there are many entities and many factors figuring into the decision process of how best to deal with specific problems in specific areas.

Looking back on what has happened in the program, it is gratifying to see the interest and effort that have been put forth. I want to take this opportunity to thank you, our Corps people (Division, District, Support Center, and Waterways Experiment Station), for your conscientious effort in the program. This year, the Baltimore District, Waterways Experiment Station, and our Support Center in Jacksonville, Florida, deserve a special thanks for their actions in response to hydrilla infestation in the Potomac.

I especially want to thank the other Federal and State agencies, private industry, and the private citizens that have cooperated with us in the program over the years, as it takes us all working together to get the job done in aquatic plant management and control.

We all face what appears to be a continuing challenge in dealing with aquatic plants in the next several years. There is indication that additional aquatic plant control may be needed in areas of the country where we have previously experienced little trouble. At the same time, most public agencies are faced with a problem of limited funding. It is imperative that we find ways to curtail the expanding cost of control operations. Our research program is critical in identifying more efficient methods of aquatic plant control and I am confident our research professionals will be equal to the challenge.

USAE DIVISION/DISTRICT PRESENTATIONS AQUATIC PLANT PROBLEMS—OPERATIONS ACTIVITIES

Lower Mississippi Valley Division, New Orleans District

by
Frank J. Cali*

The New Orleans District is responsible for aquatic growth control throughout the State of Louisiana. Operations are funded under two authorizations: the Removal of Aquatic Growth Project and the Aquatic Plant Control Program.

Operations are accomplished using 11 two-man crews at eight duty stations. During FY 84 crews continued to operate on a 10-hr day/4-day week from April through October, resulting in a 4-percent increase in productivity over FY 83. Control operations were aimed mainly at waterhyacinth; other species treated included alligatorweed, waterlettuce, hydrilla, Eurasian watermilfoil, and salvinia. The total area treated during FY 84 was 43,349 acres.** Of that total, 5,105 acres were treated by aerial application using contract helicopters. The herbicide 2,4-D was used for 95 percent of the treatment. Sonar, Diquat, Aquathol K, Cutrine Plus, and Rodeo herbicides were used for the remainder. Rodeo herbicide was used to successfully treat waterhyacinth in certain limited areas where phenoxy herbicide use was banned.

The New Orleans District has had a cooperative agreement with the State of Louisiana, Department of Wildlife and Fisheries, since 1959. Under this cost-reimbursable contract, the State treated 47,602 acres of aquatic growth in FY 84.

The Aquatic Growth Control Section, New Orleans District, attended the Louisiana Pesticide Applicators Association annual meeting in November 1984. All field employees are certified by the US Environmental Protection Agency (EPA).

Water samples were collected by Corps personnel and analyzed by private contractor for 2,4-D residues. Samples were collected at various intervals of time after spraying occurred. Results have indicated that residues decline to acceptable levels within a few days of application.

Hydrilla infestation continues to increase. It is estimated that hydrilla infests more than 30,000 acres in south Louisiana. Acceptable methods of treatment are available; however, funds are not available to treat the entire area. The New Orleans District and the Louisiana Department of Wildlife and Fisheries are cooperating to determine more accurately the extent and location of hydrilla infestations in the state.

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* US Army Engineer District, New Orleans; New Orleans, Louisiana.

** A table of factors for converting non-SI units of measurement to SI (metric) units is presented on page xiv.

USAE DIVISION/DISTRICT PRESENTATIONS AQUATIC PLANT PROBLEMS—OPERATIONS ACTIVITIES

North Atlantic Division, New York District Lake Champlain, Vermont

by
David J. Papp*

Lake Champlain is the sixth largest freshwater lake in the United States; as such it is an important regional recreational, economic, and natural resource. The lake serves as a state boundary between the States of Vermont and New York, and as an international boundary with 5 miles of the northern tip of the lake extending into the Province of Quebec, Canada. Since the establishment of the earliest canoe routes to the construction of the Lake Champlain Barge Canal, the lake has provided the major link between the Hudson and St. Lawrence Rivers.

Two species of aquatic macrophytes have reached nuisance proportions in Lake Champlain. One of these is waterchestnut (*Trapa natans*), an introduced species that has inundated several hundred acres in southern Lake Champlain. The plants infested through the barge system from the Hudson River and have steadily progressed northward to infest an ever greater area of southern Lake Champlain. In northern Lake Champlain, in particular St. Albans Bay, another introduced species, Eurasian watermilfoil (*Myriophyllum spicatum*), has become a severe nuisance.

A Reconnaissance Report, approved in November 1979, recommended that a General Design Memorandum (GDM) to include an Environmental Assessment be prepared. The State of Vermont entered into an agreement in August 1980 with the New York District under which the State developed a GDM. The GDM was approved in March 1982 with commencement of control operations in July 1982. Recommendations in the GDM included the following:

- Institution of a 10-year program to control obnoxious aquatic plant growth.
- The accomplishment of two goals: (1) progressively reduce waterchestnut and eventually confine them to south of 43°44′00″N latitude in the South lake and (2) control Eurasian watermilfoil in St. Albans Bay.
- Mechanical harvesting supplemented by handpulling with disposal at an upland site.
- A public participation program to encourage public awareness of the problem.

The benefit-cost ratio for the control program is 32.2 for the South Lake and 2.4 for St. Albans Bay.

* US Army Engineer District, New York; New York, New York.

SOUTH LAKE

The South Lake's 1984 waterchestnut harvesting project commenced on July 11th and was terminated on August 22nd. Considerable gains were made in the efficiency of the South Lake operation this year, through improvements to existing equipment and through the use of the harvesting computer program, written by Bruce Sabol of the Waterways Experiment Station. The use of the HARVEST computer program has allowed for creation of the optimum usage sequences for each piece of harvesting equipment in the various situations that are present on South Lake.

Continued improvements to the high speed transporter barge, which was specially developed and utilized for the South Lake harvesting project during the 1983 season, have again increased the efficiency of the weed disposal process. New pontoons were added in order to provide more flotation, thus allowing the barge to carry increased load weights. The transport barge's control tower was moved toward the rear of the machine to permit the bow of the vessel to ride higher while carrying the increased loads.

Although more of the control area was harvested this year than last, the lack of sufficient funds has again prevented the complete harvesting of all the waterchestnut infestations within the designated control area.

Table 1 provides a summary of activities in the South Lake with some additional facts comparing the first 3 years of harvesting.

Harvesting in South Lake was undertaken for approximately 6 weeks during 1984, a week less than the previous year, and resulted in the cutting and removal of 758 loads. This represents a 21-percent increase over the number of loads cut and removed in 1983. The percent increase is more significant than it first appears because the increased output occurred even though chestnut density was much reduced in the northern section of the control area.

The most significant reduction in weed densities continues to be in the Fort Ticonderoga area. There has been a steady decline in weed densities in this area since the inception of the project in 1982. Load numbers have decreased from 79.5 loads in 1982 to 35 in 1983 to 29 loads in 1984 which represents about a 17-percent reduction in loads. Roughly 85 percent of each load harvested in 1984 was Eurasian watermilfoil and only 15 percent was waterchestnut.

The handpulling effort this year concentrated on the northern section of the control area in an effort to keep the small coves and bays in that area from becoming infested with chestnut. Handpullers also concentrated on cleaning up areas that had been harvested or areas that were too shallow for harvester operation. The high water conditions this year allowed the harvesting equipment to cut much closer to shore, reducing the amount of weeds that the handpullers would normally have harvested.

Consideration is being given to the development of an onboard processing system for the South Lake harvesters. The purpose of the processor would be twofold: (a) reduce the bulk of the chestnut plant and (b) reduce the harvested plants into a form that might be usable as cattle feed or farm field mulch. It is envisioned that the

Table 1
1984 South Lake Champlain Harvesting Project

Equipment	*1984 Billing Period, Hours* 11 Jul-20 Jul	21 Jul-5 Aug	6 Aug-18 Aug	20 Aug-22 Aug	*Total* Hours	Cost
Two H650 Harvesters at $266/hr	64.4	91.8	82.7	21.8	260.7	$69,346.20
One H650 Harvester at $160/hr	2.7	6.0	5.7	1.1	15.5	2,480.00
One H220 Harvester at $60/hr	—	—	25	—	25	1,500.00
High speed transport at $106/hr	—	90.8	82.7	21.8	195.3	20,701.80
Handpullers at $32/hr	69	80	55	—	204	6,528.00
1 H650 Harvester (alone) at $106/hr	—	—	—	17.6	17.6	1,865.60
Biweekly billing period total ($)	19,770.40	37,563.60	34,936.40	10,151.20		$102,421.60
TOTAL LOADS	67	246	355	90		

	1982	*1983*	*1984*
Weeks harvested	7.5	7.0	6.0
Total loads	771.0	624.0	758.0
Acres	—	76.0	87.5
Total harvesting hours	—	408.7	579.5
Contract award	$118,532.50	$100,000.00	$102,421.60

processing device would cut and grind the plants to remove water and bulk and would also crush the nuts which have been a problem in using the plants as mulch on grazing lands. The plants to be used as a cattle feed would be harvested before the nut forms on the chestnuts. If a successful design for a processor can be implemented, projections are for an increase of nearly 30 percent in harvested material.

Several considerations that the State feels could further improve the project in future years are:

a. Sufficient funding should be appropriated on an annual basis so that the entire project area can be harvested in a single season. No year since the project inception has the entire project area been totally harvested. When areas are left uncut during a given year, increased densities in these areas can be anticipated in future years. Further, these uncut areas serve to reinfest areas of the Lake which have been cleared earlier.

b. A multiyear contract for the project would be beneficial for a number of reasons. Potential contractors for the project have stated that given a multiyear contract, they could reduce by 15 to 25 percent the costs per hour of equipment, thus allowing more production for less money. A multiyear contract would allow for a decreased amount of administrative work for both the State of Vermont and the Corps of Engineers. The involved bid process would not have to be undertaken each year. Additionally, the employment of one contractor from year to year gives the contractor a good familiarity with the project area, thus allowing him to make constant improvements in his techniques as well as ensuring a uniform procedure for harvesting the areas.

ST. ALBANS

For a number of reasons, the Eurasian watermilfoil in St. Albans Bay did not grow until very late in the summer. Accordingly, the harvesting scheduled to begin in early July was postponed until July 24th. Most of the weed beds which had grown so thickly last year were very sparse in most areas this summer. When the project ended in early October, the entire circumference of the bay had been harvested and some areas had been cut twice, especially around the Hathaway Point area. A new access site at Hathaway Point greatly improved the efficiency of the operation in that area and in adjacent sites.

A flyover of the Bay at the onset of the harvesting season provided valuable knowledge of the size and locations of the weed beds. The heaviest infestations of weeds occurred in the Lazy Lady Island area and around Hathaway Point.

Table 2 provides a summary of activities in St. Albans Bay with some additional facts comparing the first 3 years of harvesting.

Table 2
1984 St. Albans Harvesting Project

	1984 Billing Period, Hours					
Expense	*4 Aug*	*17 Aug*	*31 Aug*	*15 Sep*	*6 Oct*	*Total Cost*
Harvester operator						
at $15.10/hr	63.0	66.0	72.0	56.5	40.0	$ 4,492.24
at $20.36/hr	16.0	12.0	5.0	11.5	8.0	1,068.90
Dump truck w/operator						
at $23.50/hr	61.0	66.0	72.0	56.5	44.0	7,038.25
at $28.70/hr	16.0	12.0	5.5	11.5	8.0	1,521.10
Maintenance Admin.						
at $15.10/hr	21.0	9.0	18.0	33.0	30.5	1,683.65
at $4.00/hr	8.0	9.0	—	16.0	12.0	180.00
Other expenses (-$303.60, billed twice)	470.82	908.29	374.16	177.33	111.98	1,738.98
TOTAL						$17,723.12
TOTAL LOADS	20-1/3	25-1/4	31	40-1/2	26	

	1982	*1983*	*1984*
Weeks harvested	5.0	11.0	6.0
Total loads	153.0	232 3/4	143.08
Total maintenance	—	89.0	111.5
Total harvester hours	223.1	333.0	350.0
Contract award	$42,322.50	$17,725.00	$17,723.12

High water, excessive turbidity levels, and a late harvesting season in 1983 are three factors which may have contributed to the light weed growth in St. Albans Bay this year. Under normal conditions the highest water levels occur in March and April after spring runoff. However, an intense storn in early June caused the lake to rise to its highest level of the season, and also increased turbidity in the bay. A weed survey conducted after the June storm revealed almost no milfoil growth. It is possible that the milfoil growth was stressed as a result of our late harvesting during 1983. This possibility will be more easily determined during the 1985 season since the milfoil harvesting again this year extended to the end of September.

With most of the mechanical problems involving the harvesting equipment resolved during 1983, the harvesting operations went quite smoothly this season.

A higher number of maintenance hours than projected can be attributed to the following reasons. Approximately 60 of the 111 hr of maintenance represents assembling and launching the equipment at the beginning of the 1984 harvesting season when it was returned after repairs from the manufacturer, and preparation for this winter's storage which did not occur at the end of the 1983 season. Most of these hours accounted for two persons performing these tasks. Normal routine maintenance and replacement of disposable parts occupied only 1 hr of harvesting time a day. A total of 350 hr were spent in actual harvesting compared to 333 last season. All the weed beds which were designated to be harvested were cut at least once. In 1983, the harvesting percentage was not nearly as high due to the density and area of coverage of weed growth.

Recreational use of the Bay appeared to increase greatly this year. The large State park at the north end of the Bay, which has been virtually unused for the past several years, was frequented by a number of swimmers. Public sentiment seemed favorable to the conditions of the Bay this year.

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USAE DIVISION/DISTRICT PRESENTATIONS AQUATIC PLANT PROBLEMS—OPERATIONS ACTIVITIES

North Atlantic Division, New York District

by
Simeon M. Hook*

The purpose of this paper is to describe the New York District Corps of Engineers' program to control Eurasian watermilfoil (*Myriophyllum spicatum* L.) and waterchestnut (*Trapa natans*) in the State of Vermont in portions of Lake Champlain. The Aquatic Plant Control Program is authorized by Section 302 of the River and Harbor Act of 1965, Public Law 89-298. This Act provides for the control and eradication of obnoxious aquatic plans in navigable waters, tributary streams, connecting channels, and other allied waters in the interest of navigation, flood control, drainage, agriculture, fish and wildlife conservation, public health, and related purposes.

In 1979 the Vermont Department of Water Resources requested assistance from the New York District in developing and funding a control program for the above-mentioned aquatic weeds in Lake Champlain. A reconnaissance report was completed by the New York District in August 1979 which recommended that a General Design Memorandum and Environmental Impact Assessment be prepared to examine the extent of the problem, delineate a control program, and determine the cost, benefits, and environmental and social impacts of such a program. This document was completed in March 1982 and control operations began in the summer of 1982.

The Lake Champlain Aquatic Nuisance Control Program is a two-part project. It involves controlling Eurasian watermilfoil in St. Albans Bay, Vermont, and waterchestnut in southern Lake Champlain in order to prevent its spread northward. The control method selected was mechanical harvesting. It was determined to be the least environmentally damaging control method and the most acceptable to those with interests in Lake Champlain.

BACKGROUND

Waterchestnut is an annual aquatic plant native to freshwater streams and ponds in temperate areas. Although the seed contains some food value and is edible, the plants are usually found in polluted waters and may be contaminated. The waterchestnut survives in mud but grows best in water depths from several inches to 5 or 6 ft. It has been found to grow in depths of up to 15 ft in some control areas. Its annual decomposition forms a muddy bottom of high organic content.

* US Army Engineer District, New York; New York, New York.

The plant is identified by its waxy floating leaves supported by swollen leaf streams forming leaf rosettes. Each new season's growth is produced entirely from the seeds of this annual plant. The seeds germinate every spring at about the beginning of May, and form a cordlike stem 6 in. to 15 ft long which reaches the surface in about the middle of June. The plant grows as late as October and is killed by the frost in the fall. It produces nuts which become hard and black. The nuts have four sharp horns and several reverse barbs. As they are about 20 percent heavier than water, the nuts sink when they become ripe and drop off the plant. It seems that most nuts will germinate within 1 to 5 years after being dropped, with longer dormancies not unusual.

The plant spreads by rerooting in another area or by the nuts being swept by currents to downstream areas. The problems caused by the plant's existence in the waterway relate to recreation activities. Thick mats hinder passage of boats for fishing, pleasure, and waterfowl hunting. The plant has crowded out such desirable duck food as wild celery and reduces open water shore habitat for desirable game fish. If stepped on, the nut can inflict a painful wound.

The waterchestnut program in the southern portion of Lake Champlain involves the control of this plant north of 43°43′0″N or in the approximate vicinity of Benson's Landing, Vermont. Here, as in the St. Albans Bay area where Eurasian watermilfoil is the target plant, the control method used was mechanical harvesting accompanied by handpulling. Disposal of the waterchestnut was accomplished by transporting the cut plants to approved shore disposal sites after unloading plants by shore conveyor to trucks. The actual harvesting operation was subcontracted by the State of Vermont to Aquatic Control Technology, Inc., of Northborough, Mass., in the South Lake area, and to New England Constructors of Essex Jct., Vermont, in St. Albans Bay.

CONTROL OPERATIONS

The basic operation in South Lake consisted of two mechanical harvesters, one high speed transport, one shore conveyor, and one truck. Gains in the efficiency of the South Lake control effort were realized as a result of the use of an experimental harvesting computer program written by Bruce Sabol of the Waterways Experiment Station and through improvements to existing equipment. The HARVEST computer program enabled each piece of harvesting equipment to be used in an optimal usage sequence for the various situations present on the South Lake.

The high speed transport built in Toronto in 1982 continues to be a large contributor to a more efficient operation. Its speed of 12 mph versus the 3- to 4-mph speed of the harvester allows the harvesters to spend more time harvesting and not lose precious time in transporting the material to the shore. Improvements to the high speed transport for the 1984 season further increased its efficiency in the disposal process. New pontoons were added to provide more flotation, allowing it to carry increased weight. The control tower on the transport was moved toward the rear to permit the bow of the barge to ride higher in the water while carrying the increased loads.

Beginning on July 11, 1984, harvesting in the South Lake continued for approximately 6 weeks. During this time 758 loads of waterchestnut were cut and removed. The 21-percent increase over the number of loads cut and removed in 1983 is significant since the increased output occurred even though the density of the plant was much reduced in the northern portion of the control area. Of the 758 loads, 620 came from a 60-acre patch at the south end of the control plot. Each load weighed approximately 4,000 lb and all were removed from this area over a period of 4 weeks. It is unfortunate that funds ran out before completing harvesting in all the designated control areas. Even though about 90 percent of the 60-acre patch was harvested, leaving only a narrow strip along the New York shore, this patch is most likely responsible for seeding a good portion of the South Lake, and if not completely controlled, continued infestations will occur. Handpulling efforts concentrated in the northern section of South Lake in order to keep small coves and bays from becoming infested with waterchestnut. Handpullers were also used to clean up areas already harvested or areas that were too shallow for proper operation of the harvester. The handpulling effort was reduced this year since high water conditions allowed the harvesting equipment to cut close to the shoreline.

The waterchestnut was disposed of in an upland site in Benson Landing about a mile and a half from the control area. The plants were transferred from the harvesters to the transporter which carried the waterchestnut about 1 mile to the shore access site. The material was then loaded on a truck by a shore conveyor and driven the short distance to the disposal area where it was dumped. Another access site is being considered for use in 1985. This land is owned by the US Coast Guard and is 1/3 to 1/2 mile closer to the 60-acre patch than the Benson Landing site. By using this site a reduction of traveling time to and from the patch will result, allowing the removal of a greater number of loads in less time.

The Eurasian watermilfoil harvesting in St. Albans Bay did not begin until late in July. Most of the weed beds were sparse in much of the area this summer. High water, excessive turbidity levels, and a late harvesting season in 1983 are factors which might have contributed to the light weed growth. At the project's end in early October, the entire circumference of the bay had been harvested and some areas cut twice. A flyover of the Bay at the beginning of the harvesting season provided information as to the size and locations of the weed beds. A total of 143 loads of plants were harvested in the summer of 1984.

FUTURE OPERATIONS

Modification to the existing equipment is being considered for 1985. The State of Vermont and a consultant are exploring the development of an onboard processing system for the harvesters in South Lake. The purpose of this processor would be to reduce the bulk of the waterchestnut plant and remove water by cutting and grinding the plants. The nuts would also be crushed, eliminating them as a problem if the plants were used for mulch on feed land. If the plants were used as cattle feed, they would be harvested before the nut forms. It is estimated that if a successfully designed and contructed processor can be implemented, harvested material would increase by about 30 percent over present techniques.

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USAE DIVISION/DISTRICT PRESENTATIONS AQUATIC PLANT PROBLEMS—OPERATIONS ACTIVITIES

North Central Division, St. Paul District

by
Wayne Koerner*

The St. Paul District's major emphasis in the Aquatic Plant Control Program has been Buffalo Lake, a 2,500-acre impoundment on the upper Fox River in Wisconsin. The States of Wisconsin, Minnesota, and North Dakota are included in the District's program.

The St. Paul District has not yet reached the level of assistance to the states that we would like due in part to limited funding in the past and difficulty in obtaining approval on our reconnaissance report. We have, however, gained substantially in the funding of our program and have obtained valuable information through the assistance of the Waterways Experiment Station (WES) in obtaining and analyzing data on Buffalo Lake which has problems similar to those of many lakes in the midwest.

Based on the WES computer program HARVEST and results presented in the WES report "Simulated Mechanical Control of Aquatic Plants in Buffalo Lake, Wisconsin," and comments received from the Aquatic Plant Control Support Center, Jacksonville, Florida, we have revised our reconnaissance report and are in the process of resubmitting the report for review and approval.

We have considered other alternatives to the problem on Buffalo Lake but feel that mechanical harvesting is the most appropriate alternative. Heterogeneous plant populations such as those in Buffalo Lake exist in most of our lakes. Some 20 plant species were found in Buffalo Lake during the summer of 1982.

Although we are not presently involved in any active control operations, we have provided some assistance to Inland Lake Protection and Rehabilitation Districts. As a result of our efforts on Buffalo Lake, the Montello Lake District purchased a H650 harvester and a shore conveyor to perform their own harvesting. Montello Lake is located in the immediate study area of Buffalo Lake. Montello Lake is a smaller lake (less than 300 acres) but has the same heterogeneous plant populations and similar problems (100 percent plant infestation) as Buffalo Lake. The entire lake was harvested this past summer and costs obtained from the Lake District indicate an annual cost of about $20,000 including the cost of the equipment. These costs do appear on the low side, however, because some of the labor was paid for by CETA funds and does not show up in their costs.

The pattern considered for the area of Buffalo Lake in which plants would be maintained below the nuisance level selected consists of two 150-ft-wide swaths

* US Army Engineer District, St. Paul; St. Paul, Minnesota.

running parallel to the shore on either side of the lake, 300 ft offshore; and 100-ft-wide channels perpendicular to shore every 2000 ft which would act to connect the offshore swaths. This pattern covers 276 acres or about 20 percent of that portion of the lake where plants would be maintained below nuisance level.

We have found that an accurate cost estimate is difficult to obtain because of two main reasons:

a. Plant densities are known to vary appreciably from year to year in response to varying environmental factors.

b. The effects of early summer harvesting in Buffalo Lake on late summer plant conditions are unknown.

During FY 85 we plan to gain approval of our reconnaissance report and complete the State Design Memorandum. We also plan to perform mechanical harvesting for selected areas on Buffalo Lake this summer which will allow for improved planning of harvesting operations for mechanical control of Buffalo Lake as well as other lakes in our District.

USAE DIVISION/DISTRICT PRESENTATIONS AQUATIC PLANT PROBLEMS—OPERATIONS ACTIVITIES

North Pacific Division, Seattle District

by
Robert M. Rawson*

We have just completed our fifth year of control operations for Eurasian watermilfoil in Washington State. The control efforts include both mechanical and chemical treatments.

Also this year, we contributed to the funding of a 4-year study on the triploid grass carp in Washington waters. The study is being conducted by the Cooperative Fisheries Research Unit at the University of Washington and is also funded by the Washington State Department of Ecology and the US Fish and Wildlife Service. Lewis Decell of the Waterways Experiment Station has provided technical support. Gil Pauley and Gary Thomas from the University will talk about this work in detail later in the program.

Milfoil continues to expand its range in Washington State, primarily down the Columbia River. It is entering the Walla Walla District area now, and will soon be in Portland District's reservoirs. All the reservoirs impacted so far are operated by Public Utility Districts and are eligible for funding under the Aquatic Plant Control Program. The remainder of the river is made up of Corps reservoirs, which would have to do control work under project operation and maintenance funding.

We have several problems with our cost-share program; the first is an old problem, the difficulty the local sponsors have in raising the required matching funds. This is more of a problem in the more sparsely populated eastern Washington counties.

The second problem is one that became apparent only over the last several years—the lack of treatment options for flowing water. This problem has prevented any treatment in the Columbia River for the past 2 years.

Finally, this is a problem that may impact our program in the future. I am referring to the Federal Court decision that suspended all chemical spraying of forest land by the Forest Service and the Bureau of Land Management in Oregon pending the completion of worst case analysis for the chemicals being used. The Department of Interior appealed the decision based on the fact that all chemicals were Environmental Protection Agency approved and were used according to label restrictions. They lost the appeal and are still shut down.

This supports our belief that we cannot look at chemical control as a long-term solution to our aquatic plant problems. We believe that, at best, it is a stop-gap measure until milfoil comes into a natural balance or until biological controls can be developed. We will continue to support WES in their biological control research.

* US Army Engineer District, Seattle; Seattle, Washington.

USAE DIVISION/DISTRICT PRESENTATIONS AQUATIC PLANT PROBLEMS—OPERATIONS ACTIVITIES

South Atlantic Division, Charleston District

by
John L. Carothers*

The Charleston District has a variety of aquatic plant problems including Brazilean elodea, hydrilla, waterhyacinth, waterprimrose, watersmartweed, and alligatorweed. Elodea is now our worst problem but hydrilla is rapidly expanding from its recent introduction in Lake Marion and has the potential of becoming our biggest problem. Our largest infestation is in Lake Marion, which is a state hydroelectric impoundment of almost 100,000 acres. This lake is one of the two lakes in the Santee Cooper system and one of the premier fishing lakes in the country. Here the area of infestation is on the order of 30,000 acres.

The District began its cooperative aquatic plant control program in 1960 to control alligatorweed in the Santee Cooper Lakes. Our present program provides for work on additional species in any public water of South Carolina. We work closely with the South Carolina Aquatic Plant Management Council which consists of representatives of nine State agencies and a representative of the Governor's office. Each year the Council prepares an aquatic plant management plan which identifies problem areas, prescribes management strategies, and ranks each problem area by priority. Recently, all of our work has been confined to three lakes with most of the money being allocated to Lake Marion. However, higher funding for FY 85 will enable us to work on many other water bodies.

In FY 84, we treated 1,700 acres of aquatic plants with herbicides including Diquat, Aquathol-K, Rodeo, and 2,4-D. The total cost of this work was $466,000. The Federal contribution was $196,000 so the State spent far more than the required 30 percent.

In the area of biological control, we restocked flea beetles on alligatorweed as needed and as we could obtain beetles from the Jacksonville District. Stem borers were introduced years ago on alligatorweed and restocking has not been required. We planned to introduce hyacinth weevils but our source failed to produce weevils as expected, so we will try again this year.

* US Army Engineer District, Charleston; Charleston, South Carolina.

USAE DIVISION/DISTRICT PRESENTATIONS AQUATIC PLANT PROBLEMS—OPERATIONS ACTIVITIES

South Atlantic Division, Jacksonville District

by
Edward Knight*

STATE OF FLORIDA

In cooperation with the Florida Department of Natural Resources, the Jacksonville District conducts aquatic plant control under two authorizations. One is the Removal of Aquatic Growths Project (RAGP) authorized by the River and Harbor Act of 1899. The other is the Aquatic Plant Control Program (APCP) authorized by Public Law 89-298 dated 1965. The RAGP is funded 100 percent by the Corps of Engineers and is the original eradication operation and maintenance program for the control of aquatic vegetation in Federal navigation projects. The APCP is a cooperative program for the control of aquatic vegetation in other public navigable water bodies not included in the RAGP. This program is funded 70 percent by the Corps and 30 percent by the state or local government.

The majority of actual control operations are handed under contract with the Florida Department of Natural Resources (DNR), which in turn has subcontracted the work to five water management districts and seven counties. Jacksonville District spray crews are responsible for control operations in the St. Johns River between Jacksonville and Lake Washington. Waterhyacinths are still the number one target plant for District in-house crews. The St. Johns River continues to be under maintenance control. Sustained efforts have set the example for control of waterhyacinths throughout the State of Florida. There were 6,147 acres of waterhyacinth treated on the river during FY 1984.

Orange Lake, which is under contract for control operations through the Florida DNR, developed major problems with waterhyacinths this year. On several occasions the plants restricted or blocked access to fish camps on the lake. Extensive control efforts by the St. Johns River Water Management District has brought the lake back under maintenance control.

Waterlettuce created no significant problems on the St. Johns with only 34 acres treated. Lake Okeechobee, however, had a more significant problem with the plant. During the early growing season, diquat use was temporarily suspended, which compounded the waterlettuce problem. With the suspension of diquat, waterlettuce control became difficult since no other labeled herbicide seemed to be effective. Endothall products were used for a while with mixed results. Once the suspension was lifted, waterlettuce was able to be effectively controlled and the lake is now close to maintenance control.

Except for the increases in vegetation that occurred on Orange Lake and Lake

* US Army Engineer District, Jacksonville; Jacksonville, Florida.

Okeechobee, floating vegetation remained under maintenance control throughout most of the state. During the year a total of 22,713 acres of waterhyacinth, 1,008 acres of waterlettuce, and 21,657 acres of waterhyacinth and waterlettuce mix were treated in the state.

Hydrilla continues its prolific growth throughout most of the state with a few exceptions. Acceptable control of hydrilla was obtained in 1983 using Sonar in several lakes in Polk and Orange Counties and in Orange and Lockloosa Lakes. Subsequent Sonar treatments in these lakes during 1984 have maintained hydrilla at low levels. The upper river basin from Puzzle Lake south holds a tremendous amount of hydrilla. Jacksonville District crews treated a total of 67 acres on the St. Johns. Treatments were limited to navigation and access trails in areas of heavy public usage. The total acreage of hydrilla treated in the state his year was 7,209.

The District treated 619 acres of minor plants on the St. Johns in FY 1984. This number reflects a significant growth of frogsbit (*Limmobium spongia*) which caused problems in the upper basin of the St. Johns. The plants were treated with a mixture of diquat and 2,4-D. Statewide, a total of 2,006 acres of minor plants were treated under the APC and RAG programs.

PUERTO RICO

Aquatic Plant Control in Puerto Rico is also within the Jacksonville District's jurisdiction and is performed under Public Law 89-298 in cooperation with the Puerto Rico DNR.

The program officially began in FY 1982 after a cooperative agreement was signed between the Jacksonville District and the Puerto Rico Department of Natural Resources. Because of several environmental concerns about using 2,4-D, the Environmental Quality Board (EQB) would not permit control operations until sufficient monitoring was performed. The EQB consented to allow DNR to treat a small area on the La Plata River for the monitoring which was started in July 1982. Results of the monitoring showed no significant effects, and nine sites were approved initially in 1983. In 1984 four more sites were approved, making a total of thirteen. Currently most of the approved canals are under maintenance control for waterhyacinths. However, paragrass is now encroaching in some of the areas originally cleared of waterhyacinth and will be an increasing problem. A request has been submitted to EQB by DNR to use Rodeo on the encroaching grasses and on waterhyacinths. Approval is expected soon.

SUMMARY

In summary, floating vegetation is under maintenance control throughout most of the State of Florida. Hydrilla continues to make its spread with new infestations continuously being found. This plant is still regarded as a serious threat to navigation in the State and sustained efforts of control remain a high priority with the Jacksonville District. Control operations in Puerto Rico are progressing slowly due to environmental issues. Thirteen sites are now approved by EQB for treatment and at this time most of the canal sites are under maintenance control.

USAE DIVISION/DISTRICT PRESENTATIONS AQUATIC PLANT PROBLEMS—OPERATIONS ACTIVITIES

South Atlantic Division, Jacksonville District Aquatic Plant Control Operations Support Center

by
James T. McGehee*

The Aquatic Plant Control Operations Support Center (APCOSC) was formally established in FY 81. The Center is located within the National Resource Management Section, Construction-Operations Division, of the Jacksonville District.

CENTER RESPONSIBILITIES

The policies, functions, and procedures for the use of Center services are set forth in Engineer Regulation 1130-2-412. The regulation describes the relationship between the APCOSC; the Office, Chief of Engineers (OCE); and the US Army Engineer Waterways Experiment Station (WES) and establishes the following functions of the Center:

- Provide technical guidance to Corps Districts in planning phases of aquatic plant control program.
- Provide technical guidance to Corps Districts in the operational phases of aquatic plant control programs.
- Provide technical expertise and/or operational personnel and/or equipment to respond to localized short-term critical situations created by excessive growths of aquatic plants.
- Provide assistance to OCE for the training and certification of Corps application personnel.
- Upon request, assist WES in field application and evaluation of newly developed control techniques or procedures.
- Provide assistance to OCE in the development of a comprehensive, Corps-wide aquatic plant control program.

FISCAL YEAR 1984 ACTIVITIES

Table 1 provides a listing of the services performed by the Center and the types of users to which these services were provided during FY 84. A total of 133 requests for assistance were received and responded to by the APCOSC. Since established, the Center has responded to a total of 500 requests.

The primary purpose of the Center is to help Corps Districts and their state sponsors through the problems of planning and operations in the Aquatic Plant Control (APC) program. During FY 84 Corps Districts accounted for 51 of the

* US Army Engineer District, Jacksonville; Jacksonville, Florida.

Table 1
Aquatic Plant Control Operation Support Center, FY 1984
Support Assistance Through 30 September 1984

Type Assistance	*Corps* *OCE*	*WES*	*DIV.*	*DIST.*	*Other Federal*	*Other Country*	*State/ Local*	*Industry*	*Private*	*Total*
Planning	10	1	4	35	3	0	2	6	2	63
Operations	2	3	2	11	14	0	20	3	1	56
Research	0	3	0	0	1	0	1	0	0	5
Training	1	0	1	5	0	0	2	0	0	9
Totals	13	7	7	51	18	0	25	9	3	133

services provided. The next most frequent user was state and local governments with 25. Collectively these two categories comprise 57 percent of the services performed. Total services provided to Corps organizations were 78 or 59 percent of the total. Seventy-two percent of all services were provided to Federal agencies including the Corps.

PLANNING

There were 63 planning services performed during the year. The most detailed effort was in assisting the Baltimore District with the planning of control operations for hydrilla on the Potomac River. Jim McGehee from the Center spent several days with Baltimore District personnel to orient them with the Corps' APC program and assist in commencement of the planning process. The Center also hosted an onsite visit by Baltimore personnel to demonstrate the administration and actual field control methods. We have provided considerable specific information to Baltimore to help in the planning effort. Three Corps project lakes were evaluated for two separate Districts. Recommendations were given on the types and degree of control needed to maintain the intended water body uses. Seven Districts are being assisted in the preparation of cooperative agreements for use between the Corps and local sponsor. The Center is frequently called upon to help in deciding upon the methods of data acquisition and computation for benefit-cost ratios. Likewise, assistance in the determination of water body eligibility and allowable costs is frequently requested.

OPERATIONS

Operationally oriented services accounted for the second largest portion of work for FY 84. Operational services vary from year to year by the requesting organization and the specific assistance required. However, there are basic similarities in the work.

Many of the issues concerning aquatic plant control are highly technical and very specific to the field. Those new to the field may find it very difficult and time-consuming to find answers or solutions to seemingly simple problems. The Center, through its broad base of experience in the field, has been able to quickly provide answers to these questions and workable solutions to the problems. The types of plant and equipment used for aquatic plant control are not the usual for Corps contracts. Establishing rental rates and estimating rental periods for this equipment are therefore unusual to most Corps Districts. A procedure has been established by the Center to compute rental rates for this equipment and we have a broad database for estimating time requirements for particular jobs. Units of payment and technical provisions for aquatic plant control contracts may likewise be difficult for the uninitiated. The Center has provided guidance to WES and Corps Districts in the preparation of these contracts.

An annual program for the reintroduction of insect control agents for alligatorweed was established by the Center. Insects are field collected in the spring in Florida and shipped to more northerly areas where they are unable to successfully overwinter. There were some difficulties with the collection of insects this year. Florida experienced an unusually sever winter in 1984. The insects' numbers were drastically reduced and the usual early spring population buildup was late. The shipment of insects was therefore deferred until later in the year. A total of 70,500 flea beetles were collected and shipped to 12 separate agencies for operational release on alligatorweed. Four states received a total of 11,000 alligatorweed thrips to help in the control effort. Although the effects of the thrips have not been as spectacular as the flea beetle, they do appear better able to survive over the winter. It is hoped that the thrips' early spring buildup will help in the biological control efforts in the colder areas of alligatorweed domain.

RESEARCH

The Center's authority and expertise do not include conduct of research. However, we are called upon to provide other research organizations with data or information of an operational nature to assist with research. This year's assistance included information on the legal use of products not registered by EPA for tests, input of insect redistribution information to the study of alligatorweed biocontrol, providing field-collected insects for research purposes, and evaluation of a possible transfer of new terrestrial weed control technology to the aquatic environment.

TRAINING

This year's training assistance consisted primarily of providing materials or referral to others for their use in training programs. The Center has also been working with the Mobile District in the development of a course to be taught by Center personnel next fiscal year.

STATUS OF THE APCOSC

Expenditure by the Jacksonville District to support the Center function for FY 84 was $44,637.20. These funds covered personnel costs for time expended for minor services and general maintenance of the Center functions. All expenses for travel and significant commitment of manpower for services were paid by the using Corps element.

The Center was able to complete most service requests during the fiscal year in an adequate and timely manner. However, the Center was handicapped by the loss of a portion of its expertise and an increase in the intensity of involvement required by many of the requests. This caused considerable delays in some of the more detailed responses.

USAE DIVISION/DISTRICT PRESENTATIONS AQUATIC PLANT PROBLEMS—OPERATIONS ACTIVITIES

South Atlantic Division, Wilmington District

by
Charles R. Wilson*

Aquatic plant control in North Carolina is governed by the North Carolina Interagency Council on Aquatic Weed Control. This council is composed of representatives from State and Federal resource agencies, conservation groups, private industry, and local universities. The Wilmington District and the North Carolina Department of Natural Resources and Community Development are the lead agencies charged with the coordination and funding of control operations. Aquatic weed control is performed under two authorities: Removal of Aquatic Growth in Corps Projects, funded 100 percent by Federal operations and maintenance, and general funds; and the Aquatic Plant Control Program (APCP) funded by 70 percent construction general funds and 30 percent by the State of North Carolina. Treatment species presently under the Aquatic Plant Control program are hydrilla and alligatorweed.

Hydrilla has been identified at 15 sites in North Carolina, which have all been investigated for inclusion under the North Carolina APCP. Of those sites, seven provide public benefits, which were lost or reduced due to excessive growth of hydrilla, and were added to the program. Three of the proposed sites are water supply sites and have not been treated due to a reluctance by health agencies within the State and the city of Raleigh to use herbicides in water supply reservoirs. This issue is still unresolved; however, coordination is ongoing with the North Carolina Aquatic Weed Council and we expect to treat those areas in the future. Public access to Fred Bond Lake was opened late in FY 84 and treatment is proposed for FY 85. We have just completed our second year of hydrilla control at the remaining three lakes, Big Lake, Sycamore Lake, and Reedy Creek Lake, all of which are located within William B. Umstead State Park.

Reedy Creek Lake is a 20-acre impoundment with a 50-percent hydrilla infestation. Aquathol-K (liquid) was applied to 6 acres of hydrilla, which totally covered the lake's headwaters in June of FY 84. Weed control was temporary and a repeat application was required in August. Four acres of hydrilla fringing the lake's shoreline were also treated in June with a granular formulation of Aquathol-K, providing control throughout the growing season in those areas.

Big Lake impounds 60 surface acres and had 10 acres of hydrilla. Sonar was applied on two 5-acre plots, one in October and one in May. Presently, no hydrilla is known to be growing in Big Lake and it is expected that control will be long term. Big Lake will be monitored over the next few years for hydrilla regrowth.

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* US Army Engineer District, Wilmington; Wilmington, North Carolina.

At Sycamore Lake, a 20-acre impoundment, hydrilla covered 15 acres. Sycamore was drawn down last fall and Fenac was applied to 4-1/2-acre test plots. Herbicide application was by a truck-mounted sprayer, and the treatment area was limited due to bad access. This method provided good seasonal control at the treatment sites. The lake was drawn down again this fall and the entire infested area was treated with Fenac by aerial application.

Alligatorweed control under the North Carolina APCP began in 1960. Early treatment was by various chemicals in an effort to find a herbicide that was the most effective and dependable. By 1963, Silvex, commercially known as "Kuron," was the primary herbicide used by the Wilmington District. Alligatorweed control was stopped in 1970 due to uncertainty concerning the environmental and health effects of Silvex. Since alligatorweed was no longer occurring at problem levels, the program was temporarily discontinued. Since that time, alligatorweed has spread and has grown to problem proportions in many areas where it had previously been held under control. Preliminary results from an alligatorweed survey conducted by Ken Langland, of the North Carolina State University Crop Science Department, showed that the range of alligatorweed extends from the northern to the southern state line and from the coast to Piedmont. Four thousand acres of alligatorweed were identified from 16 counties in North Carolina. In addition to excess growth and associated problems, recent development of safer herbicides and an increased interest in weed control in North Carolina have renewed interest in alligatorweed control. The Wilmington District completed an environmental assessment for the control of alligatorweed in June of this year and began herbicide applications in early August in the Scuppernong River Basin. About 10 acres of alligatorweed in the Scuppernong River and Mauls Creek were treated with a Rodeo and X-77 (surfactant) mixture. Two applications were required to provide seasonal weed control. The District is supporting the North Carolina Department of Agriculture in a project to develop a cold-tolerant flea beetle. This project is just beginning to gear up; however, if all goes well, we plan to start supplementing chemical treatments of alligatorweed with flea beetle releases next spring.

No aquatic plant control is presently being undertaken at any Wilmington District reservoir; however, several of our reservoirs are threatened by hydrilla. Our primary efforts at this time have been toward education of both the general public and reservoir management staff. Project biologists have been trained to recognize potential problem plants and have been supplied with sample bottles and preaddressed mailers so that suspect plants can be sent to a State expert for positive identification. All boat ramps have been posted with signs instructing users to clean their boats and trailers prior to launching. Through an effort of the North Carolina Aquatic Weed Council, non-Corps lakes that have hydrilla have also been posted, alerting users to the potential for the spread of noxious aquatic plants.

The District Office was recently notified of excess waterprimrose growth in Falls Reservoir and has begun to monitor the weed's spread. So far, boat channels remain open and the weed is not significantly interfering with recreation or project operation and no treatment is planned at this time. If waterprimrose begins to cause problems, a control program will be developed.

For FY 85 and in the future, we plan a continuation of the existing control program for hydrilla and alligatorweed with some expansion to cover new treatment sites. Reports of waterprimrose and elodea in water bodies around the state may lead to additional treatment species being added to the program.

USAE DIVISION/DISTRICT PRESENTATIONS AQUATIC PLANT PROBLEMS—OPERATIONS ACTIVITIES

South Atlantic Division, Mobile District

by
Michael J. Eubanks*

Aquatic plant control activities within the Mobile District were carried out in four areas: Mobile Delta, Coffeeville Lake, Tennessee-Tombigbee Waterway (TTW), and Lake Seminole. Since project personnel from Lake Seminole will address that area, this presentation will summarize the activities in the other three areas.

Target species in the Mobile Delta include Eurasian watermilfoil, waterhyacinth, and alligatorweed, in that order. Aquatic plant control in the Delta has been carried out for the last 4 years by the Alabama Department of Conservation and Natural Resources (ADCNR) via an Aquatic Plant Control Program contract. Prior to initiation of the 1984 growing season, an informational meeting was held with local environmental and conservation organizations and commercial fishing interests to display the control activity plans for the upcoming growing season and to solicit their comments. Over the years these meetings have proven to be an excellent public relations mechanism. Eurasian watermilfoil, which infests approximately 3,000 acres, was treated with a liquid 2,4-D-DMA solution (Cidekick and Poly Control adjuvants used) on approximately 300 acres. These treatments were carried out by airboat with trailing hoses from mid-May until early September. The expansion of waterhyacinth in the Delta necessitated the treatment of several "hot spots" during the growing season (approximately 30 acres) in order to maintain a low population of this rapidly growing species. Liquid 2,4-D-DMA with Big Wet was used to treat these hot spots from early April to late July. Approximately 2 acres of alligatorweed were treated with Rodeo in mid-April. Also, as in the past several years, alligatorweed flea beetles were released at numerous sites in early June, thanks to the Jacksonville District. One of the most visible beneficial effects of the aquatic plant control work in the Delta is the expansion of the native eelgrass (*Vallisneria americana*) which is valued by local fishermen and duck hunters. The herbicidal treatment of the milfoil has given the 2,4-D-resistant eelgrass a competitive edge.

The hydrilla infestation on Coffeeville Lake appears to be well under control. Following discovery of the infestation in 1978, extensive control efforts with endothall products have been carried out. Hydrilla reached its maximum coverage in 1980 with about 15 acres; however, the repeated treatments have reduced the infestation significantly. An extensive underwater survey in September 1984 revealed only a few clusters of hydrilla. These patches and other suspected areas were treated in an effort to minimize the threat of expansion of hydrilla to other susceptible areas such as the Mobile Delta, Choctaw National Wildlife Refuge, and the newly impounded lakes of the Tennessee-Tombigbee Waterway.

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* US Army Engineer District, Mobile; Mobile, Alabama.

The TTW, scheduled for completion in June 1985, will consist of approximately 44,000 water surface acres, impounded by 10 locks and dams. Much of this area will provide excellent habitat for establishment of aquatic plants. Observation of the plant growth on already completed portions of the TTW at Gainesville and Aliceville Lakes, which have been impounded for 6 and 4.5 years, respectively, gives a better picture of anticipated plant growth and helps in the formulation of aquatic plant management strategies. Estimates of submersed plants in these lakes for 1982 and 1983 are 2,300 and 1,400 acres, respectively, while floating-leaf plants increased from 40 to 60 acres. The big reduction in submersed plants is thought to be related to higher lake levels and increased turbidity in 1983; however, the 1984 estimates will help discern the basis for this reduction. Major plant species include coontail, southern naiad, cabomba, muskgrass, American lotus, yellow waterlily, water-primrose, and various duckweeds. Chemical control was carried out in 1983 and 1984 to aid small boat navigation and fishermen access in the lower Aliceville Lake area. Approximately 45 acres of predominantly submersed plants have been treated with Hydrothol 191 each year. Based on review of the 1983 treatment, the configuration of the treatment area was changed to larger block areas rather than long narrow boat channels. As a result, the 1984 treatment provided more effective and longer lasting control than the previous year.

USAE DIVISION/DISTRICT PRESENTATIONS AQUATIC PLANT PROBLEMS—OPERATIONS ACTIVITIES

Southwestern Division, Galveston District

by
Joyce Johnson*

The Galveston District is located along the Texas Coast; however, the District is responsible for aquatic plant control in the entire state, except for project lands where each District is responsible for their particular areas. Texas is so large that parts of four Districts are within the state boundaries. The control of noxious aquatic plants has been a part of the District mission since about 1968. A General Design Memorandum and Environmental Statement were published in the early 1970s describing the control of waterhyacinth and alligatorweed. We use primarily 2,4-D (dimethylamine salt of 2,4-dichlorophenoxyacetic acid) for the control of waterhyacinth. Alligatorweed is controlled by flea beetles (*Agasicles hygrophila*).

During this 16-year period, the Galvestion District has had three successive contracts with the State, represented by the Texas Parks and Wildlife Department, who also do the actual field work. The Texas Parks and Wildlife Department plays a very instrumental role in the District program. The chemical spray work is accomplished primarily by boat crews; seven crews are located throughout the State during the growing season. However, aerial spraying by helicopter is done in areas of excessive and dense infestations. During the past year the state crews sprayed about 12,000 acres of waterhyacinth. Most of the biological control work has been done by the Corps, although early releases were made by the State. Since 1980 the Corps and the US Army Engineer Waterways Experiment Station (WES) have made releases of five biocontrol agents: *Neochetina bruchi*, *Neochetina eichhorniae*, and *Sameodes albiguttalis* on waterhyacinth and *Agasicles hygrophila* and *Vogtia malloi* on alligatorweed colonies. This work has been done as part of a technology transfer from WES, primarily by Dr. Al Cofrancesco and Mr. Mike Stewart. Setbacks during this past year to the biological agent establishment occurred due to high water levels in the eastern part of the state, a drought in the Neches River Basin near Corpus Christi (Figure 1), and a cold, severe winter over most of the state. It is anticipated that the incorporation of biological control agents into the State program will continue to be a major focus of the District effort through implementation of the recommendations made in the management report completed by WES in FY 84.

Work continued during 1984 on Supplement No. 1 to the General Design Memorandum and Environmental Assessment for the inclusion of submersed species, hydrilla and Eurasian watermilfoil, in the program. As part of this effort, a statewide survey of aquatic plants was completed in August. This survey, conducted by the Texas Parks and Wildlife Department, showed that several species have

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* US Army Engineer District, Galveston; Galveston, Texas.

made great inroads into Texas waters since the last major survey in the early 1970s. The most spectacular increase was in hydrilla, which in 1970 was not a problem; in fact it had not been identified in the state, and now infests about 23,000 acres. Eurasian watermilfoil increased from about 10,000 acres to more than 32,000 acres throughout the state. During 1984, also as part of the effort to complete these documents, we used video taping from aircraft to estimate the acreages of surfaced and submerged aquatic plants.

The Aquatic Plant Control Program has a secure future in the Galveston District workload. We anticipate treating hydrilla and watermilfoil this spring for the first time, provided the Supplement and Environmental Assessment are approved. Although the proposed program is not extensive, we are going to treat selected boat ramps, access lanes, and swimming areas. The biggest problem we are experiencing is finding treatments suitable for some Texas lakes which are seriously infested and used as potable water supplies.

During this past year, the State Program Director, Mr. Lou Guerra, retired. He had been with the program since the beginning and has been the primary influence in the Texas work for the past 16 years. Therefore, in summary, the Texas program will start this year with a new program Director, Mr. Bob Bounds, a new contract, new treatment areas, and new methods of treatment.

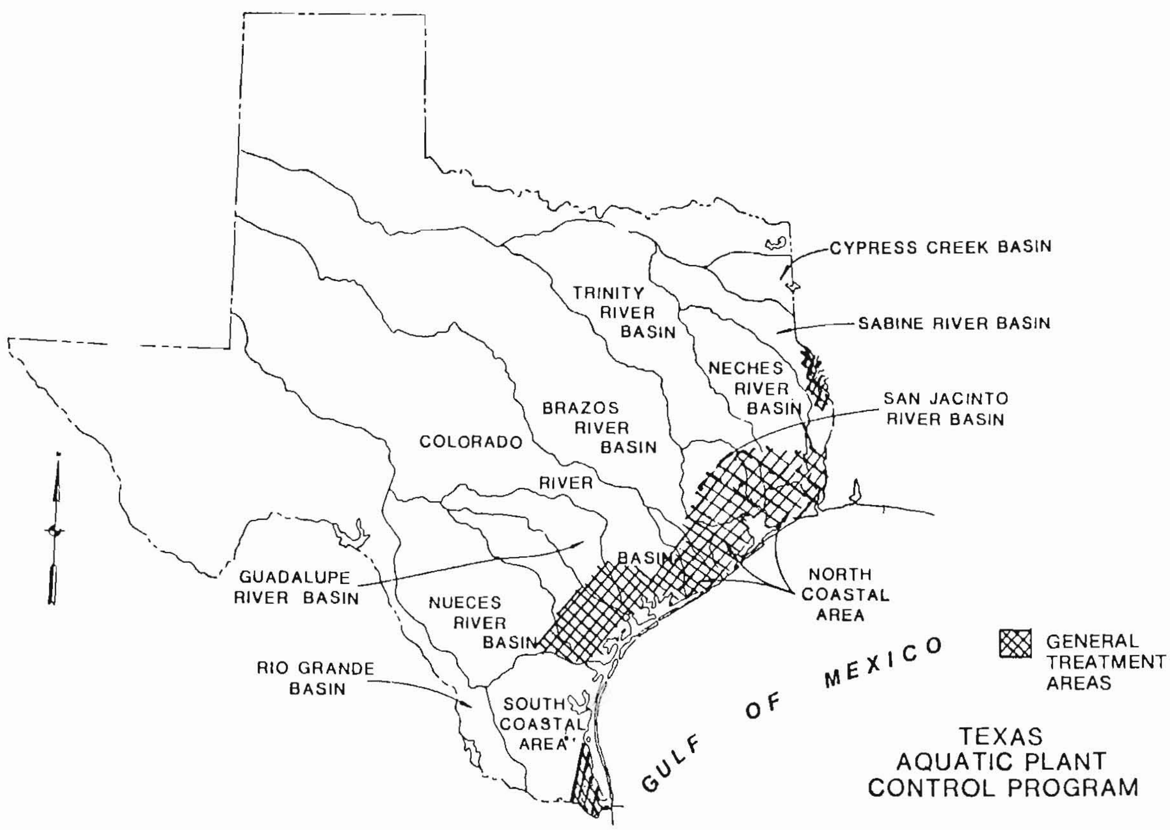

Figure 1. Texas Aquatic Plant Control Program, November 1984

USAE DIVISION/DISTRICT PRESENTATIONS AQUATIC PLANT PROBLEMS—OPERATIONS ACTIVITIES

Southwestern Division, Tulsa District

by
Loren M. Mason*

No work was performed in cooperation with the State of Oklahoma under the State Design Memorandum during calendar years 1983-1984. Contributing factors were declining State funds and lack of demonstrable problems related to aquatic plant infestations.

As to aquatic plant problems, the District has only one project which is experiencing infestation of Eurasian watermilfoil (*Myriophyllum spicatum*), Pat Mayse Lake, Texas. In the summer of 1983, a chemical treatment program was conducted within Pat Mayse Lake with Aquathol-K (granular) to control heavy infestations occurring in and around developed recreation shoreline areas. The total treated acreage was 93 acres out of an existing infestation of 600 acres. Results of an intensive monitoring study conducted in conjunction with the control program showed a 100-percent kill of the milfoil in the treated areas and no other detectable impacts occurred in the treated or the untreated areas. With the successful demonstration that milfoil could be controlled effectively and safely within Pat Mayse Lake, the District's attention was again directed to the development of information which could be used to forecast operational needs relative to aquatic plant growth and control.

1984 POSTTREATMENT MONITORING

The objective of the District's research program is to develop informational criteria which can be utilized to forecast aquatic plant growth and determine which combinations of treatment are the best for long-term control in Pat Mayse Lake, thereby allowing for more effective allocation of District and project resources. To accomplish this objective, the following questions needed answering:

a. Will milfoil continue to be a problem in the future as it has been in the past?

b. Could the problem worsen, and, if so, what might be the underlying causes?

c. Will other aquatic species replace milfoil and become an equal or greater problem?

d. What caused the decline of milfoil over the winter of 1983-1984?

e. Are there any detectable residual effects of the 1983 treatment program?

These and other related questions were investigated during 1984 under a contract with North Texas State University's Institute of Applied Science and Department

* US Army Engineer District, Tulsa; Tulsa, Oklahoma.

of Biological Sciences. The purpose of the contract was to (a) monitor changes of the plant community within the treated areas; (b) determine the overall abundance and distribution of aquatic plants during the 1984 growing season; (c) determine sediment types, quality, variances, distribution, and sedimentation rates; and (d) determine water quality variations and alterations which may occur with the increase of milfoil coverage and biomass.

STUDY SUMMARY

A series of sampling stations were established throughout the lake with water samples collected and analyzed for a variety of water quality parameters. The lake shoreline was surveyed on several occasions during the summer and aquatic vegetation was mapped (Figure 1). Samples were collected to ensure proper identification and to determine the quantity of biomass present. Sediment particle size and carbon content were determined from samples collected at several stations throughout the lake. Sediment traps were positioned within the reservoir to estimate sedimentation rate.

During the study, the overall water quality in Pat Mayse Lake was good and was typical of a meso-oligotrophic southwestern reservoir. No residual effects of the 1983 aquatic plant management program were detected.

Although Eurasian watermilfoil was still present in the reservoir during the summer of 1984, the density or amount of milfoil was greatly reduced from quantities observed during previous years. At least temporarily, *Najas* and *Chara* have replaced milfoil as the dominant aquatic plant species in Pat Mayse Lake. The factor or factors responsible for reduction of milfoil biomass have not been identified. If an unusually cold winter and high turbidity were responsible, then the milfoil should return rapidly. The biological variety of milfoil in Pat Mayse Lake

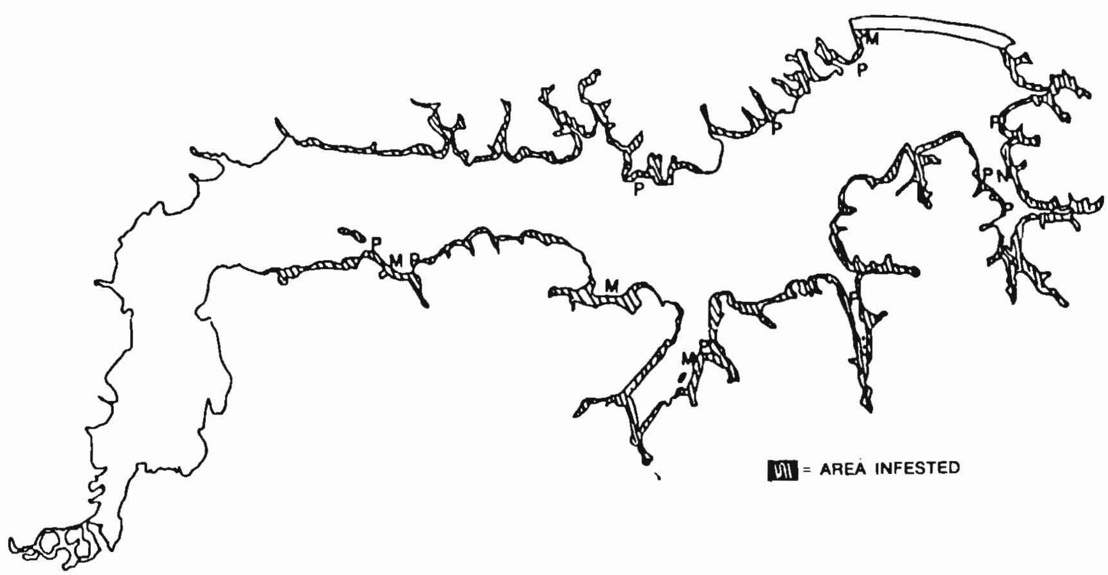

Figure 1. Areas of plant infestation at Pat Mayse Lake. Predominant species are *Chara braunii* and *Najas minor*. M stands for milfoil, P for *Potamogeton*, and N for *Nelumbo lutea*

may be different from the northern variety that survives and grows under ice in the winter. If a biological or viral agent was responsible for the density reduction, then the milfoil in the lake should remain at the current low density or even decline further.

The study of milfoil's compensation point indicated that the infestation level of 1983 was approximately 74 to 80 percent of the maximum level of infestation that would be expected for this species in Pat Mayse Lake.

The sediment nutrient content and organic carbon analyses indicated that Pat Mayse Lake should be a suitable habitat for a variety of submersed, rooted aquatic plants as well as milfoil. The sediments should not limit the growth of aquatic plants in the reservoir (Figure 2). Measured sediment accumulation rates in Pat Mayse Lake in the summer of 1984 ranged from 2.56 in./year (442 tons/acre/year) to 0.87 in./year (143 tons/acre/year) with a mean of 1.31 in./year (225 tons/acre/year). These measurements were three to four times the estimates used in the design document. During the study, turbidity in the reservoir was twice the values found during studies of the two previous years, possibly explaining the high rate of sedimentation observed.

Both *Chara* and milfoil will be observed during the 1985 growing season. The density of *Chara* approached 49 tons/ha (14.8 tons/acre) during the 1984 sampling period. The decline of milfoil biomass is believed to be only temporary.

CONCLUSIONS

As a result of the 1984 study, reasonable estimates can now be made concerning the following criteria for Pat Mayse Lake:

- Maximum depths that milfoil will grow in a worst case situation.
- Maximum milfoil biomass densitites per acre and correlated surface acreage infestation.

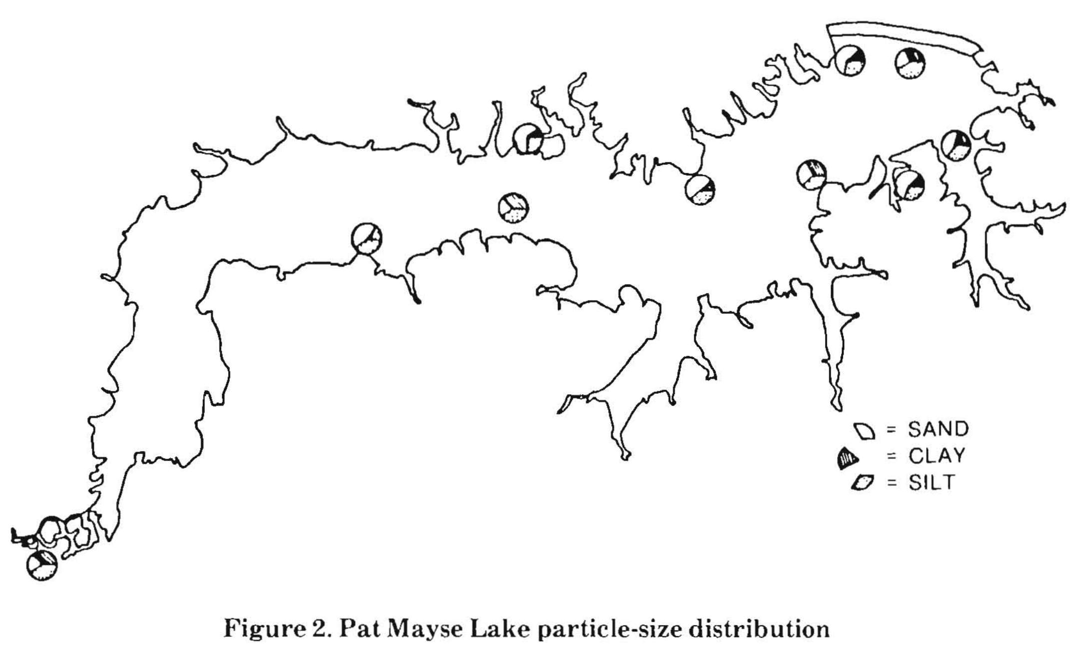

Figure 2. Pat Mayse Lake particle-size distribution

- Sediment quality composition and lake bottom configuration. As to future control programs and studies, the District has acquired and rehabilitated a small mechanical harvester that will be utilized in keeping the swimming beaches and boat ramps open to public use. Chemical control will be utilized only in extreme cases where infestations become excessive and/or project purposes, human health, or safety are threatened.

Objectives of the 1985 study will be focused upon the following:

- Source of high sedimentation rates and impacts upon the aquatic plants.
- Evaluation of mechanical harvesting, regarding depth of cut and rate of regrowth, number of cuttings required, as well as time of each cutting and overall growth rates of cut vegetation relative to undisturbed milfoil.
- Water quality monitoring of selected parameters and treatment locations.
- Impact of environmental variations, frequencies, and hydrology correlations as well as nutrient storage and usage by plants when highly stressed.

USAE DIVISION/DISTRICT PRESENTATIONS AQUATIC PLANT PROBLEMS—OPERATIONS ACTIVITIES

Pat Mayse Lake, Texas, Aquatic Plant Management Program: Continuing Studies

by
John H. Rodgers, Jr.,* and Phillip A. Clifford*

INTRODUCTION

During 1984, a study was conducted to monitor water quality in Pat Mayse Lake to assess the densities and distribution of aquatic plants in this reservoir and to determine sediment quality and accumulation rates. Water quality monitoring was conducted to ensure that there were no residual effects of the treatment program with endothall in 1983 (Rodgers, Reinert, and Hinman 1983). In order to determine the distribution and abundance of aquatic plants in Pat Mayse Lake, the shoreline was surveyed and plant densities were determined. Sediment samples were collected and analyzed to determine sediment quality. Sediment accumulation rates were estimated from sediment traps.

Pat Mayse Lake (PML), a 5,990-acre reservoir located in Lamar County, Texas, is operated by the Tulsa District as a water supply for the City of Paris, Texas, and for recreation, wildlife habitat, and flood control (Figure 1). Eurasian watermilfoil (*Myriophyllum spicatum*) was probably first introduced to PML in 1976 and became apparent in June 1978 when approximately 74 acres of the lake were occupied by the plant. By November of the same year, milfoil was estimated to cover 351 acres. The severe winters of 1979 and 1980 reduced the milfoil infestation to less than 200 acres at the end of summer 1980. By the fall of 1981, over 1,000 acres were infested, involving about 80 percent of the 67-mile shoreline. Following a period of heavy rains in the spring of 1982, the milfoil infestation covered about 474 acres by August (Figure 2) (Rodgers, Hinman, and Reinert 1982). This reduction in coverage was temporary and the plant increased its distribution during the 1983 growing season.

Heavy milfoil infestation contributes various problems for PML. Visitation to the lake decreased during the period of September 1980 through August 1981 to 42 percent of the previous year.** Damage to boat motors increased with increasing milfoil infestation. In the summer of 1980, a drowning occurred in a milfoil-infested area near Lamar Point.†

In the summer of 1983, endothall (Aquathol Granular Aquatic Herbicide-Pennwalt Corporation) was used to treat six areas within PML which were infested with milfoil. A seventh sampling station was established at the City of Paris, Texas,

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** Personal Communication, J.J. Harmon, District Engineer, Tulsa District, 25 January 1982.

† Personal Communication, Paul Gray, CE Project Manager, Pat Mayse Lake, 1982.

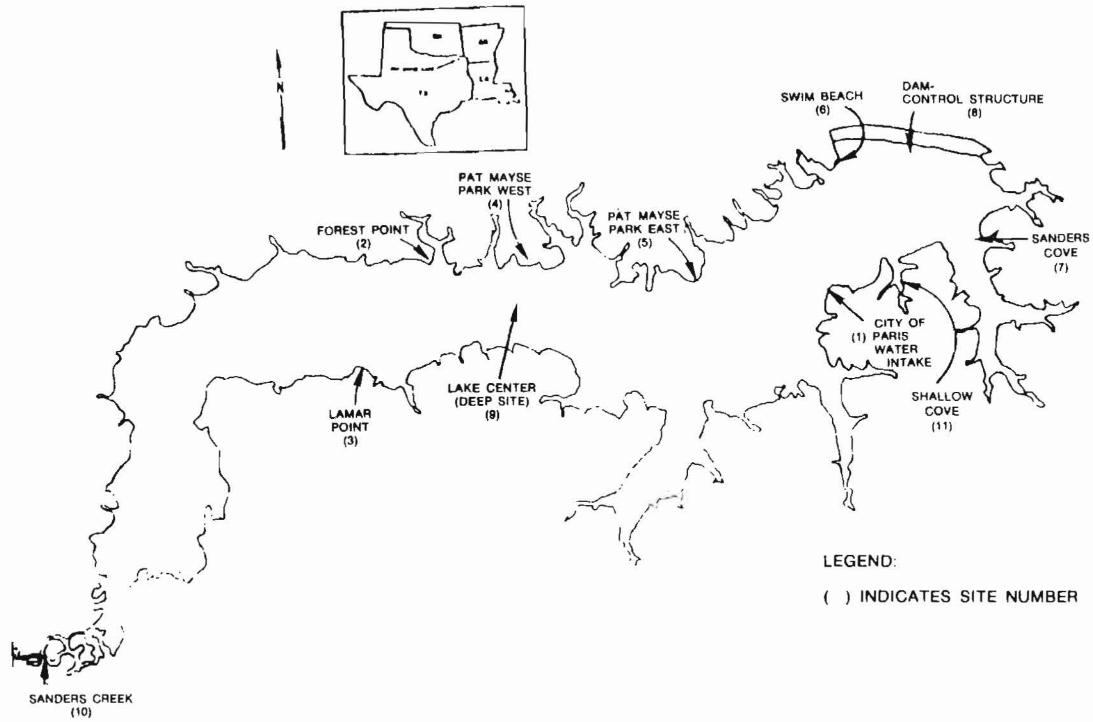

Figure 1. Pat Mayse Lake, location and sampling sites for 1984 program

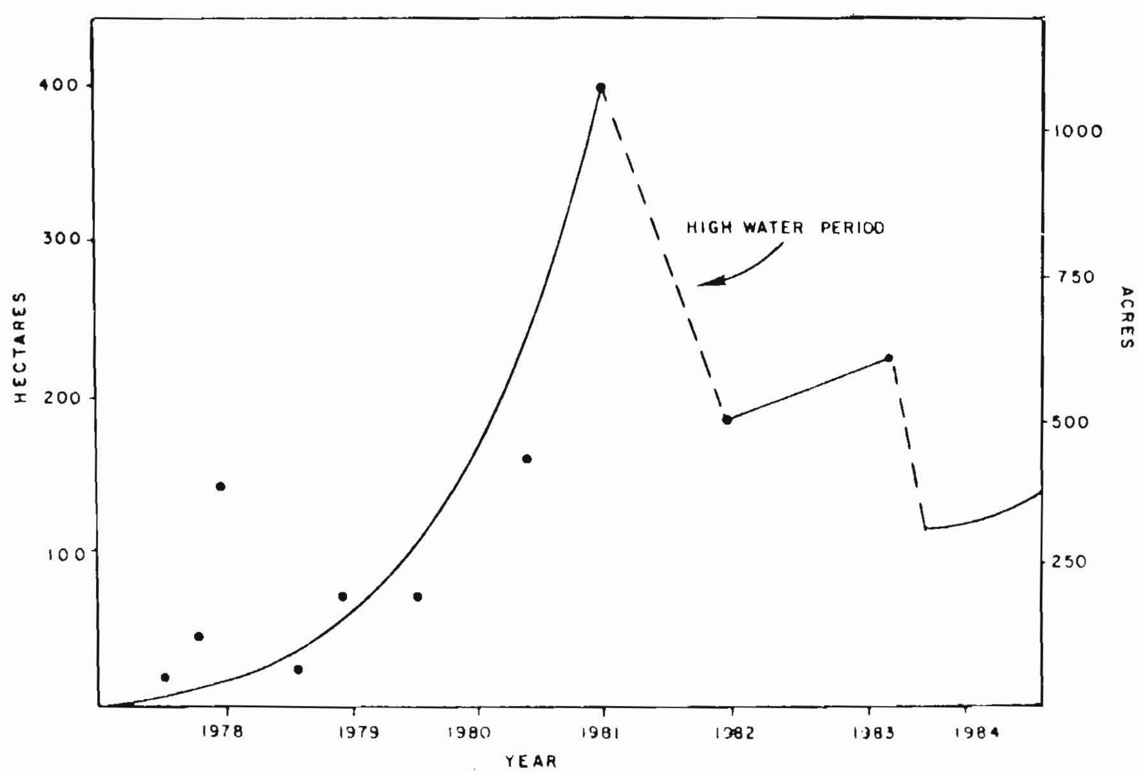

Figure 2. Surface area of Pat Mayse Lake infested with milfoil through time

water intake as a control site. Water, sediments, and fish were collected and analyzed for endothall by gas chromatography. In addition, water samples were analyzed for temperature, dissolved oxygen, pH, turbidity, and chlorophyll *a*. The herbicide was applied on June 14, 1983, by crews from the Texas Department of Parks and Wildlife. Sampling occurred on the following dates: June 7, 1983 (7 days prior to treatment); June 14, 1983 (treatment day); June 17, 21, 24, and July 14, 1983 (days 3, 7, 10, and 30 after treatment).

Endothall was not detected in the vicinity of the City of Paris water intake and the drinking water standard for endothall was not exceeded. No endothall was detected in the water, sediments, or fish samples that were collected 30 days after herbicide treatment. None of the water quality parameters were significantly altered due to herbicide use. No impacts on nontarget aquatic organisms were detected. Endothall rapidly dissipated within 72 hr from the treatment areas. Both dispersion (dilution) and biodegradation probably contributed significantly to rapid transfer and degradation of endothall.

The treatment program was apparently very successful and approximately 100 percent control of milfoil was obtained in the treatment areas with no adverse effects observed. The aquatic weed problem was alleviated and the lake was returned to normal usage capacity. There was an apparent increase in visitation to Pat Mayse Lake and usage of the treated sites. Many lake users were strongly supportive of the aquatic plant control program. People with vested interest in the lake (Health Department, City of Paris, local press, etc.) were very cooperative. Local boat dealers and bait shops reported that their revenues had been affected and they attributed the increases to "ripple" or "spin-off" from the treatment program. The program was responsive to an immediate and critical need. However, regrowth of milfoil and milfoil growth around the Paris water intake may have to be addressed in the future.

The aquatic plant management program of 1984 had multiple objectives. In addition to plant distribution and biomass studies, the compensation depth for milfoil was determined using the light-dark bottle method to project future infestation potential for this species. From a management perspective, some estimate of the anticipated magnitude of milfoil infestation in PML would be useful. Sediments were characterized as to nutrient concentrations. The sediment characteristics may be correlated with macrophyte densities and distributions (Barko 1982) and we wanted to determine whether sediment quality would limit growth of milfoil in PML.

MATERIALS AND METHODS

The abundance of aquatic plants (biomass) was assessed by removing all above-sediment plants from 1/8-m^2 replicate plots using a sharp-edged rake. These samples were taken at intervals during the study period at several sampling sites throughout the lake. The plants were weighed to give biomass/unit area estimates. Since the plants grow only to certain depths in the lake, a depth contour map was used to estimate total area covered. From this, total plant biomass in the lake was estimated.

Plant distribution was visually estimated by shoreline surveys to determine the location of plant beds in the lake. Monthly boat surveys of the shoreline were conducted to determine species present in each area and to determine the relative area covered by each species.

The water quality monitoring effort of the 1984 program had two objectives. First, as a continuation of the previous year's monitoring, the sampling stations of the 1983 treatment with Aquathol K were closely monitored to ensure that the previous assessment of no serious effect is valid in the long term as it was shown to be in the short term. Secondly, since different species of plants were dominant this season (1984), monitoring was conducted to determine concurrent differences in water quality.

In both the previous and present water quality monitoring programs, the parameters, ammonia, nitrate, total, and orthophosphates, were assessed through time using *Standard Methods* (American Public Health Association 1980). Values from this study were compared to the previous year's values to follow the quantities of nutrients present through time. Organic compounds from species replacing milfoil or nutrient release from decaying vegetation might cause taste and odor problems due to algal blooms. These organic compounds were analyzed as nitrogen, phosphorus, and carbon. Simultaneously, chlorophyll ***a*** was assessed as an indicator of such blooms, should they exist.

Turbidity is an indirect measure of the amount of suspended solids in the water. Increased solids can cause increased treatment costs. Also, high turbidity can be an indicator of increased sedimentation rates, influencing the expected or useful life of a reservoir. Dissolved oxygen, conductivity, and temperature were also routinely monitored.

Sedimentation rates were determined using sediment traps designed by Hargrove and Burns to estimate net sediment accumulation rates. Each trap was retrieved and the depth, volume, and dry weight of sediment were measured. Sediment samples were also collected using an Eckman dredge. Analyses were conducted according to procedures in Black (1965).

RESULTS AND DISCUSSION

Aquatic plant abundance and distribution in PML

Based on observations from the previous year, milfoil was not present in expected quantities in PML during this growing season (Figure 2). Recent observations indicate that the decline in milfoil density was only temporary and we do not think a biological factor was involved. Rather, the unusually cold winter and high turbidity may have contributed to the reduction in milfoil biomass (Figures 3 and 4). Milfoil biomass declined from a maximum of 344 g dry weight/m^2 (1.4 tons/acre) in 1983, to a maximum of 3.83 g dry weight/m^2 (0.0016 tons/acre) in 1984.

Shoreline mapping revealed that milfoil has been at least temporarily replaced by *Chara braunii*, *Najas minor*, and *Nitella hyalina*. These species were relatively evenly mixed and distributed throughout the shallow areas of PML. The mixture

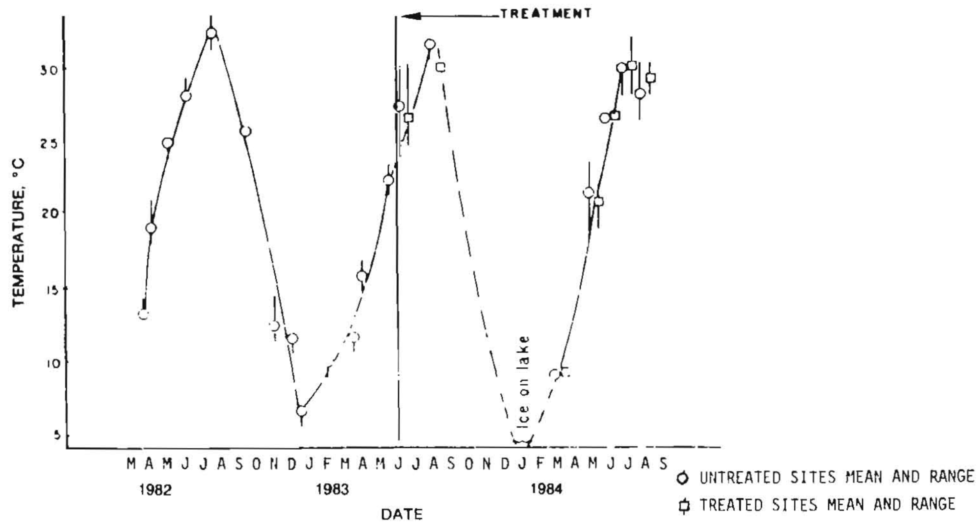

Figure 3. Water temperature in Pat Mayse Lake at treated and untreated sites. All values are at 1-m depth

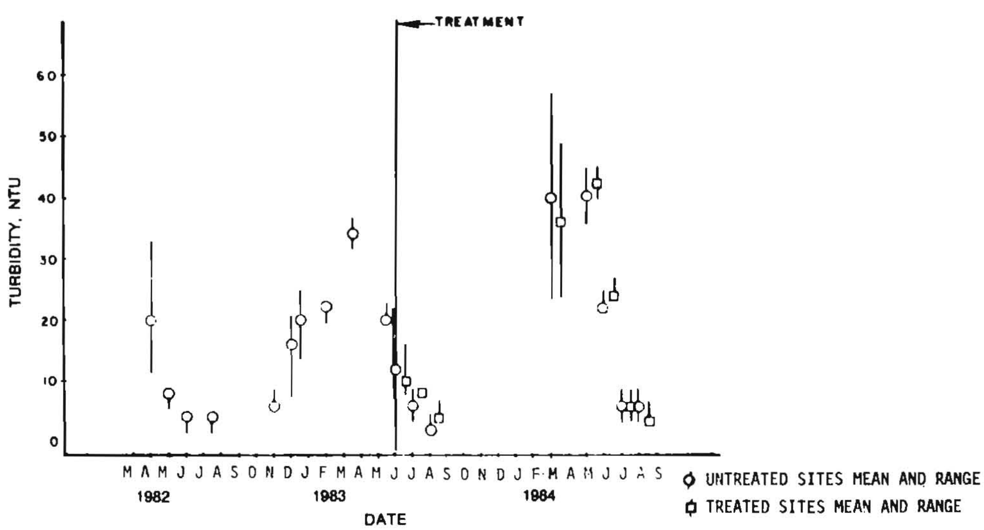

Figure 4. Turbidity at treated and untreated sites

was found in average quantities of about 13.3 tons/ha (5.38 tons/acre) with biomass ranging from almost zero to the maximum observed in Sanders Cove of 49.0 tons/ha (14.8 tons/acre). Estimates of surface coverage approached 220 ha (544 acres) or 9.08 percent of the lake surface (Figure 5). Other genera present included *Potamogeton* (two species), *Sagitaria* (two species), milfoil, *Ludwigia*, and *Elecoharis*. With the possible exceptions of *Chara* and *Najas*, none of the species appear to be present in sufficient quantities to pose any management problems in the lake.

Milfoil compensation, depth, and habitat potential

The compensation depth of milfoil was determined using the light-dark bottle technique. Data were collected during the peak photoperiod of the day (0900-1500 hr) and, therefore, do not directly reflect maximum growth potential since they do not consider dark periods when respiration is depleting photosynthate. These reported values probably more accurately represent a worst case estimate. Based on results of replicated experiments (Figure 6), the compensation depth for milfoil in PML is estimated to be about 3 m. The average encompassed by this depth in PML is about 330 acres or about 10 percent of the lake surface. This value is similar to maximum previous milfoil infestation levels except during the low water or drought year, 1981. It would appear that management plans can be based on an average infestation of 350 to 500 acres with a maximum of about 1,000 acres of rooted milfoil in PML. However, special attention may have to be given to rafts of milfoil that may grow unattached and drift with the wind.

Water quality

None of the water quality parameters were significantly different when values from treated stations of the 1983 program were compared with values from untreated stations. As previously mentioned, the most notable water quality parameters were turbidity and temperature (Figures 3 and 4). Turbidity in 1984 was almost twice the values observed in 1983. The cold winter temperature produced ice cover on this reservoir for the first time in the lake's history.

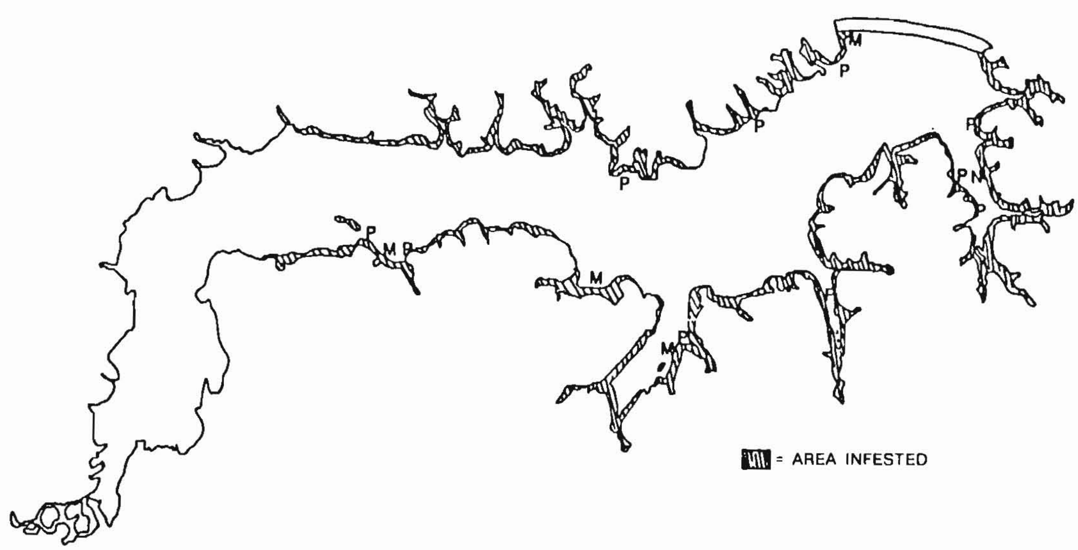

Figure 5. Aquatic plant distribution in Pat Mayse Lake, 1984

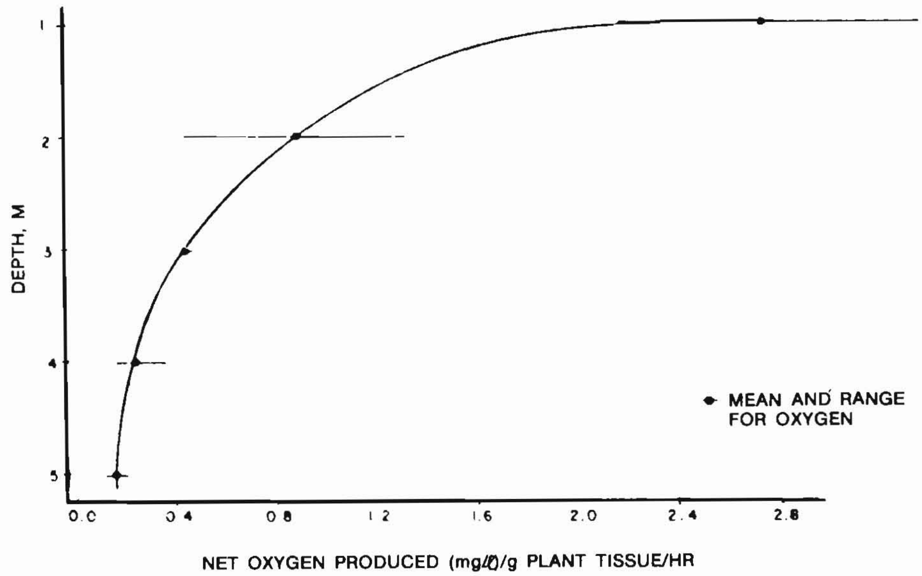

Figure 6. Milfoil compensation point (dissolved oxygen - light-dark bottle) in Pat Mayse Lake

Chlorophyll *a* varied inversely with nitrogen and phosphorus. Chlorophyll *a* in the water increased sixfold in the spring relative to winter values and nitrogen and phosphorus decreased fourfold and fivefold, respectively. Currently, PML appears to be nutrient limited with respect to algal blooms.

Sediment character and accumulation rate

Sediment particle size (sand, silt, and clay) was similar throughout the lake with no one fraction dominating (Figure 7). No differences were observed between areas with milfoil and areas with no plants.

The nutrient content of the sediments may influence plant distribution. The deep water sites near the old river bed of the reservoir had the greatest nitrogen and phosphorus concentrations (Figure 8). These sites have no aquatic weed growth potential as does Sanders Creek which currently has no aquatic weeds. Barko (1982) indicated that high organic carbon (10 to 20 percent) in sediments adversely affects macrophyte growth rates with as much as 30 to 70 percent reduction in biomass/shoot. None of the sites in PML had sufficient volatile matter to inhibit milfoil growth.

Sediment accumulation rates were measured using sediment traps. Data collected in 1984 indicated maximal sedimentation rates of 2.56 in./year (442 tons/acre/year) and minimum rates of 0.87 in./year (143 tons/acre/year). The mean rate was 1.31 in./year (225 tons/acre/year). These data were collected during 1 year and should be supplemented with further information for verification since they indicate more sediment should have accumulated than actually has. The turbidity values and sedimentation rates indicate that 1984 was an exceptional year. Knowledge of sediment accumulation through time may be a beneficial tool for reservoir management and future design criteria.

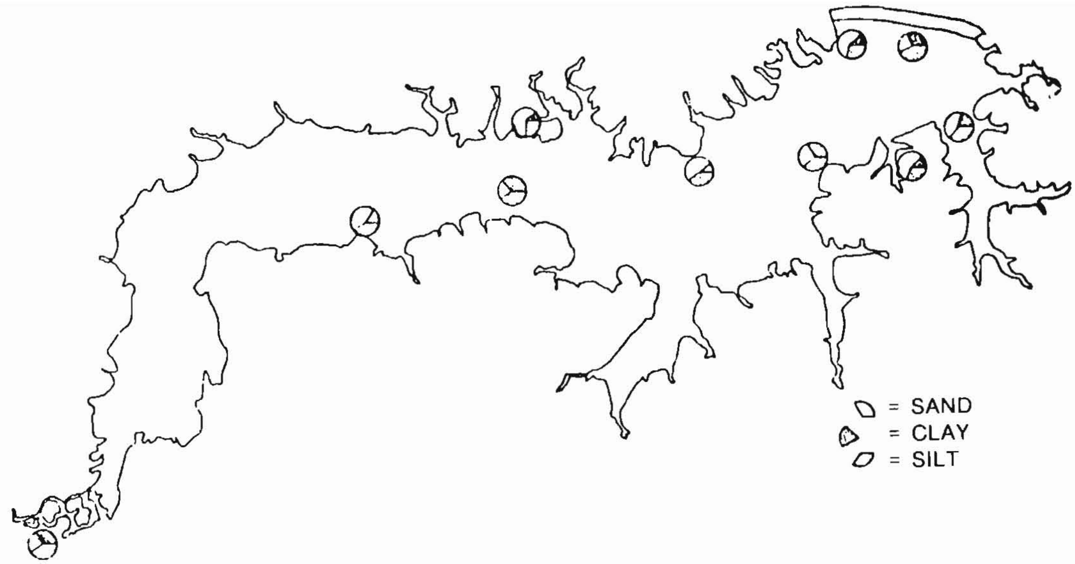

Figure 7. Sediment particle-size distribution at sites in Pat Mayse Lake

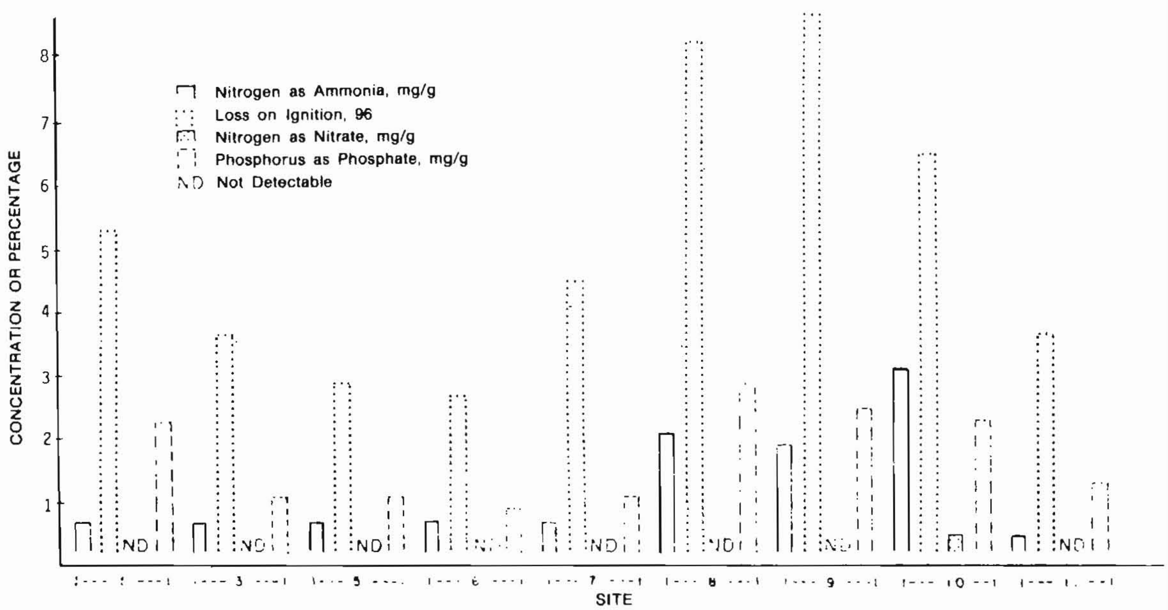

Figure 8. Sediment characteristics at sites in Pat Mayse Lake. Site 1 is City of Paris water intake; site 3 is Lamar Point; site 5 is Pat Mayse Park east; site 6 is swim beach by dam; site 7 is Sanders Cove; site 8 is dam control structure; site 9 is lake center; site 10 is Sanders Creek; and site 11 is shallow cove near water intake

CONCLUSIONS

Results from the 1984 study support the following conclusions:

a. Water quality parameters were not affected by the 1983 treatment programs. The overall water quality was good and typical of a meso-oligotrophic southwestern US reservoir.

b. Milfoil biomass in PML declined from a maximum of 344 g dry weight/m^2 in 1983 to a maximum of 3.83 g dry weight/m^2 in 1984.

c. The milfoil decline has been tentatively attributed to unusually cold weather and high turbidity.

d. Milfoil has been replaced at least temporarily by *Chara*, *Najas*, and *Nitella*.

e. Using milfoil's compensation point for estimations, the infestation level of 1983 was approximately 75 to 80 percent of the expected level for this species in PML.

f. Sediment nutrient content and organic matter analyses indicated that PML should be a suitable habitat for a variety of submersed rooted aquatic plants as well as milfoil. Sediments should not limit the growth of aquatic plants in PML.

g. Measured sediment accumulation rates in PML in the summer of 1984 ranged from 0.87 in./year (143 tons/acre/year) to 2.56 in./year (442 tons/acre/year) with a mean of 1.31 in./year (225 tons/acre/year). These measurements are some three to four times the estimates used in the reservoir design document. During this study, turbidity in PML was twice the values found during studies of the two previous years, possibly explaining the high rate of sedimentation observed.

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ECOLOGY OF AQUATIC PLANT SPECIES

Effects of Sediment Composition: Growth Regulating Mechanisms

by
John W. Barko* and R. Michael Smart*

BACKGROUND

Sediments provide an important source of nutrients, principally nitrogen, phosphorus, and micronutrients (Barko and Smart 1981; Smart and Barko 1985) that are relatively less available to submersed macrophytes in the overlying water of most aquatic systems. The physical and chemical composition of sediments markedly affects macrophyte growth rates, and may have an important bearing on species succession and distribution in aquatic systems (Barko 1982, 1983; Barko and Smart 1983). In the investigation of Barko and Smart (1984) including 40 different sediments from 17 geographically widespread North American lakes, the growth of both *Hydrilla verticillata* (L. f.) Royle and *Myriophyllum spicatum* L. was demonstrated to decline exponentially with increasing sediment organic matter content, and was also relatively poor on very sandy sediments. That study provided useful indices for macrophyte growth in relation to sediment composition, but did not experimentally address mechanisms of growth regulation.

The purpose of this article is to examine results of recent experiments designed to elucidate possible mechanisms underlying the influence of sediment composition on the growth of submersed macrophytes. These experiments represent extensions of previous work (Barko and Smart 1983, 1984), with current methodology fundamentally the same.

RESULTS AND DISCUSSION

In the earlier investigation of Barko and Smart (1984) macrophyte growth as total dry weight biomass varied approximately tenfold and twentyfold in *Hydrilla* and *Myriophyllum*, respectively, but nearly all of this variability occurred on sediments with an organic matter content of less than 20 percent (Figure 1). Within this range sediment density declined sharply with increasing organic matter content (Figure 2). With increasing organic matter beyond 20 percent, both macrophyte growth (Figure 1) and sediment density (Figure 2) remained essentially unchanged.

On sediments excluding "sands" (i.e., sediments with >75 percent sand by weight) the growth of both species increased with increasing density of sediments over the range of 0 to 20 percent organic matter, and over the same range decreased with increasing organic matter content (Figure 3). Whereas sediment density and

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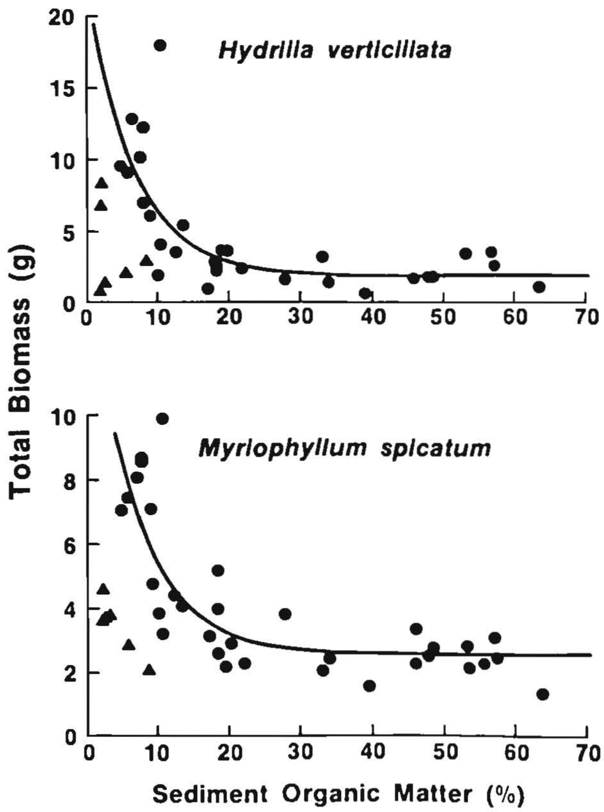

Figure 1. Relationship between growth, as total dry weight biomass (n = 4), of *Hydrilla* and *Myriophyllum* and sediment organic matter content (n = 2). Triangles designate sediments containing > 75 percent sand, which were excluded from curve fitting

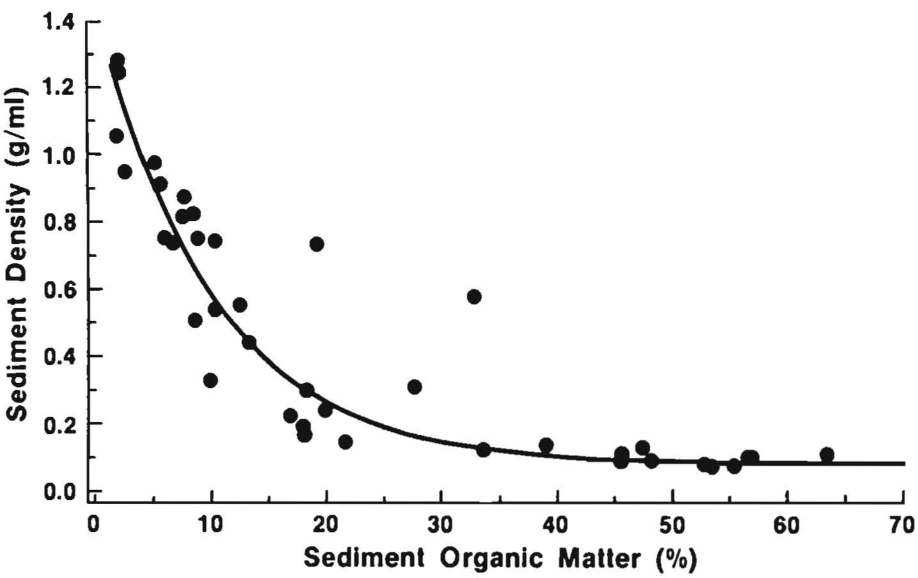

Figure 2. Relationship between sediment density (n = 2) and sediment organic matter content (n = 2) for 40 sediments from North American lakes

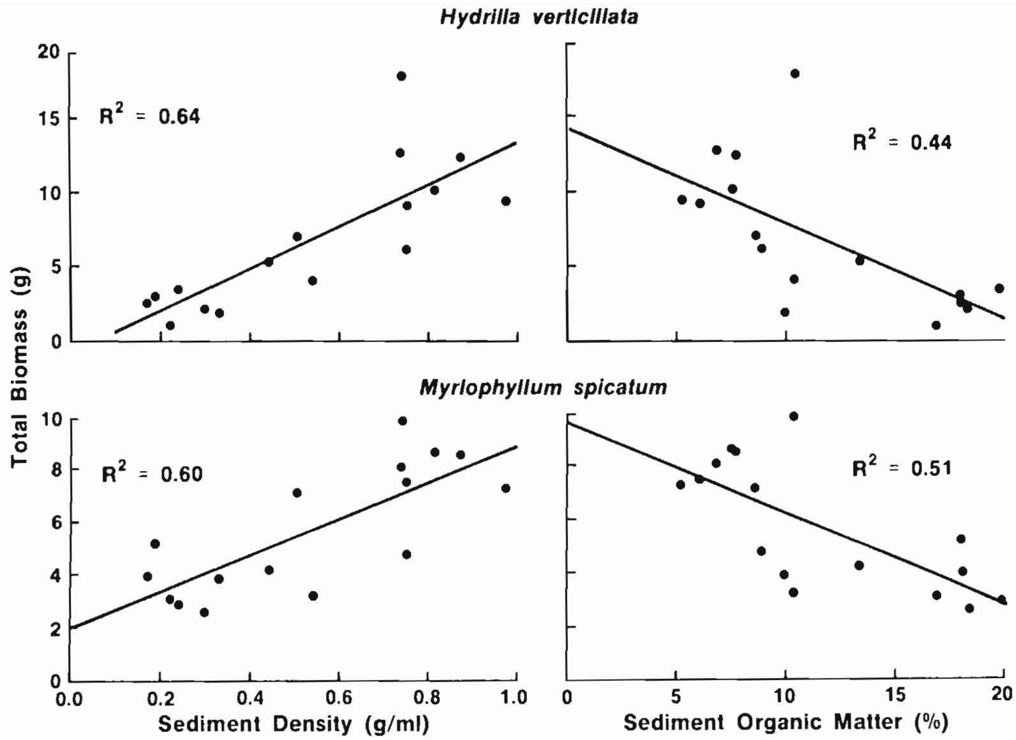

Figure 3. Relationships between growth, as total dry weight biomass (n=4), of *Hydrilla* and *Myriophyllum* and both sediment density (n=2) and sediment organic matter content (n=2) on sediments containing <20 percent organic matter excluding "sands" (i.e., those with a sand fraction >75 percent)

organic matter content were highly correlated with one another ($R^2=0.81$), macrophyte growth was somewhat better related statistically to sediment density than to organic matter content (Figure 3).

Macrophyte growth on "sands" was diminished at high sediment densities (ca. 0.9 to 1.3 g/ml), while growth on highly organic sediments was diminished at low sediment densities (ca. <0.25 g/ml) (Figures 1 and 2). These observations suggest commonality between the effects of sand and organic matter on macrophyte growth, mediated discontinuously by sediment density.

In order to more directly examine the influences of sand (high density) and organic matter (low density) on macrophyte growth, a separate experiment involving sediment manipulations was conducted. Manipulations included additions of an inorganic, nonsandy sediment from Lake Washington, Washington State, in increments of 20 and 40 percent by volume, to a washed builders sand (97 percent sand) and to a composite organic sediment (53 percent organic matter). At the maximum level of addition, the organic matter content was reduced to 25 percent in the organic sediment, and the sand fraction to 75 percent in the sand. With additions of Washington sediment, the growth of *Hydrilla* increased overall dramatically, threefold on the organic sediment and sevenfold on the sand (Figure 4). These increases in growth accompanied an increase in sediment density from 0.10 to 0.23 g/ml in the former and a decrease in density from 1.43 to 1.23 in the latter. Notably, the growth of *Hydrilla* was stimulated in this experiment even though the organic

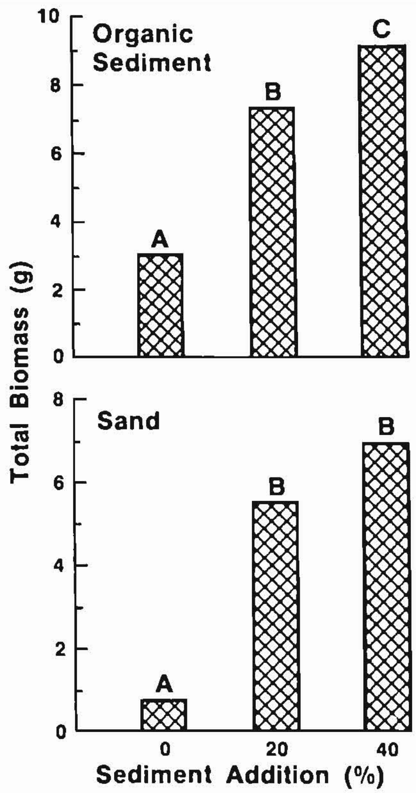

Figure 4. Effect of additions of fine-textured inorganic sediment to both an organic sediment and to a sand on the growth, as total dry weight biomass, of *Hydrilla*. Refer to text for experimental details. Vertical bars represent means (n = 4). Values sharing the same letter within sediments do not differ significantly from one another (Duncan's Multiple Range Test)

sediment remained "organic" and the sand remained a "sand."

In the sediment addition experiment, changes in sediment mineralogy and overall nutrient content (unmeasured) were undoubtedly coupled with changes in measured sediment variables (density, texture, and organic matter). Accordingly, results were potentially influenced by nutritional factors. This possibility, and in addition an inability due to design to separate the effects on *Hydrilla* growth of sediment organic matter content and sediment density, prompted the conduct of an additional experiment designed to evaluate the effects of sediment density more directly.

The composite organic sediment (without sediment additions) was differentially centrifuged to remove interstitial water, thus providing four statistically discrete fractions with respect to sediment density. Centrifugation with concomitant increases in sediment density resulted in a significant increase overall in the growth of *Hydrilla*; however, this effect was somewhat less than that achieved over a similar range in density by additions of fine-textured inorganic sediment (Figure 5). Increased growth on centrifuged sediments was presumably independent of changes in sediment mineralogy and mass nutrient content (nutrient mass per sediment mass), ignoring minor losses in interstitial water removed since the sediment matrix remained unchanged. Nutrient density (nutrient mass per sediment volume) did change however, increasing directly with increasing sediment density. Sediment organic content (measured) was unaffected by centrifugation. From this experiment it is apparent that sediment density, and not organic matter content per se, is important in regulating *Hydrilla* growth.

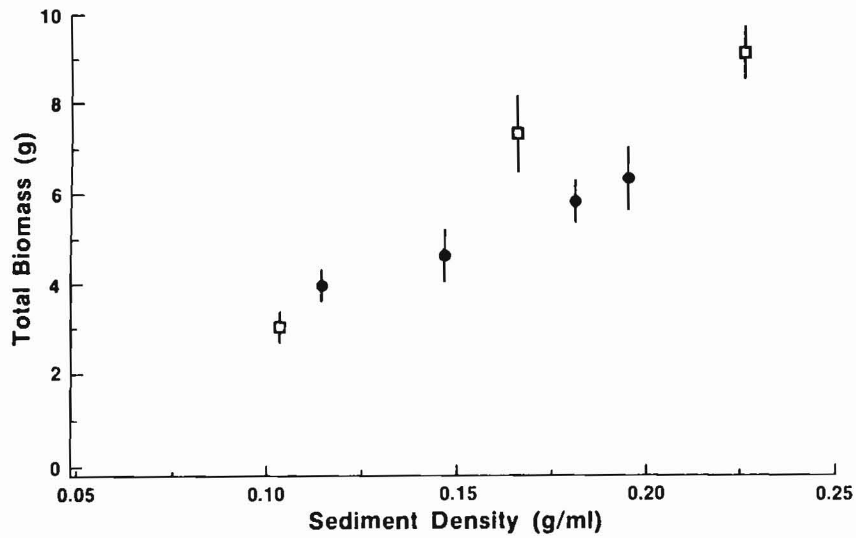

Figure 5. Relationship between growth, as total dry weight biomass, of *Hydrilla* and the density of amended organic sediment. Amendments included additions of fine-textured inorganic sediment (open squares) and centrifugation (closed circles). Refer to text for experimental details. Values are means (n = 4) ± one standard deviation

Density, in connection with texture, can physically mediate root development (Sculthorpe 1967); however, there were no indications in this investigation that root development was impaired where macrophyte growth was poorest on either low density organic sediments or on high density sands. Indeed, the proportionately greater amount of root mass to shoot mass of both species on these unfavorable sediments compared to others (Barko and Smart 1984) suggests the contrary. The possibility remains, however, that sediment density mediated growth through its effect on sediment nutrient densities and macrophyte nutrition. Diminished macrophyte growth on high density sands, due to their inherently infertile nature, was probably caused by a general nutrient inadequacy. Accordingly, the increased growth of *Hydrilla* on sand, amended by additions of inorganic sediment (Figure 4), likely reflects an effect of fertilization. Increased growth on the organic sediment, amended identically as above, suggests a possibly similar effect.

In order to directly examine the possibility that nutrient availability limited macrophyte growth on organic sediments, a series of experiments involving additions of phosphorus and iron to organic sediments were conducted. These elements were selected for addition because of their possibly reduced availability in organic sediments (Sikora and Keeney 1983). Organic sediments used here were obtained from separate collections at original sites in Lakes Buckhorn, Chemung, Chenango, and Seminole (cf. Barko and Smart 1984). Phosphorus and iron were added separately and in combination as $CaHPO_4$ at 0.1 g/ℓ wet sediment and as Fe_2O_3 at 5.0 g/ℓ wet sediment, respectively. Added P approximated 10 times that, which as the only source, would be required to sustain 10 g of *Hydrilla* growth. Added Fe was approximately equivalent to 20 percent of that in Lake Washington sediment. In these experiments neither sediment organic matter content nor sediment density was affected by nutrient additions.

Table 1
Effects of Nutrient Additions to Organic Sediments on the Growth of *Hydrilla*

Organic Sediment	*Treatment Addition*	*Growth Increase**
1	P	No
2	P	No
3	P	No
4	P	Yes
5	Fe	No
6	Fe	No
5	Fe + P	Yes
6	Fe + P	Yes

* Yes indicates significantly greater growth (total biomass) at $P < 0.05$.

Hydrilla did not respond to the addition of Fe alone, and responded to the addition of P alone on only one of four sediments so treated (Table 1). The growth of this species increased significantly, however, on both sediments amended by P in combination with Fe (Table 1). In another separate experiment the effect on *Hydrilla* growth of P and Fe additions to an organic sediment (as above) was examined in a nitrogen-free solution. In that experiment, the combined addition of P and Fe had no effect on *Hydrilla* growth (data not presented), strongly suggesting a general nutrient inadequacy, i.e. multiple nutrient limitation on low density (organic) sediments.

The positive response of *Hydrilla* to "fertilization" by addition of fine-textured inorganic sediment to both sand and organic sediment (Figure 4), in combination with results achieved by specific nutrient additions above, indicates that the regulating effect of sediment sand and/or organic matter content on the growth of submersed aquatic vegetation, while mediated by sediment density, directly involves nutrition. In this regard, the availability in sediments of N, P, Fe, and possibly other micronutrients is of critical importance because of the importance of sediment rather than the open water as the predominant source of these elements in most aquatic systems (cf. Smart and Barko 1985).

CONCLUSIONS AND RELATED IMPLICATIONS

The growth of submersed macrophytes is relatively poor on both highly organic sediments and sands compared to that on fine-textured inorganic sediments. Poor growth on sands is related to high sediment density, while poor growth on organic sediments is related to low sediment density. Mechanisms of growth regulation on sand and organic sediments are similar, both involving nutrition. High concentrations of organic matter in sediments exert a negative influence on the growth of submersed macrophytes by reducing sediment density and the associated availability of essential nutrients (notably N, P, and Fe).

From its greater range in growth, *Hydrilla* appears to be more sensitive than *Myriophyllum* to sediment composition. In nature, individual differences in the ability of aquatic macrophyte species to cope with sediment infertility or other factors associated with high sand and/or high organic matter fractions may be important in determining the species composition of aquatic macrophyte communities. In this connection, it is notable that submersed aquatic vegetation is replaced in lakes by emergent vegetation as sediment organic matter accumulates (Walker 1972; Wetzel 1979; Carpenter 1981).

We are aware of one documented occurrence of a decline in rooted submersed aquatic vegetation following a major loading of organic matter due to watershed disturbance (Kight 1980; cf. Barko 1982). Conversely, the growth of submersed aquatic vegetation on organic sediments may be stimulated by additions of inorganic sediment (Figure 4). Sediment composition may be modified by aquatic plants themselves directly, by sediment nutrient uptake (e.g. Barko and Smart 1980) and contributions of their own remains to the sediment (Walker 1972; Wetzel 1979; Carpenter 1981), and, indirectly, by collecting externally loaded materials (Mickle and Wetzel 1978a, b, 1979; Patterson and Brown 1979). In view of these findings, we suggest that watershed disturbances, direct mechanical disturbances of bottom sediments, or autogenic processes affecting the inorganic/organic composition of sediments (and thus, sediment density and nutrition) may contribute fundamentally to vegetational changes in aquatic systems.

ACKNOWLEDGEMENTS

Co-workers D. G. McFarland, M. S. Hennington, and R. H. Warren provided technical assistance. Support for this research effort was provided by the US Army Corps of Engineers Aquatic Plant Control Research Program (APCRP). This article was written using some of the data from a much more comprehensive source scheduled to be published as an APCRP technical report in 1985.

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Effects of Water Chemistry on Aquatic Plant Species: Photosynthesis and Growth of *Myriophyllum spicatum* L.

by

R. Michael Smart* and John W. Barko*

It has long been realized that the chemical composition of water is of primary importance in affecting the distribution of aquatic plants (Moyle 1945; Hutchinson 1975). Various authors have attempted to relate the species distribution of submersed macrophytes to general water chemistry conditions, using parameters such as hardness, alkalinity, conductivity, pH, total dissolved solids, or salinity (see reviews by Moyle 1945; Hutchinson 1975). In this regard, *Myriophyllum spicatum* L. has been characterized as generally occurring in waters that are hard (particularly with respect to Ca), moderate to high in alkalinity, neutral to high in pH, high in electrical conductivity, and eutrophic (Moyle 1945; Spence 1967; Hutchinson 1970; Seddon 1972; and Kadono 1982). Many exceptions to these general patterns of occurrence have been noted (Hutchinson 1970; Giesy and Tessier 1979; Kadono 1982), however, and it remains difficult to explain observed distributions on the basis of general water chemistry conditions.

As noted by Hutchinson (1957, 1970), many water chemistry parameters, particularly pH, Ca concentration, alkalinity, and total electrolyte content, are correlated. Although these parameters may act independently in affecting plant distribution, it is difficult to discern their effects from distribution data (Hutchinson 1970; Seddon 1972; Kadono 1982). Another difficulty is that sites differing in water chemistry are likely to differ in other environmental parameters (notably sediment composition) as well (Pearsall 1920; Moyle 1945). For these reasons it is of interest to determine the effects on growth of independently varying specific water chemistry parameters under otherwise uniform environmental conditions.

While considerable attention has been given the macronutrients N, P, and K (see review by Smart and Barko 1984a), relatively little attention has been devoted to the effects on macrophyte growth of other water chemistry parameters such as alkalinity, dissolved inorganic carbon (DIC), and major cations. Huebert and Gorham (1983) demonstrated that *Potamogeton pectinatus* L. required Ca, Mg, and HCO_3^- in solution to achieve normal growth. Barko (1983) indicated that, at low levels of Ca and DIC in solution, growth of *M. spicatum* was depressed.

While little is known of the effects of DIC, alkalinity, and major cations on the growth of submersed macrophytes, there is considerable information on the effects of these on photosynthesis. Among the many published reports on photosynthesis in

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relation to water chemistry, the most notable concerning *M. spicatum* are Steemann Nielsen (1947); Adams, Guilizonni, and Adams (1978); and Titus and Stone (1982). These authors indicate that *M. spicatum* can use HCO_3^- in photosynthesis and that such use follows Michaelis-Menten kinetics. Steemann Nielsen (1947) demonstrated the promotive effects of cations, particularly Ca. The stimulatory effect of Ca on photosynthesis was also noted by Stanley (1970).

The objective of this investigation was to determine the effects of independently varying major cation concentrations and DIC levels on the growth and photosynthesis of *M. spicatum*. In this presentation we report on the effects of DIC on growth and photosynthesis. We also examine the relationship between growth and photosynthesis to determine the utility of photosynthetic DIC uptake as an indicator of the growth potential of a particular water composition.

METHODS AND MATERIALS

Growth and photosynthesis experiments were conducted using a 3- by 3-factorial design with three levels of DIC — 3.5, 10.5, and 21.0 mg DIC · ℓ^{-1}. At each DIC level we provided three levels of major cations (Na, K, Ca, and Mg) in proportion to their occurrence in bicarbonate lake waters (Hutchinson 1957; Smart and Barko 1984b). Concentrations of both DIC and cations in the high level solutions were 6 times those in the low level solutions, with the intermediate (mid) level solutions being 3 times the concentration of the low level solutions. Solutions were formulated by additions of reagent-grade chemicals to deionized (reverse osmosis) water. Gaseous CO_2 was administered to solution prior to adding required amounts of $CaCO_3$ to achieve solubility (Smart and Barko 1984a). Concentrations of Ca and DIC in the high level solution were near the upper limit for solutions of this type in equilibrium with atmospheric pCO_2.

Two growth experiments were conducted under conditions differing in the relative magnitudes of atmospheric gas exchange (aeration) and solution volume in an attempt to evaluate the relative importance of the atmosphere and solution as sources of inorganic carbon. The first experiment was conducted in lucite columns 150 cm tall by 15 cm OD (outside diameter) in an environmental chamber. The second experiment was conducted in 150- by 90- by 90-cm-deep, white fiberglass tanks in the greenhouse facility of the Environmental Laboratory (WES). Additional experimental details are available elsewhere (Smart and Barko 1985).

Photosynthetic DIC uptake was measured in each of the experimental solutions using an apparatus that also allowed for measuring uptake of amtospheric CO_2. The apparatus, associated methodology, and results of atmospheric CO_2 uptake studies will be described elsewhere (Smart and Barko 1985).

RESULTS AND DISCUSSION

Results of the first experiment, described earlier in detail (Smart and Barko 1984b), are briefly summarized here. Ash-free total biomass accrual was significantly affected by cation level only under low DIC conditions. Similarly the effect of increasing DIC was observed only under low DIC conditions. Growth of *M.*

spicatum was increased by increasing DIC from 3.5 to 10.5 mg·ℓ^{-1} but was unaffected by further increase to 21.0 mg·ℓ^{-1}. In the second experiment (Figure 1) growth increased with an increase in DIC between 3.5 and 10.5 mg DIC·ℓ^{-1} but did not increase between 10.5 and 21.0 mg DIC·ℓ^{-1}.

Photosynthetic uptake of DIC from solution fit Michaelis-Menten kinetics with a half-saturation constant (K_m) of 24.5 mg DIC·ℓ^{-1} (Figure 2), indicating that photosynthetic rate was half maximal at 24.5 mg DIC·ℓ^{-1}. Therefore, unlike growth which was apparently saturated at 10.5 mg DIC·ℓ^{-1}, photosynthetic DIC uptake was only half saturated at the highest level of DIC examined (21 mg DIC·ℓ^{-1}). This difference between the responses of growth and photosynthesis to DIC indicates that there may not be a simple relationship between the two measures of plant response. Plotting growth of *M. spicatum* in each solution against photosynthetic uptake of DIC measured in the same solutions (Figure 3) confirms the lack of a clear relationship between the two in either of the experiments. These results suggest that other sources of carbon may have been moderating the effect of DIC on growth. To examine the influence of other sources of carbon on the growth of *M. spicatum*, we constructed a carbon budget for each of the experimental treatments in the second growth experiment (Figure 4).

Total carbon uptake during growth was the sum of plant organic carbon (0.465 × ash-free dry weight) and plant inorganic carbon ($CaCO_3$ encrustation). Plant inorganic carbon was determined by measuring weight loss of ashed plant samples (550° C) after recombustion at 1100° C. The quantity of C originating from solution was determined from the difference between the initial and final DIC. The change in solution Ca was used as a measure of $CaCO_3$ precipitation. Precipitation of $CaCO_3$ was further partitioned between $CaCO_3$ encrustation (measured) and precipitation in the tanks (by subtraction). The remainder of the total carbon uptake was considered to have been supplied by atmospheric CO_2 exchange. We considered heterotrophic respiration in the water column as insignificant in this experiment due to the lack of N, P, and organic C in solution (Smart and Barko 1984a). Sediment respiration was also considered an insignificant source of inorganic carbon due to the small quantities of sediment used in the experiment. Likewise, root uptake of CO_2 from sediment has been shown to be insignificant in *M. spicatum* (Loczy, Carignan, and Planas 1983) and was not included in our budget.

Much of the carbon used for growth in these experiments was apparently derived from atmospheric exchange with the solution. The quantity of carbon derived directly from solution increased with DIC and, to a lesser extent, with increasing solution cations. The latter effect is due to the increase in $CaCO_3$ precipitation with increasing solution Ca and DIC. Although carbon derived from solution increased with increasing DIC between 10.5 and 21.0 mg·ℓ^{-1}, this increased uptake from solution was offset by decreased CO_2 exchange from the atmosphere.

Uptake of CO_2 or HCO_3^- from solution decreases the partial pressure of CO_2 (pCO_2) in solution and establishes a gradient in pCO_2 across the air-water interface. The rate of entry of atmospheric CO_2 into solution is dependent on this gradient. In low alkalinity (low DIC) solutions a relatively small uptake of DIC results in a substantial gradient in pCO_2 (Figure 5). For this reason, atmospheric CO_2 supply may be initially higher under low alkalinity conditions. As DIC depletion continues,

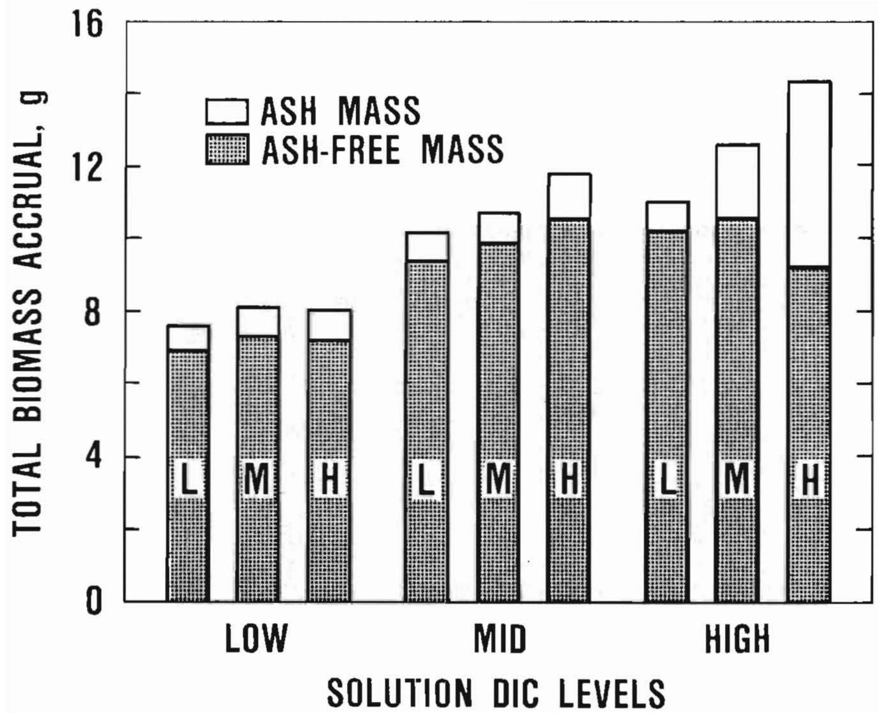

Figure 1. Biomass response of *M. spicatum* to increasing levels of DIC and major cations (Na, K, Ca, Mg). Cation levels are indicated within bars: L = low, M = mid, H = high

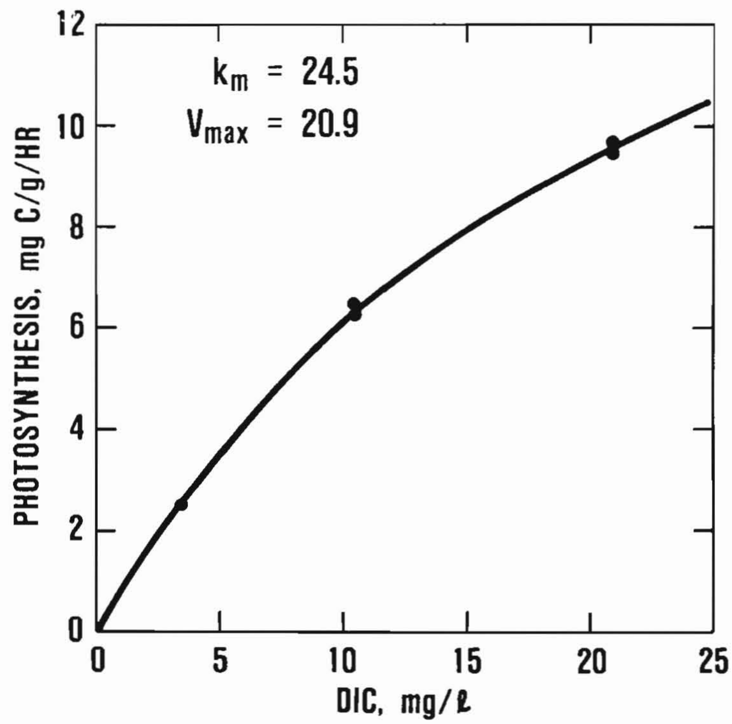

Figure 2. Photosynthetic DIC uptake of *M. spicatum* in relation to solution DIC. Kinetics (K_m = half-saturation constant, $mg \cdot l^{-1}$; V_{max} = maximum photosynthetic rate, $mg\ DIC \cdot g^{-1} \cdot hr^{-1}$) are based on linear transformation of initial photosynthetic DIC uptake data

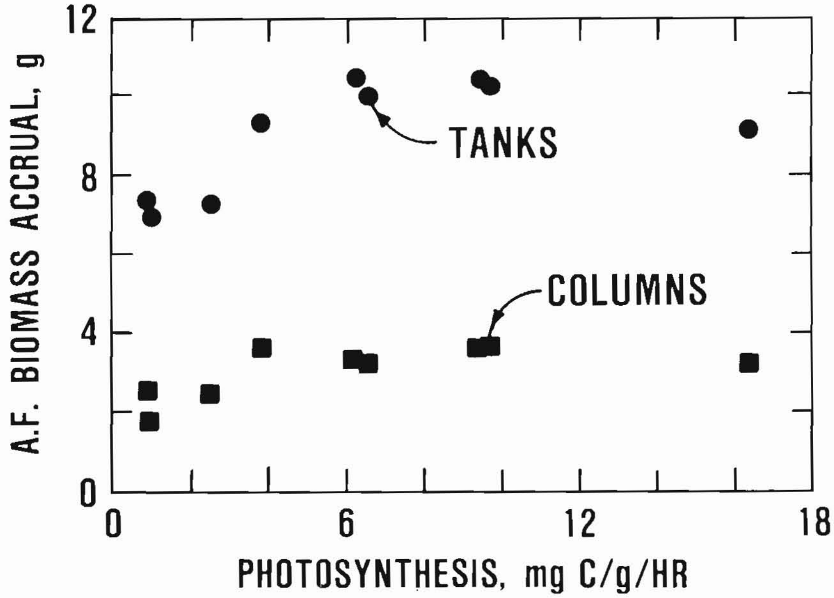

Figure 3. Biomass accrual of *M. spicatum* in the first (columns) and second (tanks) experiments in relation to apparent initial photosynthetic DIC uptake rates (A.F. = ash free)

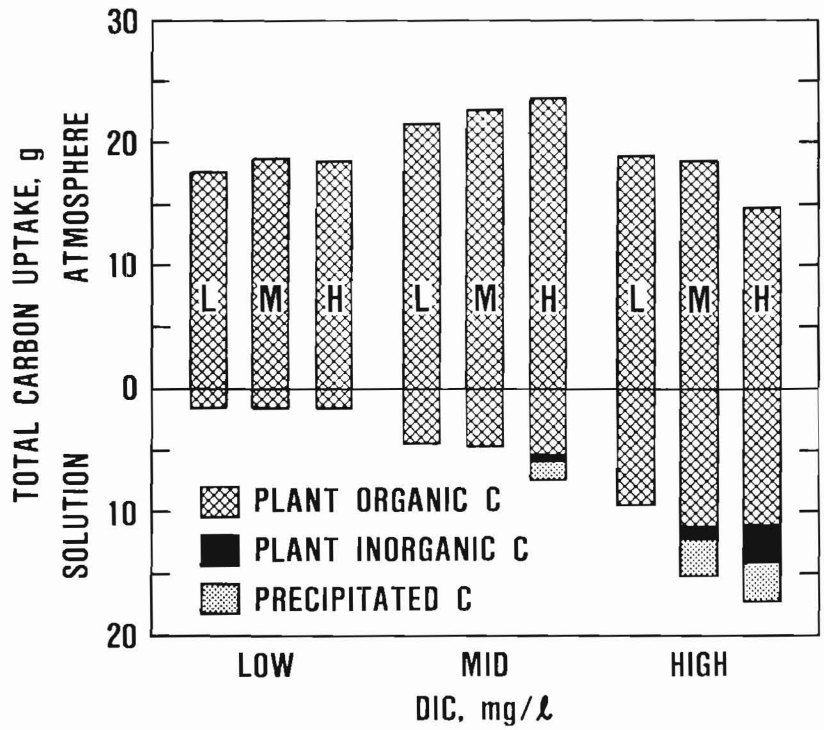

Figure 4. Carbon balance for *M. spicatum* growth under different DIC and cation conditions in the second experiment. Cation levels are indicated within bars: L = low, M = mid, H = high

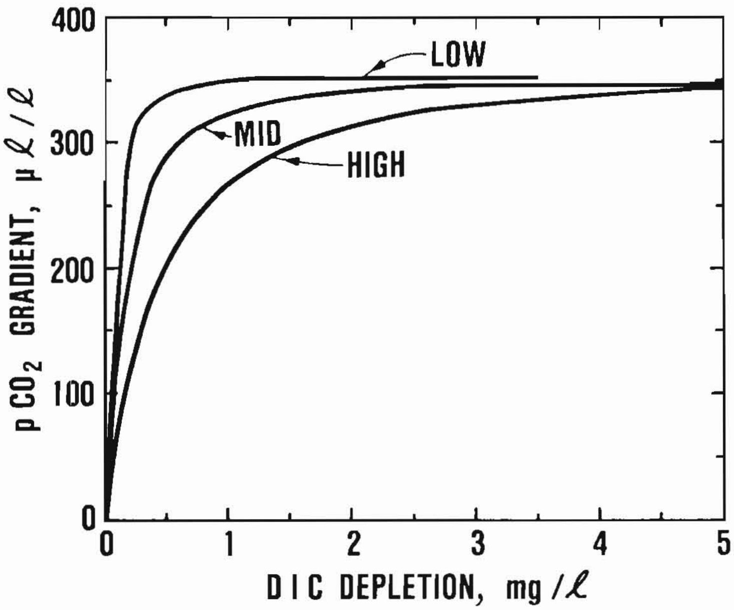

Figure 5. Relationship between the gradient in pCO_2 across the air-water interface and the depletion of solution DIC due to plant uptake of CO_2 or HCO_3^-. Curves are calculated for low (3.5 mg DIC·ℓ^{-1}), mid (10.5 mg·ℓ^{-1}), and high (21.0 mg DIC·ℓ^{-1}) carbon solutions

the gradient in pCO_2 approaches an upper limit depending on the atmospheric pCO_2. Precipitation of $CaCO_3$, which occurred in the solutions containing higher levels of DIC and Ca, increases solution pCO_2, thus lessening the gradient and subsequently atmospheric CO_2 supply. For these reasons the magnitude of atmospheric CO_2 supply is not easily predicted from the initial solution composition.

Our inability to relate growth of *M. spicatum* in these experiments to photosynthetic DIC uptake rates measured in the same solutions is due to the moderating influence of atmospheric CO_2 exchange on growth. Thus, while photosynthetic DIC uptake rates were roughly proportional to the quantity of solution DIC used for growth, they were not good predictors of plant growth.

CONCLUSIONS

Many of the environmental parameters known to affect the relative availability of atmospheric and solution C were controlled in these experiments. These parameters include temperature, mass transfer coefficients, mixing, and water exchange. In addition, other sources of C including water column respiration, sediment respiration, diffusion of HCO_3^- from the sediment, and dissolution of sedimentary carbonates were minimal. In view of the much greater complexity of natural systems, the use of photosynthetic rates as a diagnostic tool for assessing the submersed macrophyte growth potential of natural systems seems unwarranted without a more complete understanding of the interactions of the various carbon sources.

The relative importance of the various sources of C for supporting the growth of submersed aquatic plants needs to be determined. These sources include DIC, atmospheric exchange of CO_2, water column respiration, and sediment respiration. Understanding the relationships among the various C sources is necessary to an understanding of the effects of water chemistry on submersed aquatic plants.

ONGOING AND FUTURE RESEARCH

We are conducting similar studies on the effects of water chemistry on the growth of other species of submersed aquatic plants. These studies will consider both introduced and native species (*Egeria, Hydrilla,* and *Potomogeton*). This information should be useful in identifying possible competitive relationships among problem and nonproblem species.

We plan to initiate preliminary studies this fiscal year in an attempt to quantify the relative importance of atmospheric, solution, and sedimentary sources of inorganic carbon for macrophyte growth in different aquatic systems.

ACKNOWLEDGMENTS

We gratefully acknowledge the diligent technical efforts of Susie Hennington, Dwilette McFarland, and Ramona Warren.

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DEVELOPMENT OF COMPUTER-AIDED EVALUATION SYSTEMS

by
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SELECTING AN OPTIMAL CONTROL STRATEGY

The field of aquatic plant control has evolved from early efforts to totally eradicate "nuisance" aquatic vegetation over broad areas to more recent efforts to "manage" vegetation at an acceptable level within specific areas. Through this evolution, an increasing number of constraints have been imposed on typical aquatic plant control operations. Now, in addition to the usual objectives of minimizing operational costs and achieving at least temporary control of the vegetation, it is important to minimize undesirable environmental side effects and to achieve vegetation control only within specifically defined target areas. In order to meet these constraints, resource managers need to be diligent in evaluating and selecting a control technique and determining when, where, and to what degree treatment should be applied.

At the current state of development, numerous aquatic plant control techniques are available for operational use, and additional techniques are in the last stages of developmental research. Table 1 contains a list of these techniques. While multiple candidate techniques may not be available for each aquatic plant problem site, many sites will have several feasible control options. Given these choices, as well as the selection of time schedule, location, and level of treatment, the selection of an optimum (or even a workable) control strategy can be a difficult task.

The determination of an optimum control strategy involves a sequence of evaluations and decisions (Figure 1). The first step is to verify that an aquatic plant problem does exist. A dense stand of aquatic plants does not, by itself, constitute a problem. To be a problem, it must interfere with (or threaten to interfere with) a use of a water body. Once a real problem has been identified, the project goals may be defined, i.e., what level of vegetation control, when, and at what location would alleviate the problem. Additionally, the budgetary, environmental, and political constraints associated with the prospective operation need to be defined. The complete set of goals and constraints then defines the requirements for a successful control operation. Next, a list of control techniques which may be effective at this specific problem site needs to be developed. Probable effectiveness, cost, and environmental effects of each candidate technique must be estimated. Matching these estimates with project goals and constraints for each candidate technique will identify the best technique for the situation. Finally, the specific means of applying the selected technique must be determined. For example, if mechanical harvesting were selected as the best technique, it would be necessary to determine what specific machine to use, how the harvested material would be handled and disposed, how

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Table 1
Operational and Near-operational Control Techniques

Type	*Control Agent*	*Target Species*
Mechanical	Harvesters	Most types Aquatic vegetation
Chemical	2,4-D	Waterhyacinth Alligatorweed Milfoil
	Endothall	Most SAV
	Diquat	Hydrilla Milfoil Waterhyacinth
	Copper compounds	Most SAV
	Fluridone	Most SAV
	Glyphosate	Hyacinth
Biological	White amur	Certain SAV
	Waterhyacinth weevils	Waterhyacinth
	Waterhyacinth leaf spot fungus	Waterhyacinth
	Argentine waterhyacinth moth	Waterhyacinth
	Alligatorweed thrips Alligatorweed flea beetle }	Alligatorweed

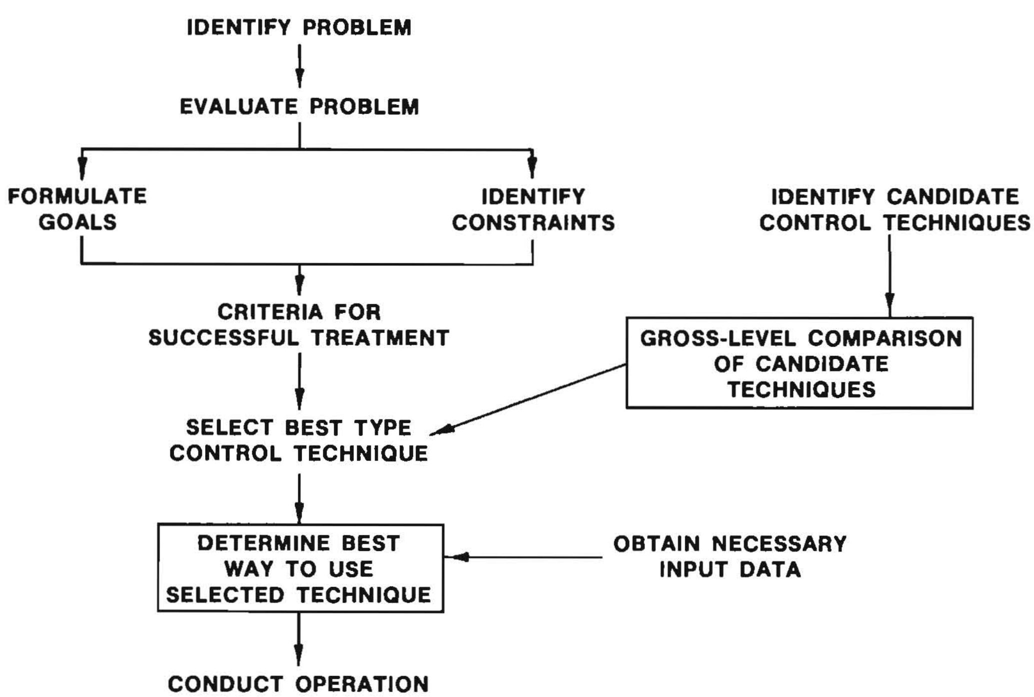

Figure 1. Procedure for determining optimal control strategy

deep to cut and in what specific areas, what is the time schedule, and what is the frequency of harvesting. This evaluation requires very specific information on the target site. Numerous specific strategies for applying the technique would be evaluated in terms of cost, effectiveness, and environmental effects, and would be compared with project goals and constraints. Based on this comparison, the best specific strategy would be identified.

PROCEDURES TO AID DECISIONMAKERS

The procedure described above requires that a methodology be available for each control technique whereby it would be possible to quantitatively estimate effectiveness, cost, and environmental effects for any site-specific set of conditions. For most available control techniques, such quantitative methodologies are nonexistent. Technical documents on newly developed techniques, prepared for use by operational personnel, tend to be either long technical treatises or overly simplistic user manuals, neither of which provides direct information to enable the user to quantitatively estimate the factors needed for evaluation of the prospective control strategy. This problem is more a limitation of the written medium rather than the fault of the documents' authors. The effectiveness of most techniques is a function of the simultaneous interaction of numerous environmental and operational factors which tend to be difficult to intelligibly describe in a written format.

A far more effective means to transfer this technology to the operational user community is in the form of computer models and evaluation systems. Structured in a well-conceived computer program, a system for effectiveness evaluation can rapidly provide precise estimates of the effects of candidate control strategies by allowing the computer to handle the involved mathematical computations, while the user need only be concerned with specifying operational and environmental variables specific to the candidate operation. The user need not be knowledgeable of the mathematical operations of the model. Two successful examples of aquatic plant effectiveness evaluation systems (developed at WES) are the mechanical control simulation model HARVEST (Sabol and Hutto 1984)* and the white amur stocking rate model STOCK (Miller and Decell 1984).** Each of these models interactively queries the user for the necessary inputs, performs the computations, outputs information in a directly usable format, and runs on a desk-top-size personal computer. These models can be used most effectively by the users to play "what if" games, comparing the results of different possible control strategies, and varying a single operational input at a time to "fine tune" the strategy.

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* A. C. Miller and J. L. Decell 1984. "Use of the White Amur for Aquatic Plant Management," Instruction Report A-84-1, US Army Engineer Waterways Experiment Station, Vicksburg, Miss.

** B. M. Sabol and T. D. Hutto 1984. "Simulating Mechanical Control Operations," Aquatic Plant Control Research Program Information Exchange Bulletin, Vol A-84-1, US Army Engineer Waterways Experiment Station, Vicksburg, Miss.

DEVELOPING COMPUTER-AIDED EVALUATION SYSTEMS

The approach described above will be used to prepare additional effectiveness evaluation systems for operational and near-operational control technologies. The generalized framework for developing these new systems is illustrated in Figure 2. The first step is to develop a conceptual framework of the specific control system. Important components to be included in the model and the types of output information desired will be identified and conceptually linked together in a diagram depicting the flow of information through the system. This framework will be developed by Aquatic Plant Control Research Program researchers who are familiar with the cause/effect mechanisms involved in the system, and Corps operational personnel who are most familiar with the operational information needs. Next, all published and unpublished data available on the system will be

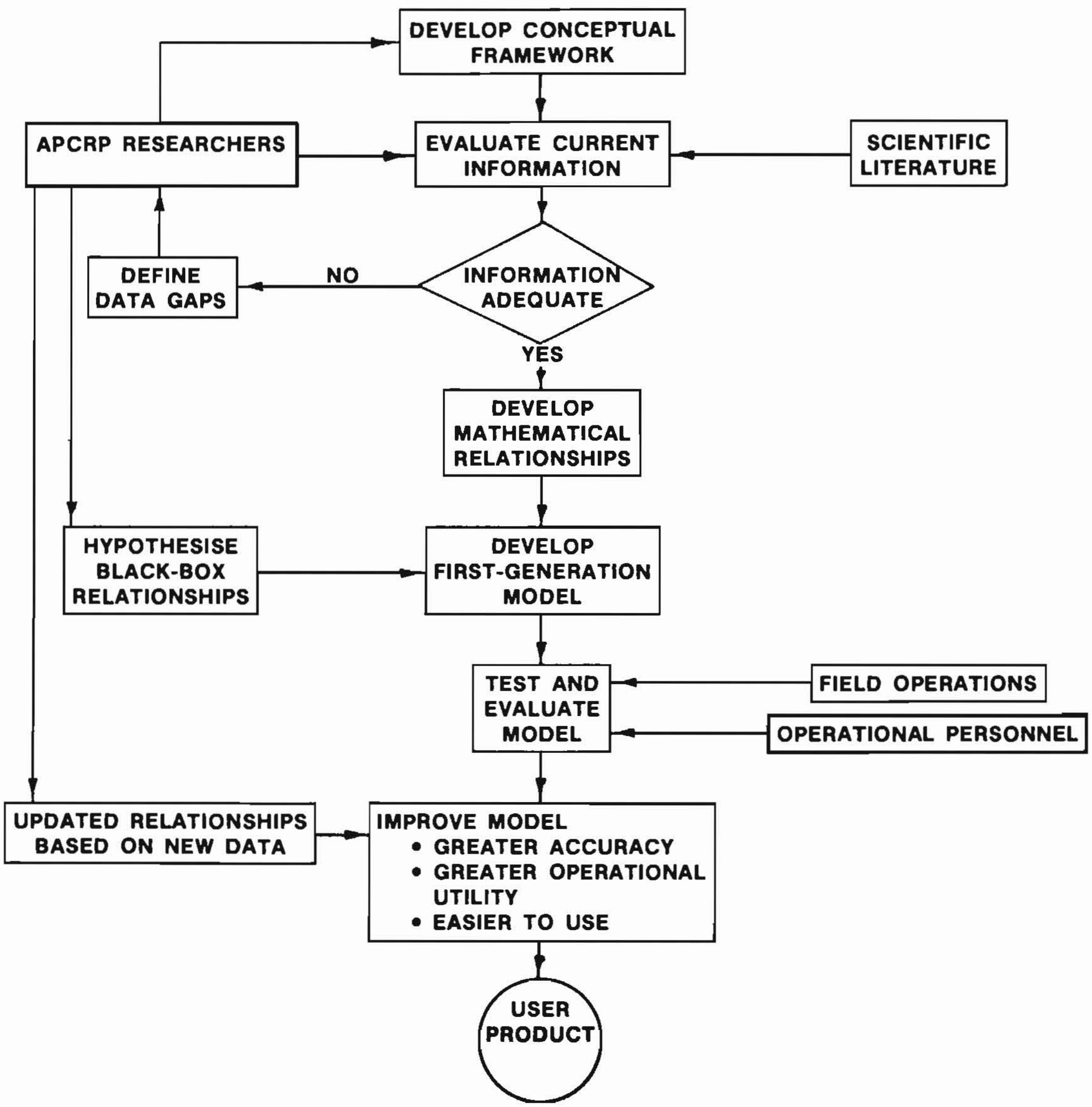

Figure 2. Framework for developing new evaluation systems

examined to develop the mathematical relationships inherent in the system and to identify critical data gaps which will need to be filled. Based on the mathematical relationships developed in this phase, a first-generation computer model will be coded and verified. Next, the model will be tested against field data to validate it and to identify needed improvements. As data gaps (identified earlier) are filled, new mathematical relationships will be developed and the code will be modified accordingly. After sufficient testing and revision have been performed to ensure the model's accuracy and operational utility, user manuals will be prepared and the model will be made available to the public.

FY 85 PLAN

During this fiscal year, we will initiate development of models on two control systems. The first model will be used for simulation of the control of waterhyacinth by the two species of *Neochetina* weevils (*N. bruchi* and *N. eichhorniae*).

This type of control system has been operational for several years, and there is a large body of data available for modeling. The framework for this model will be the basis for developing models on additional plant/insect control systems. The second model will be for simulating the control of waterhyacinth with the herbicide 2,4-D. Control by this method has also been operational for many years and also contains a large data base. This modeling effort will produce the first aquatic herbicide effectiveness model and, as such, will be useful as a framework for developing additional aquatic herbicide effectiveness models.

BIOLOGICAL CONTROL TECHNOLOGY DEVELOPMENT

An Overview

by
Edwin A. Theriot*

DIRECT-ALLOTTED RESEARCH FOR FY 1984

The FY 1984 direct-allotted biological control research was apportioned among five work units, two of which were completed. These include:

a. ***Insects and pathogens for control of waterhyacinth.*** These two work units (I and II) were completed in FY 1984 with the preparation of three reports, a final report on the large-scale operation management test with insects and pathogens from the control of waterhyacinth in Louisiana, a technical report on *Neochetina* species and their impact on waterhyacinth in the United States, and an instruction report to provide field guidance for the use of insects and pathogens to manage waterhyacinth. The last two reports were prepared in cooperation with Dr. Ted Center, U.S. Department of Agriculture (USDA), Fort Lauderdale aquatic weed center.

b. ***Insects for control of hydrilla.*** Overseas searches in Asia and Australia for candidate biocontrol agents were completed in FY 1983 (Work Unit III). Overseas host specificity and efficacy studies were initiated in FY 1984. As a result of these studies, two insect species have been approved for quarantine studies in the United States thus far: a *Bagous* weevil from India and a *Hydrellia* fly from Pakistan.

c. ***Pathogens for control of hydrilla.*** Techniques for aquarium assay were refined and candidate microorganisms were tested to determine their effects on hydrilla (Work Unit IV).

d. ***Pathogens for control of Eurasian watermilfoil.*** Combinations of the fungus *Mycoleptodiscus terrestria* and pectinolytic bacteria proved efficacious on Eurasian watermilfoil in outdoor pool studies at the University of Massachusetts (Work Unit V). Methods of inoculum production and application were developed for small-scale field studies. The first year of the domestic survey for pathogens on Eurasian watermilfoil was completed. Several hundred bacterial and fungal organisms have been isolated from symptomatic tissues.

SUPPORT PROJECTS FOR FY 1984

Three projects were addressed in support of Corps Districts and the Department of Interior, National Park Service:

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* US Army Engineer Waterways Experiment Station, Vicksburg, Mississippi.

a. ***Biocontrol of waterhyacinth and alligatorweed in southeastern Texas.*** This project, with funds provided by the Galveston District, involved the release and establishment of all available insect biocontrol agents on waterhyacinth and alligatorweed. A technical report was prepared and a guidance document was issued to provide a detailed management plan for the Galveston District.

b. ***Biocontrol of waterhyacinth in the San Joaquin-Sacramento River Delta in California.*** All three insect biocontrol agents were released and have become established and have overwintered on waterhyacinth in the California Delta. Funding for the project was provided by the State of California, Department of Boating and Waterways, through the Sacramento District.

c. ***Biocontrol of alligatorweed and waterhyacinth in Jean Lafitte National Historical Park, Louisiana.*** This project involves the intensive management of all insect biocontrol agents on alligatorweed and waterhyacinth within the park in order to keep scenic and recreation trails open. Funding for the project is provided by the Department of Interior, National Park Service.

DIRECT-ALLOTTED RESEARCH FOR FY 1985

The FY 1985 direct-allotted biological control research will be apportioned among three work units, as follows:

a. ***Insects for control of hydrilla.*** The most promising group of candidate insect biocontrol agents found in the overseas searches in Australia will be selected and host specificity and efficacy studies will be initiated. A petition will be submitted to the USDA/APHIS for the release of the insects now in quarantine which prove to be host specific on hydrilla.

b. ***Pathogens for control of hydrilla.*** Additional aquarium-level evaluation of lytic-enzyme-producing microorganisms will be conducted, and the most promising candidates will be selected for larger scale evaluation.

c. ***Pathogens for control of Eurasian watermilfoil.*** Field evaluation of *Mycoleptodiscus terrestris* in combination with pectinolytic bacteria will be conducted. The domestic survey for pathogens on Eurasian watermilfoil will be completed and candidates will be selected for biocontrol potential.

SUPPORT PROJECTS FOR FY 1985

Support projects in Texas and California will continue. Emphasis will be placed on the redistribution of insects to additional areas. The support project in Louisiana will entail the establishment of all biocontrol agents of alligatorweed and waterhyacinth in selected sites.

TECHNOLOGY TRANSFER

A total of six reports were prepared in FY 1984:

- Final report on the large-scale operations management test with insects and pathogens for control of waterhyacinths in Louisiana.
- A technical report on *Neochetina* species and their impact on waterhyacinth in the United States.
- A guidance document on biological control of waterhyacinth.

- A technical report on overseas surveys in Asia and Australia for insects on hydrilla.
- A technical report on lytic-enzyme-producing microorganisms for control of hydrilla.
- A preliminary report on biocontrol agents on alligatorweed and waterhyacinth in southeast Texas.

Two information exchange bulletins were prepared in FY 1984:

- "Alligatorweed and Its Biocontrol Agents," by Alfred F. Cofrancesco, Jr.
- "Feasibility of Applying Genetic Engineering Technology to Aquatic Plant Control," by Judith C. Pennington.

BIOLOGICAL CONTROL TECHNOLOGY DEVELOPMENT

Microbiological Control of Hydrilla with Lytic-Enzyme-Producing Microorganisms

by
Edwin A. Theriot* and Judith C. Pennington*

INTRODUCTION

The lytic-enzyme studies on hydrilla were initiated in FY 1982. The approach used in this study consisted of isolating bacteria and fungi from the microflora of hydrilla and screening them for production of enzymes that can lyse specific plant components. These microorganisms were successively subcultured on restrictive media to enhance their enzyme production and then reintroduced to hydrilla in the hope that their more powerful enzyme systems would enable them to attack plant tissues. The rationale for this approach was prompted by promising work done on Eurasian watermilfoil by Dr. Haim Gunner, University of Massachusetts in Amherst.

Candidate microorganisms have been selected and evaluated in test tube and initial aquarium assay studies (Pennington 1984). A total of twenty-two isolates demonstrated the ability to produce lytic enzymes on cellulose or pectin media, seven of which significantly damaged hydrilla tissues in test tube assays. Aquarium assays were conducted using six of the seven promising candidate microorganisms (Table 1). None of the candidates significantly damaged hydrilla in these initial aquarium studies.

Before further aquarium assays could be conducted, it was necessary to look critically at the methods employed in the first aquarium study and how it differed from the test tube assays. Inoculum for the aquarium assay consisted of filtered organisms, while whole inoculum, including nutrient growth medium (PDB) and accumulated exogenous metabolites, was used in the test tube assay. The test tube assay was conducted on first-generation, field-collected plants, whereas the aquarium assay was conducted on hydrilla grown in the greenhouse through several subcultures. Laboratory studies were conducted to determine if these factors had any bearing on the negative results of the aquarium study and to develop methods of evaluation of the candidates in large-scale tank studies in the greenhouse.

This paper presents the results of the FY 1984 studies and represents the end of the first phase or laboratory phase of the project. The next phase is the scale-up to greenhouse tank and field studies.

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Table 1
Candidate Microorganisms

Isolate Number	*Name*
56	*Aspergillus awomori* Nakazawa
156	*Humicolo* sp. with *Tricoderma* sp.
161	*Humicola* sp. with *Tricoderma* sp.
170	*
236	*Fusarium moniliforme* Sheldon
249	*Aspergillus awomori* Nakazawa

* Unidentified

METHODS

Assay 1: Greenhouse plants

A test tube assay was conducted using the six best isolates from the preliminary hydrilla test tube assay. Fungal inoculum was prepared by introducing each isolate into two 500-ml Erlenmeyer flasks each containing 100 ml of sterile PDB. Cultures were incubated for 6 days. Mycelia were broken apart and mixed with an omnimixer for 5 min. One inoculum flask for each isolate was filtered through Whatman No. 1 filter paper on a Buchner funnel under mild suction. The residue was resuspended in 100 ml of sterile distilled water. The filtrate was centrifuged to remove any visible residues.

Whole (unfiltered) inoculum, inoculum filtrate, and resuspended filtered organisms were quantified by serial dilution followed by plate counts.

Hydrilla from greenhouse cultures were selected and placed in test tubes. Fifty millilitres of sterile distilled water was used rather than macronutrient solution. The hydrilla sprigs were typical of greenhouse culture, i.e. having thin stems and leaves with a generally pale, fragile appearance.

Each hydrilla tube culture received 1 ml of inoculum at levels shown in Table 2. Untreated controls received 1 ml of sterile distilled water and treated controls received 1 ml of PDB. Each treatment and control was replicated seven times.

Damage index values were defined prior to test initiation as follows:

> Index 1—Vigorous, healthy plants with normally spaced internodes and no evidence of disease or damage.
>
> Index 2—Faintly chlorotic plants only slightly paler than healthy sprigs and exhibiting few or no damage spots.
>
> Index 3—Chlorotic plants, or plants showing less than 50 percent damage.
>
> Index 4—Markedly chlorotic plants, or plants exhibiting pronounced damage exceeding 50 percent of sprig.
>
> Index 5—Chlorotic to brown plants, plants with brown stems and most leaves transparent and disintegrating, or obviously dead plants.

A damage index value was assigned to each sprig prior to inoculation and every week for 6 weeks after inoculation.

Table 2
Inoculum Viabilities (CFU/ml)*

	Assay I		Assay II	
Isolate No.	*Whole***	*Organisms*†	*Whole*	*Organisms*†
56	5×10^4	9×10^5	1×10^5	5×10^4
156	6×10^3	6×10^3	6×10^4	1×10^5
161	8×10^2	9×10^3	8×10^6	1×10^6
170	7×10^3	2×10^4	3×10^4	8×10^4
236	4×10^6	8×10^5	2×10^6	4×10^6
249	1×10^5	4×10^5	7×10^6	5×10^5
$\bar{X}$	7×10^5	4×10^5	2×10^7	1×10^6

* Colony forming units per millilitre. Counts given are for quantification conducted at the time of inoculation.

** Whole (unfiltered) inoculum.

† Filtered organisms.

All test sprigs developing a damage index value of 4 to 5 were sampled for recovery of the isolate. Approximately 2 cm of the lower stem including a single node, when possible, were excised aseptically, sterilized by immersing for 1 min in 1 percent NaHC10, and rinsed with sterile distilled water three times. The tissue were plated for isolation on PDA plates. At the same time, isolates were taken from stock cultures and streaked in a similar manner so that parallel development could be closely observed. Mycelia or colonies suspected of being the tested isolate were transferred to slant culture for later taxonomic confirmation.

Assay II: Field plants

A hydrilla test tube assay identical to Assay I was conducted using hydrilla from the field. These plants had thick stems and leaves, were dark green, and were generally coarse and robust in appearance. Test procedures, including inoculum preparation, were the same as described above for Assay I.

Data analysis

Differences among treatment means and means of treatment type (i.e. whole inoculum, filtered organisms, etc.) were determined using DUNCAN option to the PROC GLM procedure (SAS 1982).

RESULTS

Assay I: Greenhouse plants

Mean damage index values as indicated by Duncan's Multiple Range Test by treatment types (Table 3) showed that whole inoculum produced significantly greater damage to hydrilla than was produced by filtrate, filtered organisms, or untreated controls. Whole inoculum produced significantly greater damage than was produced by PDB-treated controls in all except the sixth week.

There were no significant differences between mean damage index values of filtrates and filtered organisms except in the last 2 weeks. In the fifth and sixth

Table 3
Mean Damage Index Values for Hydrilla by Treatment Types

1st Week		*2nd Week*		*3rd Week*		*4th Week*		*5th Week*		*6th Week*	
*Treatment Type**	*$\bar{X}$***	*Treatment Type*	*$\bar{X}$*	*Treatment Type*	*$\bar{X}$*	*Treatment Type*	*$\bar{X}$*	*Treatment Type*	*$\bar{X}$*	*Treatment Type*	*$\bar{X}$*
				Assay I: Greenhouse Plants							
W	1.81^{a}	W	2.79^{a}	W	3.05^{a}	W	3.43^{a}	W	3.71^{a}	PDB	4.00^{a}
F	1.10^{b}	PDB	1.17^{b}	PDB	2.29^{b}	PDB	2.71^{b}	PDB	3.29^{b}	W	3.93^{a}
A	1.02^{b}	F	1.48^{b}	F	1.60^{c}	F	1.79^{c}	F	2.14^{b}	F	2.48^{b}
PDB	1.00^{b}	Unt	1.14^{b}	Unt	1.14^{b}	Unt	1.29^{c}	Unt	1.43^{bc}	Unt	1.71^{bc}
Unt	1.00^{b}	A	1.05^{b}	A	1.10^{c}	A	1.17^{c}	A	1.29^{c}	A	1.62^{c}
				Assay II: Field Plants							
W	1.98^{a}	W	3.19^{a}	W	3.88^{a}	W	4.31^{a}	W	4.45^{a}	W	4.62^{a}
F	1.60^{ab}	F	2.64^{ab}	F	3.38^{a}	F	3.69^{ab}	F	3.90^{a}	F	4.00^{ab}
A	1.29^{b}	A	1.88^{bc}	PDB	2.29^{b}	PDB	2.86^{bc}	PDB	3.00^{b}	PDB	3.29^{bc}
PDB	1.14^{b}	PDB	1.71^{cd}	A	2.17^{b}	A	2.45^{c}	A	2.62^{b}	A	2.76^{c}
Unt	1.00^{b}	Unt	1.00^{d}	Unt	1.00^{c}	Unt	1.14^{d}	Unt	1.29^{c}	Unt	1.29^{d}

* Key to treatment types: W = Whole inoculum; F = Filtrate; A = Filtered organisms; PDB = PDB-treated controls; Unt = Untreated controls

** $\bar{X}$ = mean damage index values of all treatments of the same type. Means in the same column followed by the same superscript are not significantly different ($P < 0.05$) according to Duncan's Multiple Range Test.

weeks means of filtrates were significantly greater than means of filtered organisms. PDB-treated controls produced significantly greater mean damage index values than filtrates except in the first 2 weeks. There were never any significant differences between mean damage index values of filtrates and of untreated controls.

Filtered organisms were never significantly different from untreated controls. PDB-treated controls produced significantly greater mean damage index values than filtered organisms except in the first 2 weeks when there were no significant differences.

PDB-treated controls produced significantly greater mean damage index values than untreated controls only after the second week.

The inoculated organisms were recovered from 79 percent of the cultured hydrilla sprigs that received whole inoculum, none of those that received filtered organisms, and one that received filtrate.

Assay II: Field plants

Duncan's Multiple Range Test by treatment types (Table 3) showed that mean damage index values for whole inoculum were significantly greater than means for filtered organisms, PDB-treated controls, or untreated controls. Mean damage index values of whole inoculum were never significantly different from those of filtrates.

Mean damage index values of filtrates were significantly different from those of filtered organisms except in the first 2 weeks. Mean damage index values of filtrates oscillated from no significant differences to significantly greater than means of PDB-treated controls (Table 3). Filtrates produced significantly greater means than untreated controls after the first week.

Mean damage index values of filtered organisms were never significantly different from PDB-treated controls, but were significantly greater than untreated controls after the first week.

PDB-treated controls produced significantly greater mean damage index values than untreated controls after the second week.

The inoculated organisms were recovered from 40 percent of cultured hydrilla sprigs that received whole inoculum, 42 percent of those that had received filtered organisms, and 30 percent of those that had received filtrate.

DISCUSSION

Results of assays with both greenhouse and field plants showed that whole inoculum was significantly more damaging to hydrilla sprigs than filtered organisms. Moreover, filtered organisms never produced damage significantly greater than untreated controls in Assay I. In Assay II this was not true, but mean damage index values for filtered organisms were consistently close to values for untreated controls. These results suggest that the organisms require nutrients or metabolites to inflict damage on hydrilla. Results of Assay I suggest that nutrients are very important since PDB treatment produced significantly greater damage after the second week than all other treatments except whole inoculum. In Assay II, PDB was less damaging compared to whole inoculum; however, filtrate treatments produced consistently high means in Assay II that were also significantly greater than all other treatments in the third and fifth weeks (except whole inoculum from which filtrate was never significantly different). These tests provide additional evidence that the organisms require nutrients and, perhaps, accumulated metabolites (cellulase) to damage hydrilla. These results are also consistent with results of the initial hydrilla aquarium assay in which filtered organisms alone produced no damage to hydrilla (Pennington 1985).

Statistical comparisons of results between the two assays could not be made because inoculum levels were not identical. However, examination of the data suggested that the greenhouse plants of Assay I were more resistant to attack by filtered organisms than the field plants of Assay II. In Assay I, filtered organisms never produced mean damage index values that were significantly different from untreated controls. In Assay II, filtered organisms produced significantly greater mean damage index values than untreated controls after the first week (Table 3). Mean inoculum levels for whole inoculum and for filtered organisms were higher in Assay II than in Assay I, but there was greater difference between means of whole inoculum levels for the two assays ($\Delta = 2000\times$) than between means of filtered organism inoculum levels ($\Delta = 100\times$) (Table 2). The significant damage exerted by whole inoculum implied that sufficient numbers of organisms were present even at the lower inoculum levels and that test results must be explained by some mechanism other than differences in inoculum levels. Nutrient levels and hydrilla resistance as determined by plant source may account for differences between effects of filtered organisms in the two assays.

CONCLUSIONS AND FUTURE RESEARCH

Conclusions

Conclusions of this study are:

a. The lytic-enzyme-producing isolates need nutrients and perhaps accumulated metabolites to damage hydrilla.

b. Hydrilla from greenhouse cultures are more resistant to filtered organisms than hydrilla from healthy field cultures.

FY 1985 research effort

Aquarium assay. Additional aquarium assays will be conducted utilizing the results obtained to better evaluate candidate organisms.

Greenhouse tank studies. Tank studies to be conducted in greenhouse facilities will be initiated. These will more closely simulate field conditions and will require development of methods for large-scale application. Formulation of inoculum and methods for application will be addressed. This effort will be beneficial to the initiation of field studies as well.

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BIOLOGICAL CONTROL TECHNOLOGY DEVELOPMENT

The Search for Insects to Control U.S. Hydrilla Populations

by
Joe K. Balciunas* and F. Allen Dray*

The expanding range of *Hydrilla verticillata* in the United States has forced increased use of mechanical and chemical methods for controlling infestations of this noxious aquatic weed. The escalating costs associated with such methods have precipitated a search for biological agents capable of controlling hydrilla populations. The ongoing project reported herein is a part of that research. The primary focus of this project is to find insects which damage hydrilla in its native range.

The first phase of this research involved three preliminary surveys of the insect faunas associated with hydrilla in Asia and Australia. During the 3 years allocated to this phase, samples were collected at 61 sites in 10 countries (Figure 1). These samples were returned to the United States where they were found to hold 2,623 specimens in 40 insect families (Balciunas 1984).

Identification of these specimens proved particularly troublesome because taxonomic information for insect families in these countries is often rudimentary or nonexistent. As an illustration, preliminary examination of weevils from overseas collections suggested that several species of *Bagous* had been captured. Representative specimens were sent to an authority on the taxonomy of this group. He determined that at least 18 species were represented. He also reported (O'Brien, personal communication) that approximately half of these were either new records for the geographical region in which collected or were undescribed to science.

Nine of the 40 families in these collections (Table 1) are described by Merritt and Cummins (1978) as including some herbivorous species. One of these, the Chironomidae, embraced about one third of the total specimens gathered in the survey. Suspected of damaging the apical meristems of hydrilla in Africa (Pemberton 1980), this group appears to play a very minor role in controlling hydrilla in Asia and Australia. While the potential for controlling hydrilla with these insects is exciting, midges often form dense swarms following synchronous emergence. This gives them the undesirable potential of becoming an annoying pest in their own right. Thus, chironomids will receive only limited attention in the upcoming studies.

Several other of the nine herbivorous families collected in Asia and Australia merit further investigation as possible biocontrol agents. One of these is the shorefly genus *Hydrellia* (Ephydridae). This genus has been reported to occasionally inflict severe damage on various host plants (Lilljeborg 1861; Lange, Ingebretsen, and Davis 1953; Deonier 1971) as a result of the leaf mining habits of the larvae. The

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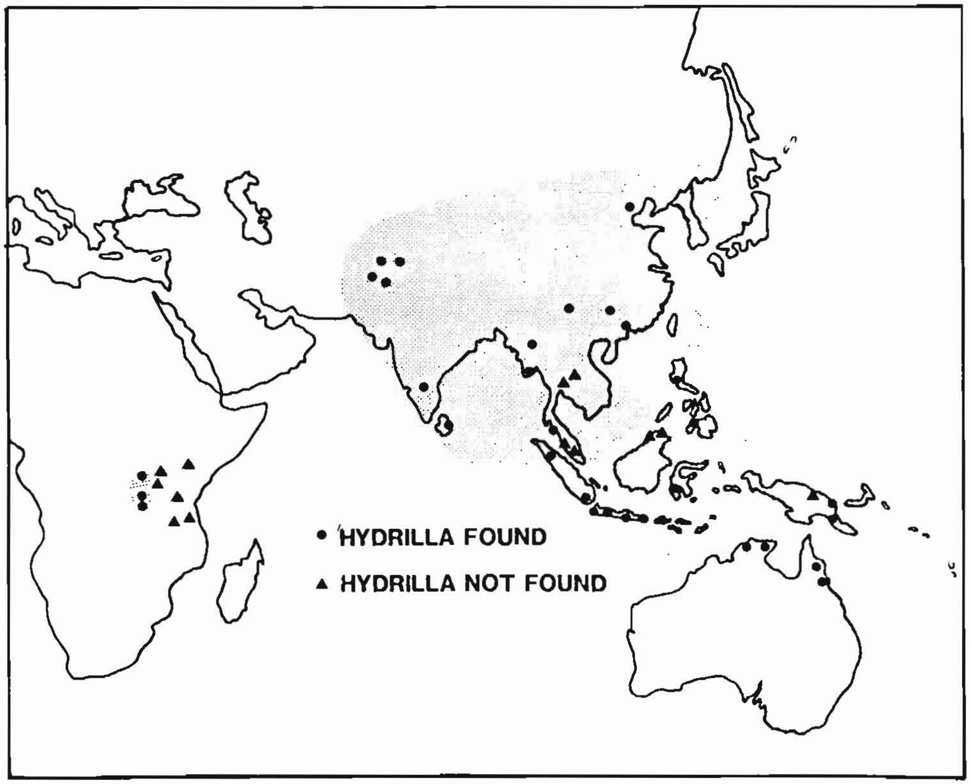

Figure 1. Map indicating range of hydrilla in Asia and Australia. Symbols indicate sites pertaining to this research examined 1980-1984 by various researchers. Note that a circle or triangle may represent more than one site

senior author has observed extensive destruction of hydrilla by *Hydrellia pakistanae* during surveys in India. Another group of importance which was very abundant is the moth family Pyralidae. Balciunas (1984, p 54) reports that aquatic larvae "... cause the most easily observed damage to hydrilla, and when present in large numbers, completely defoliate the plant." A final family of considerable interest is the weevils (Curculionidae). Members of this group are often host specific and they have been involved in several successful biocontrol projects (Julien 1982) including one for waterhyacinth. Ultraviolet light collections gathered 45 species within the above groups. Therefore, these will be the primary focus of the second phase of this project.

This second phase involves intensive, long-term studies of potential control agents in one relatively limited geographical region. These investigations will include examining the biology, life history, host specificity, and control efficiency of several weevils, moths, and shoreflies. Additionally, studies will be conducted on any promising new agents discovered during the course of this work.

Analyses of local facilities, presence of target species, available assistance, and accessibility to productive sites, as well as associated costs, have led to the selection of Queensland, Australia, as the primary region for the upcoming research. Since the key to this phase is long-term investigation, it was decided that 1984 would be used to complete identifications, draft a final report on the preliminary overseas surveys (Balciunas 1984), and make the extensive preparations necessary to

Table 1
Herbivorous Insect Families Collected from Hydrilla in Asia and Australia

Order	*Family*	*Country*	*Specimens*
Coleoptera	Curculionidae	Australia	1
		India	44
	Dryopidae	Indonesia	3
		Malaysia	1
	Helodidae	Malaysia	2
Diptera	Chironomidae	Australia	78
		Burma	10
		India	19
		Indonesia	232
		Malaysia	385
		New Guinea	144
		Philippines	32
		Sri Lanka	3
	Ephydridae	Australia	82
		India	16
		Malaysia	7
		New Guinea	1
		Philippines	1
Hemiptera	Corixidae	Australia	10
		Burma	8
		Malaysia	53
Leopidoptera	Pyralidae	Australia	118
		Burma	15
		India	68
		Indonesia	46
		Malaysia	204
		New Guinea	7
		Philippines	7
		Sri Lanka	86
		Thailand	22
Orthoptera	Tridactylidae	India	1
Trichoptera	Hydroptilidae	Australia	2
		Burma	4
		Indonesia	1
		Malaysia	3
		New Guinea	3
	Leptoceridae	Australia	9
		Burma	4
		India	1
		Indonesia	12
		New Guinea	11
		Sri Lanka	2

successfully complete this portion of the project in Queensland. This strategy has made it possible to consolidate remaining overseas funds and to devote the entire calendar year 1985 to this research. It is hoped that these studies will lead to the introduction of several species into quarantine here in the United States where they will be more intensively examined for host specificity and, ultimately, released.

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BIOLOGICAL CONTROL TECHNOLOGY DEVELOPMENT

Microbiological Control of Eurasian Watermilfoil

by
Haim B. Gunner*

INTRODUCTION

Previous work has confirmed that microorganisms derived from *Myriophyllum spicatum* and selected for specific lytic enzyme potential will (on reinoculation onto the plant) serve as pseudo-pathogens. Organisms which have been tested in previous aquarium and pool studies include: *Mycoleptodiscus terrestris* with a pronounced cellulolytic and pectinolytic potential, a *Fusarium* sp. Isolate MH3F, and two strongly pectinolytic bacteria (PB) Isolates P-8 and MS-11.

Previously reported work has provided evidence that the pathogenic effect is dependent on a threshold level of inoculum of approximately 1 by 10^8 bacterial cells and 1×10^4 to $\times 10^6$ fungal propagules per millilitre of plant medium, respectively. A second element is the prior stressing of the subject plants by environmental or metabolic interactions. To this end, evidence of interactions with plant hormones and other growth modifying agents has been reported.

The work reported below describes an extension in the scale of our experiments to pool size and had three objectives in mind:

- To assess the capacity of the test microbial inocula to reduce and control *Myriophyllum spicatum* populations.
- To determine synergistic interactions between bacterial and fungal inocula resulting in more effective control of *Myriophyllum spicatum.*
- To ascertain whether inocula provided to the plants remained as sustained populations within the aquatic ecosystem.

MATERIALS AND METHODS

Tests of phenolic compounds

Three phenolic compounds reported** to be excretion products of *M. spicatum* with demonstrable inhibitory effects on several aquatic microorganisms were tested against selected isolates derived from the surface of *M. spicatum.* The compounds selected included shikimic acid, protocatechuic acid, and ellagic acid. These were respectively diluted to final concentrations of 0.01 ppm, 0.1 ppm, 1.0 ppm, 10.0 ppm, 100 ppm, and 1000 ppm. Separate 6.0-mm disks made from Whatman No. 1 filter paper were saturated with each dilution of the various compounds and deposited on

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** D. Planas, et al. 1981. "Ecological Significance of Phenolic Compounds of *Myriophyllum spicatum*," *Verh. Internat. Verein. Limnol.*, Vol 21, pp 1492-1496.

the surface of separate TSA agar plates seeded with a lawn of the selected bacterial culture. After 48-hr incubation at 25° C the growth response of each bacterium to each compound at the various dilutions was determined by the radius of inhibition or stimulation of microbial growth around the disk.

Pool experiments: inoculum preparation*

The *Mycoleptodiscus terrestris* cultures were grown for 7 days at room temperature in large aerated glass bottles containing potato dextrose lactose broth (PDLB). On the fifth day of growth, 50 ml of 3-percent cellulose was added to the bottles for each litre of medium. This was to induce cellulase production prior to contact with the plants.

After 7 days the growth medium with culture was blended at 80 rpm for 1 min to break up the mycelial threads. Equal amounts of culture medium and 2-percent xanthan were mixed together for the final inoculum.

The *Fusarium* sp. and bacterial cultures were grown for 3 days at 26° C on a shaker table in flasks containing trypticase soy broth.

Equal amounts of culture medium and xanthan were combined, as above, for the final inoculum.

Pool protocol

Myriophyllum spicatum plants were collected from Stockbridge Bowl in Stockbridge, Massachusetts. Four-inch tips were cut from the healthy plants for use in the experiment. A mixture of sand and pond soil (3:1) was prepared for embedding the freshly collected plant tips. Twenty-five tips were planted in each seedling tray in approximately 2 in. of the sand/soil mixture.

Five plastic-lined pools (6 ft by 18 in.) (Figure 1) were filled with tap water (approximately 200 gal). The trays with plant tips embedded in soil were carefully placed in the pools. Pool B contained 23 trays, while A, C, D, and E had 22 trays.

The plants were given 1 month to adjust to the new environment and to grow new roots. During this time the water was partially exchanged on a weekly basis. No water was exchanged after inoculation of the plants.

Inoculum application

The inoculum was dispensed to each pool via a 2.5-ℓ polyethylene bottle equipped with an air supply and an outlet tube terminating with five trailing hoses (Figure 2). Application, in the concentrations shown in Table 1, was made under water at plant level; however, due to air trapped in the inoculum during its preparation, the inoculum floated to the surface at first and later settled on the surface of the plants.

Mean air temperatures during the growth period are shown in Table 2.

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* A similar protocol was employed for jar-scale experiments. PDLB was replaced by cellulose mineral salts on the fifth day of growth.

Figure 1. Pool array for pilot field study of biological control of *M. spicatum*

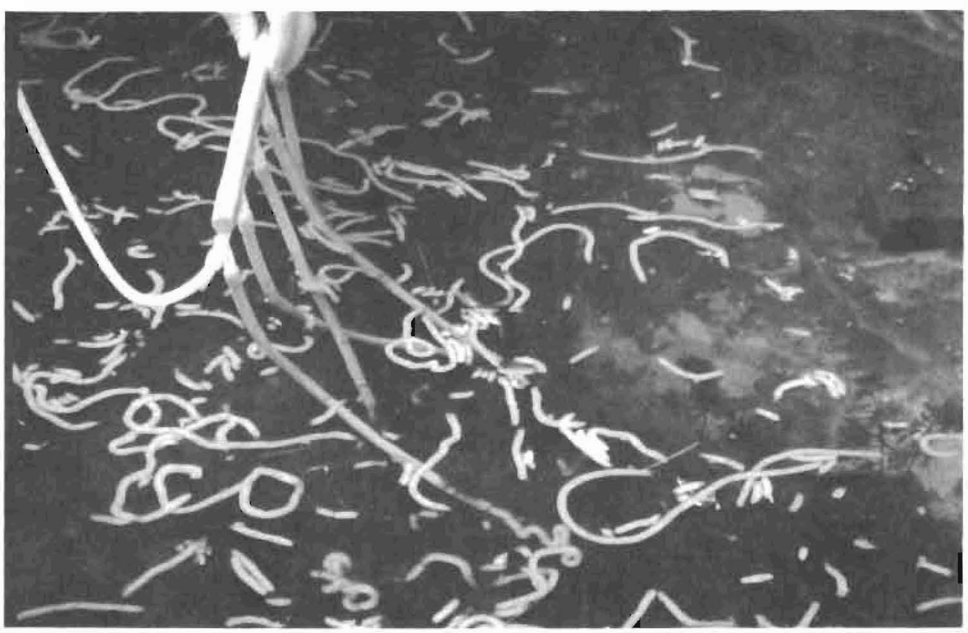

Figure 2. Manifold for distribution of inoculum in xanthan carrier

Table 1
Inoculum for Pool Experiment

Organism	*Inoculation Concentration, CFU/ml*	*Estimated Pool Concentration, CFU/ml*
	Initial Inoculation, 6 September 1984	
P-8	1.1×10^8	6.6×10^5
MS-11	4.3×10^8	2.6×10^6
MH3F	6.5×10^7	6.5×10^5
MT2F	$<1 \times 10^2$	$\sim 1 \times 10^{-1}$
MTSB	$<1 \times 10^2$	$\sim 1 \times 10^{-1}$
	Second Inoculation, 5 October 1984	
MT2F	1.3×10^3	1.1×10^1
MTSB	1.2×10^4	1.1×10^2

Table 2
Mean Air Temperature During Growth Period

Week 1		*Week 2*		*Week 3*	
9/6	54.0°F	9/13	60.0°F	9/20	67.0°F
9/7	55.5	9/14	68.5	9/21	59.5
9/8	58.0	9/15	52.5	9/22	59.0
9/9	64.0	9/16	50.5	9/23	68.5
9/10	69.0	9/17	54.5	9/24	76.0
9/11	74.5	9/18	55.0	9/25	72.5
9/12	64.5	9/19	61.0	9/26	58.5
Mean =	62.8	Mean =	57.4	Mean =	65.9

Week 4		*Week 5*		*Week 6*	
9/27	48.0°F	10/4	51.5°F	10/11	62.5°F
9/28	51.0	10/5	46.0	10/12	63.00
9/29	53.5	10/6	41.5	10/13	57.5
9/30	55.5	10/7	47.0	10/14	57.0
10/1	49.0	10/8	58.0	10/15	56.0
10/2	45.0	10/9	64.0	10/16	56.0
10/3	50.0	10/10	58.0	10/17	55.5
Mean =	50.3	Mean =	52.3	Mean =	58.2

Week 7	
10/18	60.0°F
10/19	59.0
10/20	63.0
10/21	54.5
10/22	67.0
10/23	58.5
10/24	52.0
Mean =	59.1

Biomass harvest

Trays were slowly lifted from the pools with spotters present to catch any plant material detaching itself. The plants were removed from the trays by gently washing the soil mixture from their roots. The plants from each tray were then placed in a preweighed screen drying tray. The drying trays were placed in an oven at 105° C for 24 hr, removed, and weighed. They were then placed back in the oven for an additional 12 hr and reweighed to ensure constant weight. The weight of the empty tray was then subtracted and the biomass for each tray was recorded.

Statistical analyses of the data were then performed using the "F-Test" to determine overall variance, and the "T-Test" to determine if a significant difference existed between pairs of pools.

RESULTS

As shown in Figure 3, *M. terrestris* (MH3F) is a significant producer of antagonistic substances, and a screening of randomly selected microbial isolates from the surface of *Myriophyllum* spp. showed these to be highly sensitive to these inhibitory secretions. The relationship between *M. terrestris* and the *Fusarium* sp. confirms the potential of the former as a means of *M. spicatum* control since clearly the *M. terrestris* can successfully compete for eco-sites on the plant surface.

Results of small-scale tests performed in advance of outdoor pool experiments on the efficacy of synergistic microbial inoculations in the control of *M. spicatum* are shown in Figures 4-7. In Figure 4, untreated control *M. spicatum* plants are shown after 14 days under the experimental regime. In Figure 5, we can observe a marked contrast to Figure 4 in response to inoculation with the pectinolytic-bacterial

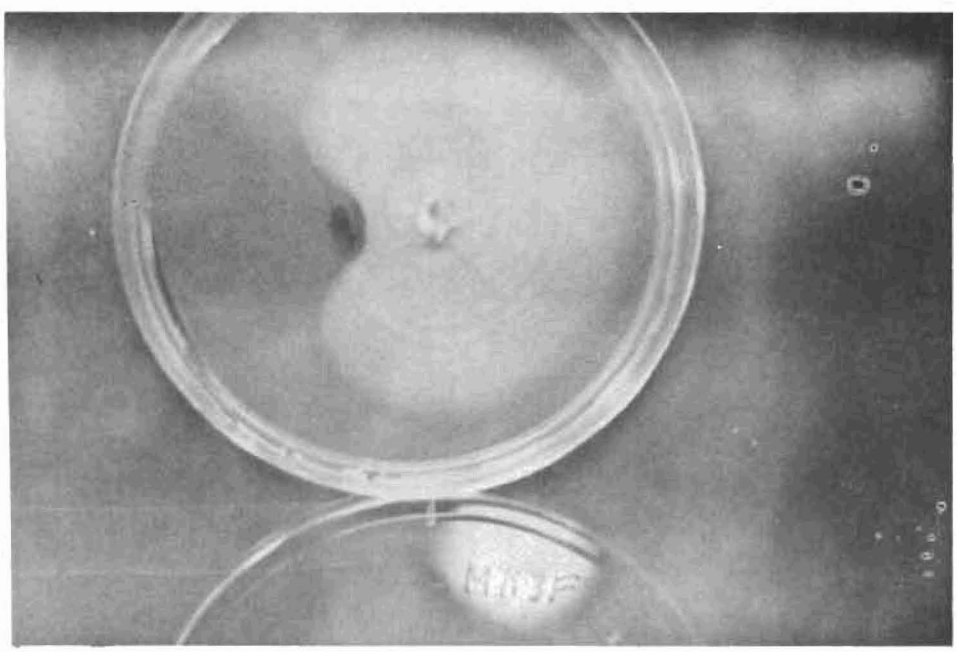

Figure 3. Demonstration of the strongly antagonistic potential of *M. terrestris* (small dark colony) against a *Fusarium* sp.

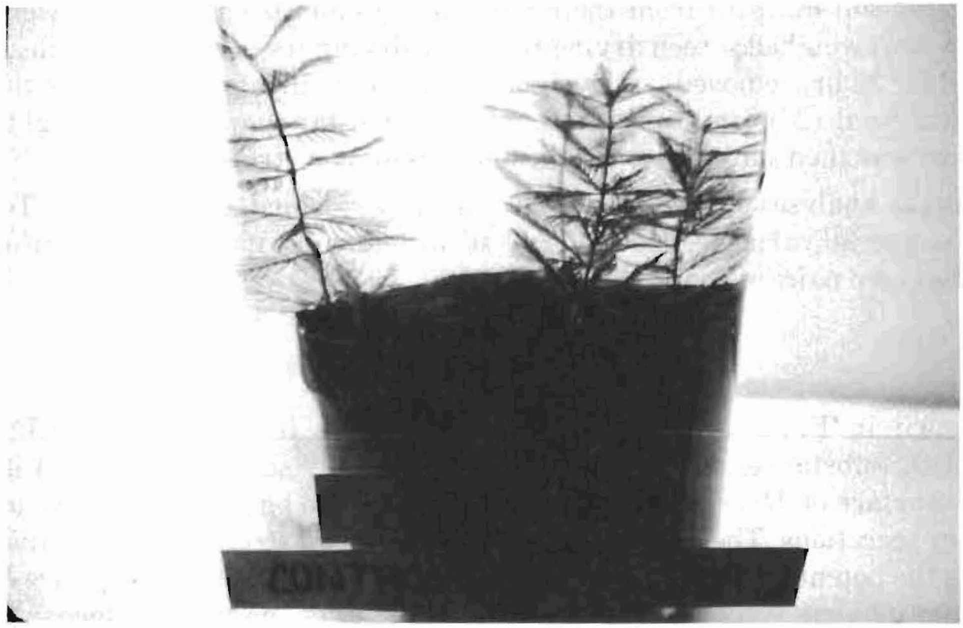

Figure 4. *M. spicatum* untreated control after 14 days

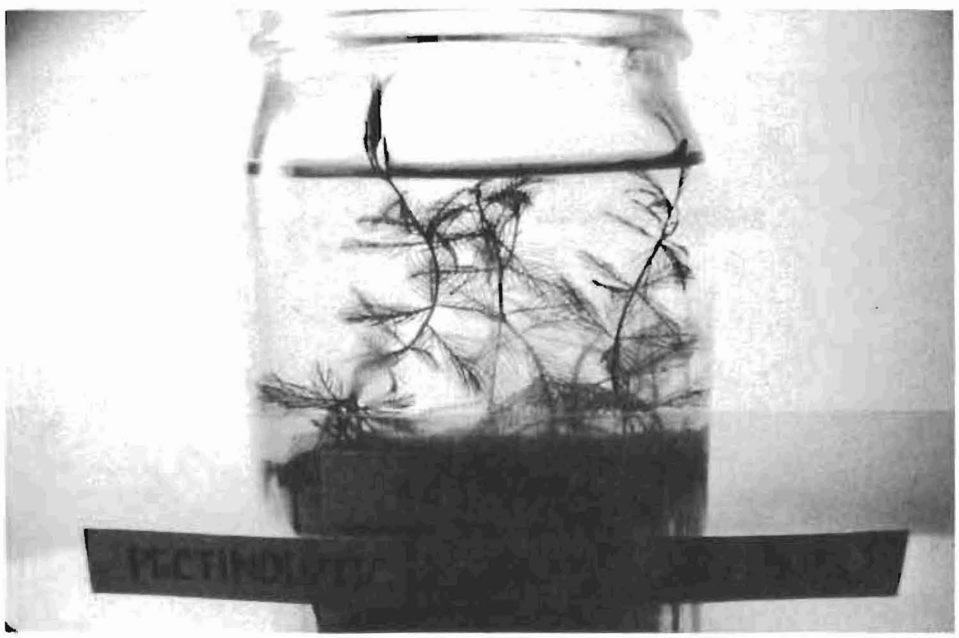

Figure 5. *M. spicatum* 14 days after treatment with pectinolytic bacterial Isolates MS-11 and P-8. Note abnormal internode elongation

Figure 6. *M. spicatum* 14 days after treatment with *M. terrestris*

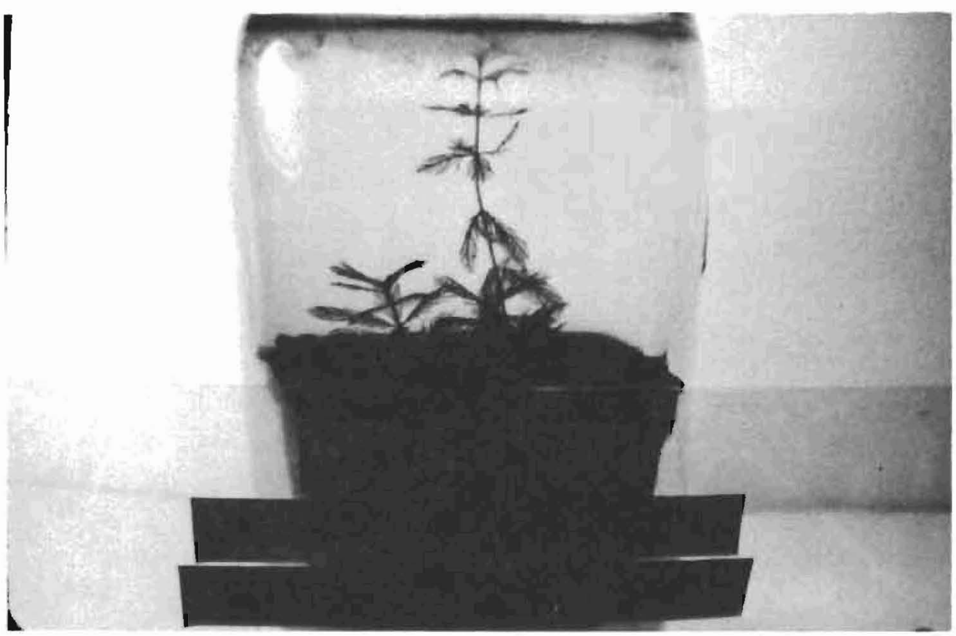

Figure 7. *M. spicatum* 14 days after treatment with *M. terrestris* pectinolytic bacterial Isolate MS-11. Note necrotic condition of plant remains

Isolates MS-11 and P-8. Here, there is a distinct elongation of the internode distance which appears similar to the effect previously observed with the plant hormone Gibberellin. A similar distortion in growth may be seen (Figure 6) in which the plants have been treated with *M. terrestris* Isolates 2F and SB for 2 weeks. Most striking are the reduced growth and deterioration of the plants, observed after inoculation with both *M. terrestris* Isolate 2F and pectinolytic-bacterium Isolate MS-11 (Figure 7). Little growth has occurred, and the plant is stunted and necrotic.

Figure 8 shows a view of the control pool treated with xanthan alone. The plants were green, bearing full healthy leaves. Leaf density, angle of growth, and size appeared normal as did the internodal distance. The plant population showed some deterioration related to tip separation and seasonal dieback typical in New England at this time. Significantly the water remained clear at all times.

In contrast, in pool B (Figure 9) inoculated with *M. terrestris* Isolate 2F and pectinolytic-bacterium Isolate MS-11, a dense brown coating was observed on all plants throughout the experiment. The water turned yellow-brown during the second week and continued to darken. By the fourth week there was a significant increase in the internodal length, and the leaves appeared abnormally developed. After the second week, green tips were only sparsely present on plants, indicating the decline of the plant population. At the close of the experiment (8 weeks) the pool gave off an odor of decaying plant material, reflecting the necrotic condition of the plant population.

In Figure 10, we see the effects of treatment with *M. terrestris* Isolate SB and pectinolytic bacterium Isolate P-8. In this instance the plants were covered with a dense brown mat of organic material for the first few weeks which slowly dissipated during the course of the experiment. As in pool B, the water appeared brown and

Figure 8. Pool A - Treatment 1. Control *M. spicatum* received only xanthan. Normal growth cycle and little necrotic tissue present

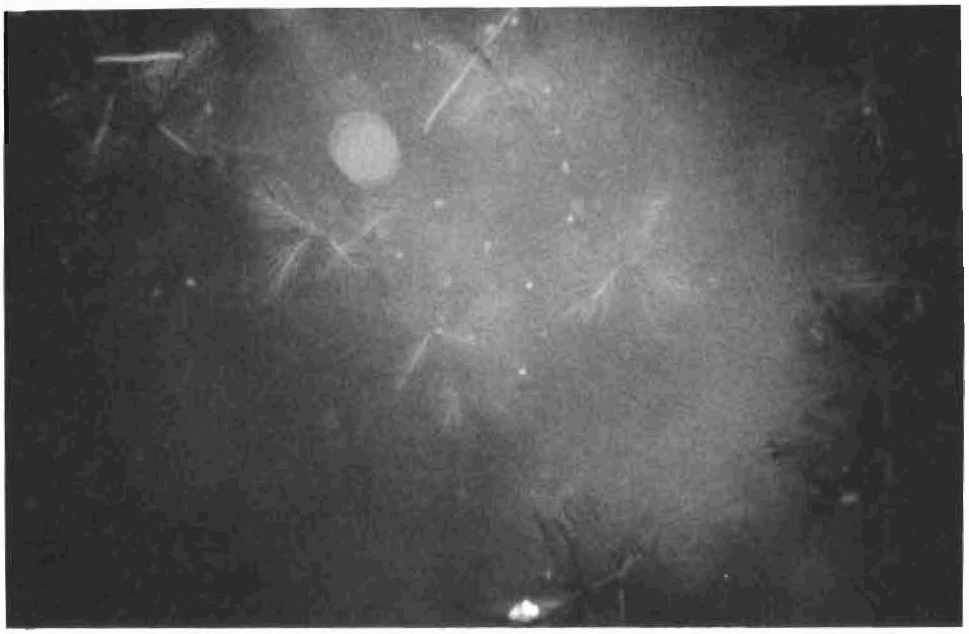

Figure 9. Pool B - Treatment 2. *M. spicatum* treated with *M. terrestris* Isolate 2F and pectinolytic bacterial Isolate MS-11. Note copious disintegration of plant material and turbidity due to plant debris

Figure 10. Pool C - Treatment 3. *M. spicatum* treated with *M. terrestris* Isolate SB and pectinolytic bacterial Isolate P-8. Note similar disruption of plant tissue and turbidity due to decomposing plant debris

darkened from the second week on, though less intensely. Again, the internodal distance was increased relative to the control plants, and leaf development was abnormal. At the termination of the experiment, the majority of the plants had been reduced to necrotic tissue and an odor of organic decay was evident.

The plants in pool D, inoculated with *Fusarium* sp. Isolate MH3F and pectinolytic bacterium Isolate MS-11, appeared green and healthy (Figure 11). The internodal distance was greater than in the control plants, and the leaves were more developed. This may be attributed to the hormone-mimicking effect induced by the pectinolytic bacterium, but the presence of *Fusarium* sp. did not result in any pathogenic effects. No dark coating of organic material was observed on the plants in this treatment. Plants had produced leaders by the second week of the experiment and new growth appeared after 4 weeks. Seasonal tip separation and decay appeared as in the control pool. No odor of decay was noted. The growth cycle of the plants appeared to be approximately 1 week behind that of the control.

The plants in pool E (Figure 12), treated with *Fusarium* sp. Isolate MH3F and pectinolytic bacterium Isolate P-8, appeared similar to those in pool D but somewhat more robust. Their growth cycle appeared in synchrony with the plants in the control pool. Leader growth did not appear until the fourth week of the experiment. A coating of organic material had dissipated by the fifth week at which time the leaves were a paler shade of green than those in the control pool or pool D. The water remained clear until the termination of the experiment at which time the plants were as healthy as the control population.

Randomly collected plants typical of the populations in their respective pools are shown in Figure 13. Control plant A appears green with short internode distances

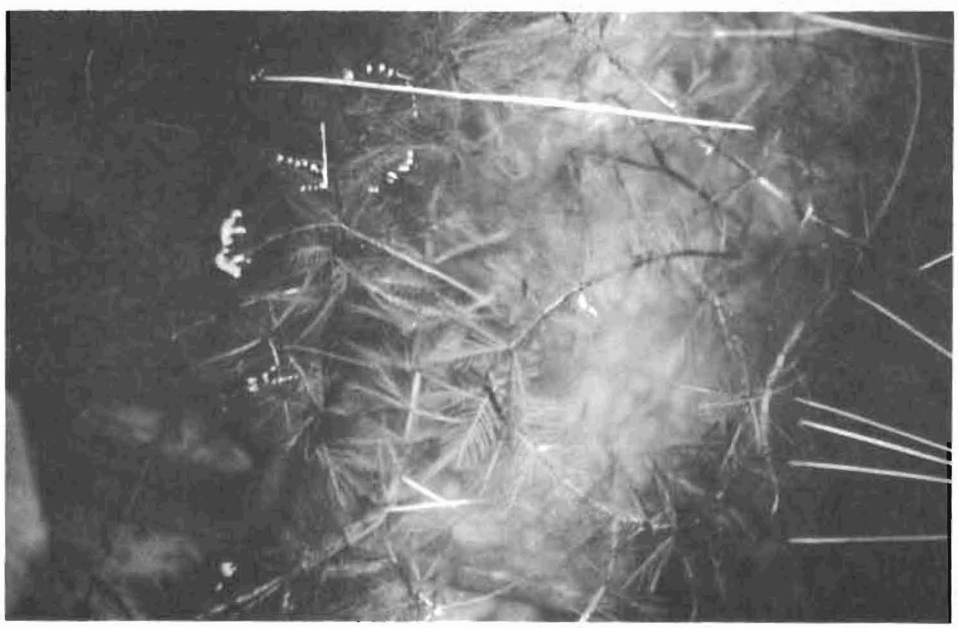

Figure 11. Pool D - Treatment 4. *M. spicatum* treated with *Fusarium* sp. Isolate MH3F and pectinolytic bacterial Isolate MS-11. Note internodal elongation and production of leaders but without significant tissue deterioration

Figure 12. Pool E - Treatment 5. *M. spicatum* treated with ***Fusarium*** sp. Isolate MH3F and pectinolytic bacterial Isolate P-8. Plant growth appears stimulated without significant tissue deterioration

Figure 13. Representative *M. spicatum* plants randomly selected from the various experimental pools. A = Treatment 1, unstressed normal growth; B = Treatment 2, internodal elongation, plant entirely necrotic and covered with organic debris; C = Treatment 3, similar response and appearance to Treatment 2; D = Treatment 4, moderate internodal elongation with little necrosis; E = Treatment 5, moderate internodal elongation with little necrosis

and little organic debris accumulated. It is significant that each of the other treatments in which a pectinolytic bacterium was present resulted in increased stem elongation. However, only the presence of *M. terrestris* (plants B and C) was associated with extensive deterioration of the plant. In these instances the tissue appeared necrotic, organic debris accumulated, and there was a general dieback of the population. The *Fusarium* sp. Isolate was unable to exercise a pathogenic role even when the plants were stressed by inoculation with pectinolytic bacteria.

Figures 14-23 provide a record of changes in the microbial populations in the test pools during the course of the experiment. In general it was observed that, as might be expected, the numbers in the water profile were about three orders of magnitude, or more, lower than the numbers recovered from the plant surface. This reflects primarily the dilution of substrate availability and corresponding reduction in population numbers. The water-profile populations, however, followed a pattern roughly similar to the plant surface microfloral distribution. This would suggest that the dominant variable in determining distributions of microbial populations in aquatic ecosystems in which *M. spicatum* is preeminent is, in fact, the nutrient substrate provided by the plant and its subsequent decomposition products.

In situ population distribution on the plant surface appears to be determined by site availability as well as by the intimate interactions of competing microbial populations. This would be reflected in the successful colonization of the plant surface by *M. terrestris* as a consequence of the ecological advantage afforded it by its antagonistic capacities.

Another major variable observed in all instances was the effect of ambient temperature (Table 2). During weeks 4 and 5 when temperatures fell significantly, there was a corresponding drop in population counts in the water profile. There was

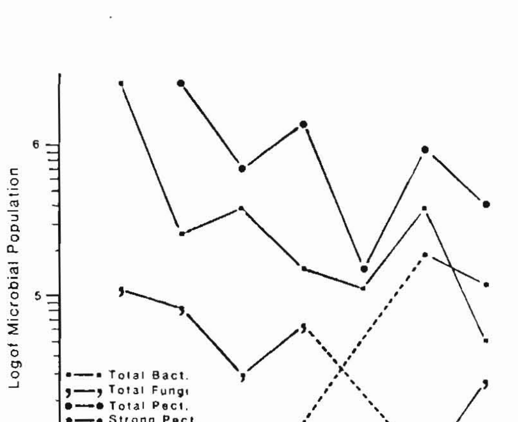

Figure 14. Microbial populations defined by selective growth media recovered from *M. spicatum* surfaces without prior inoculation

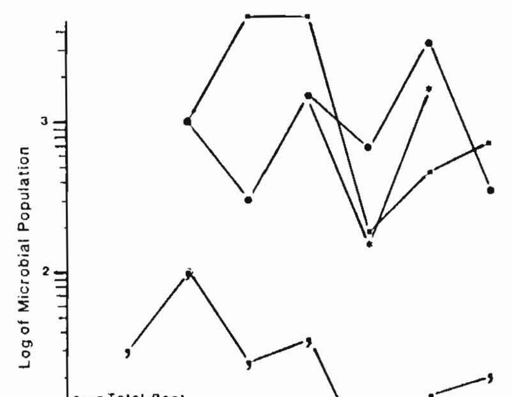

Figure 15. Microbial populations defined by selective growth media recovered from the water profile in pools containing *M. spicatum* after inoculation with MH3F and P-8

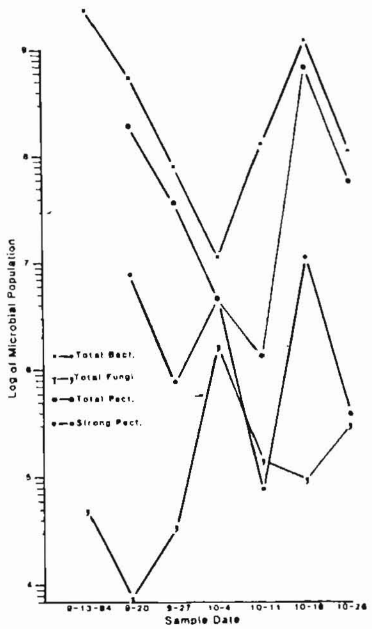

Figure 16. Microbial populations defined by selective growth media recovered from *M. spicatum* surfaces after inoculation with MT2F and MS-11

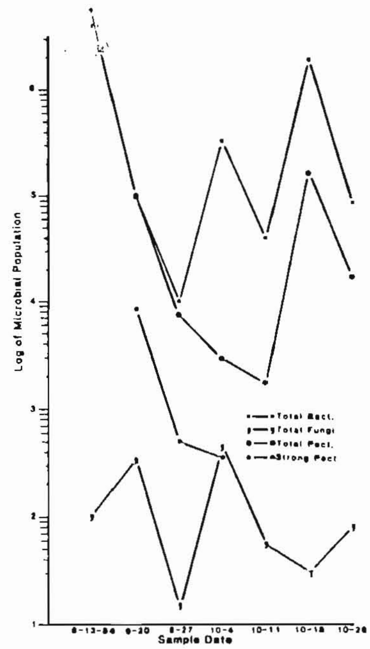

Figure 17. Microbial populations defined by selective growth media recovered from the water profile in pools containing *M. spicatum* after inoculation with MT2F and MS-11

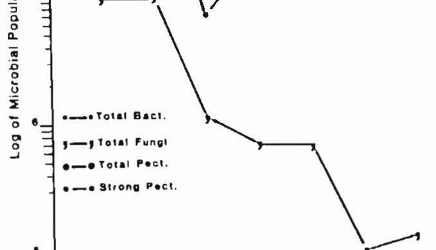

Figure 18. Microbial populations defined by selective growth media recovered from *M. spicatum* surfaces after inoculation with MTSB and P-8

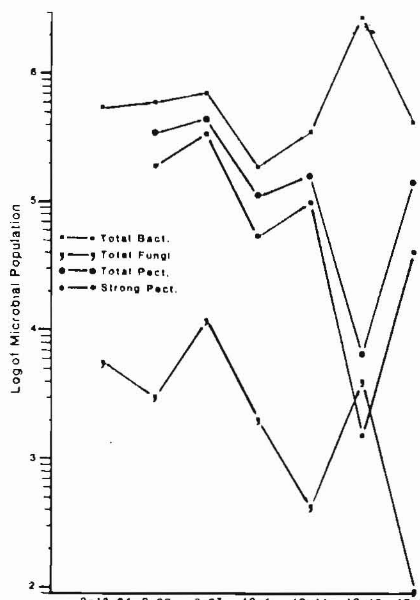

Figure 19. Microbial populations defined by selective growth media recovered from the water profile in pools containing *M. spicatum* after inoculation with MTSB and P-8

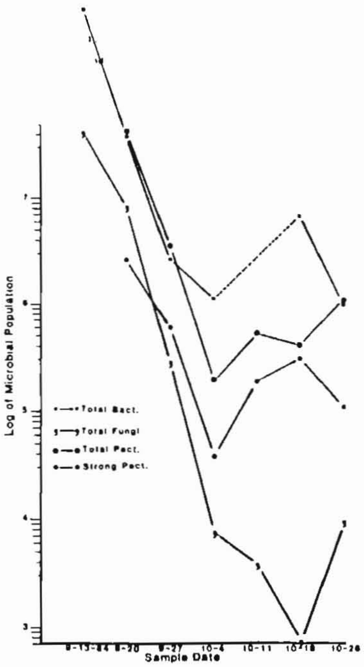

Figure 20. Microbial populations defined by selective growth media recovered from *M. spicatum* surfaces after inoculation with MH3F and MS-11

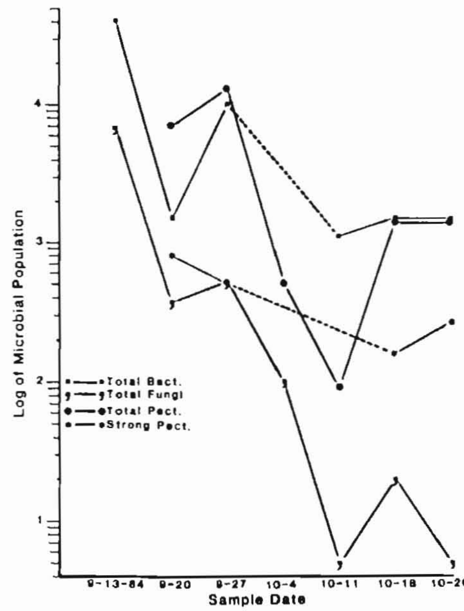

Figure 21. Microbial populations defined by selective growth media recovered from the water profile in pools containing *M. spicatum* after inoculation with MH3F and MS-11

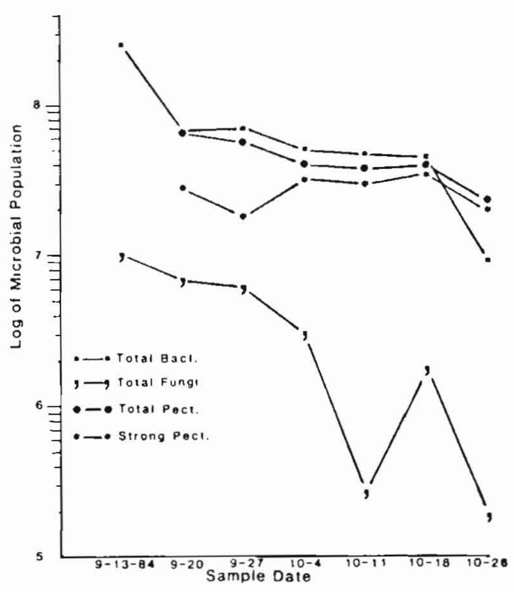

Figure 22. Microbial populations defined by selective growth media recovered from *M. spicatum* surfaces after inoculation with MH3F and P-8

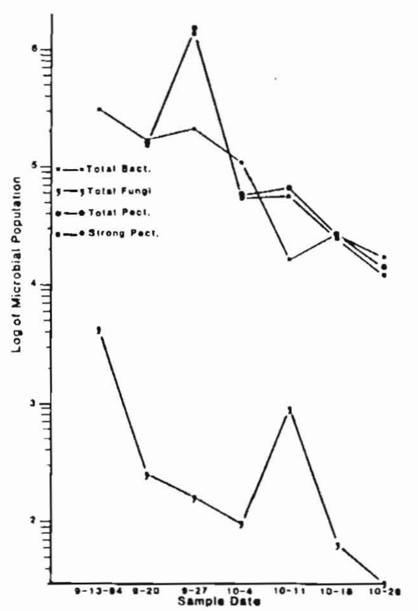

Figure 23. Microbial populations defined by selective growth media recovered from the water profile in pools containing *M. spicatum* after inoculation with MH3F and P-8

a smaller drop in the numbers recovered from plant surfaces as well, presumably reflecting the advantage provided by the sustained nutrient resource provided by the plant.

A third generalization concerning the microbial populations among the various treatments is the ultimate limitation to colonization of plant surfaces. This was reflected in the decline to stable numbers among all treatments except 2 and 3 approximating the levels observed in the control pool. The limiting mechanism may be spatial or may reflect the metabolic defense systems exercised by the plant as well.

In the control pool data (Figures 14 and 15) the dilution effect was pronounced in going from plant surface population to numbers in the water profile, a pattern which appeared in all treatments. Fungal counts corresponding to general population distribution were accordingly consistently reduced. An interesting phenomenon was the emergence of a strongly pectinolytic population on the plant surface during the concluding weeks of the experiment though the plants remained green and healthy. This may reflect the increased tip separation which took place resulting in greater pectin availability. This suggests that the appearance of this floral component reflects the seasonal change and a greater predisposition in the plants to pectinolytic attack.

Shown in Figures 16 and 17 are the population changes after inoculation with *M. terrestris* Isolate 2F and PB Isolate MS-11. Initial counts were several orders of magnitude higher on the plant surface than in the water profile. However, as noted, the final counts (particularly in the water profile) ultimately fell close to control levels. Although remaining higher on the plant surface, they showed a pattern which appeared to reflect competition for sites.

Figures 18 and 19 showing population patterns after inoculation with *M. terrestris* Isolate SB and PB Isolate P-8 reveal an interesting departure in the sustained numbers of bacteria, particularly pectinolytics, on the plant surface. This in fact reflects the rapid growth rate of Isolate P-8. Again, a strong pectinolytic flora asserted itself while fungal numbers were reduced. This may also reflect fungal incompatibility with the dominant bacterial populations.

The consistently high number of pectinolytic bacteria found may reflect the fact that pectinolytic-bacterial Isolates P-8 and MS-11 are stimulated by shikimic acid, a phenolic breakdown product of *M. spicatum*, and may therefore be selectively adapted to a relationship with the plant. Other phenolic breakdown products, protocatechuic acid and ellagic acid, which have been reported to have a selectively inhibitive effect on aquatic microorganisms, were found to have no effect on these isolates. It is significant that in both treatments with *M. terrestris*, where plant death was observed, fungal numbers remained relatively low while pectinolytic numbers remained at a sustained high level. This reflects the differential growth rate of these two populations and presumably their relative roles in effecting the decline of the plant. This mechanism of control would appear to be confirmed in the population distributions shown in Figures 20 through 23. In this instance the numbers of pectinolytic bacteria again remained relatively high while the numbers of fungi declined. This decline suggests the inability of the *Fusarium* sp. to gain access to the plant. Accordingly, unlike the treatment with *M. terrestris*, the plants remained viable, suggesting the need for appropriately interactive agents to bring about the ultimate decline of the plant.

It may also be noted from these data (Figures 14-23) that the bacterial numbers were lowest on the plant surface when the plants were healthiest. These numbers appear to reflect two divergent processes: (a) the capacity of the plant to generate its own protective mechanism; and (b) the evolution of compatibility between plant and microbial populations, as evidenced by the sustained numbers of pectinolytic bacteria present on the plant surface. It is this latter propensity that, in our estimation, provides the key to an ecologically rational biological control system.

The biomass measurements resulting from the various treatments are recorded in Table 3. In effect these data reflect the excess growth in all treatments over the control since plant stress was achieved through stimulation of abnormal plant growth. This stimulation may be the result of two factors:

- The presence of the PB P-8 and PB MS-11 with their demonstrated ability to stimulate plant elongation.
- The addition to each pool of a nutrient increment when the entire culture medium with its mineral salts base was added as inoculum.

Clearly, both these factors enhanced plant biomass, although in the case of the treatments with *M. terrestris* these factors were only a prelude to the eventual decline and death of the plants. It must be concluded, however, that under these circumstances biomass may be a misleading measure of control efficacy and another parameter (perhaps more quantitatively descriptive) applied.

Table 3
Comparative Efficacy of Various Microbial Inocula in the Control of *Myriophyllum spicatum* as Measured by Relative Biomass Harvest

	*Biomass**		*Pair T - Test†*			
Treatment	*Mean***	*STD*	*Treatment 1*	*Treatment 2*	*Treatment 3*	*Treatment 4*
Control	2.37	0.546				
MT2F + MS-11	2.591	0.541	1.39			
MTSB + P-8	3.009	0.393	4.09[a]	-3.19[a]		
MH3F + MS-11	3.091	0.578	4.72[a]	-2.72[b]	-0.48	
MH3F + P-8	2.945	0.853	3.07[b]	-1.46	0.31	0.62

NOTE: Treatment 1 = inoculated with *Mycoleptodiscus terrestris* Strain 2F, and a pectinolytic bacterium, Strain MS-11.

Treatment 2 = inoculated with *M. terrestris* Strain SB and a pectinolytic bacterium, Strain P-8.

Treatment 3 = inoculated with *Fusarium* sp. Strain MH3F and Strain MS-11.

Treatment 4 = inoculated with *Fusarium* sp. Strain MH3F and Strain P-8.

* gm dry weight (105°C 24 hr) per tray of 20 plants.

** Mean of biomass of 23 trays in Treatment 1 and 22 trays in Treatments 2, 3, 4.

† (a) Highly significant difference level at $P(t) \leq 0.01$.
(b) Significant difference level at $P(t) \leq 0.05$.

DISCUSSION

These results provide a significant demonstration of the ability of biological agents derived from the plant ecosystem to control *M. spicatum* under simulated field conditions. The complex nature of plant pathogenesis renders simplistic the notion of any direct application of a microorganism resident on *M. spicatum* as an agent of the plant's destruction. Clearly the plant's defense systems militate against such an easy assault. Moreover, the influence of environmental factors, such as temperature, may also directly and indirectly affect the dynamics of microbial populations. Indeed, Cullen and Andrews,* in a wide-ranging review of biological control strategies, emerge with a clear recognition of the importance of environmental factors, topographic features on the plant surface, and even plant exudates over competitive interactions among microorganisms in the phyllosphere.

Thus, microorganisms derived from the plant ecosystem must be specifically adapted to treat with at least three strategies of plant defense:

- The generation of inhibitory plant metabolites.
- Competition for eco-sites on the plant surface.
- The ability of the control agents to breach the integrity of the plant's structural elements.

The microbial isolates which have been successful under this testing regimen appear to meet these constraints. The *M. terrestris* with its antagonistic capacities clearly preserves for itself an explicit eco-niche on the plant surface. Similarly, the pectinolytic-bacterial Isolates MS-11 and P-8 appear selectively stimulated by the *M. spicatum*, responding to one of its breakdown products, shikimic acid, while remaining undeterred by other phenolics known to inhibit a variety of aquatic microorganisms. These microorganisms find their way through the plant's defenses by stressing its metabolism through stimulation of abnormal growth. Subsequently, the fungus and bacteria conclude their attack through the release of tissue destructive enzymes, in this instance, cellulolytic and pectinolytic. It remains now to broaden the scope of application and to refine application systems to appropriate field dimensions.

* D. Cullen and J. H. Andrews. 1984. "Epiphytic Microbes as Biological Control Agents," *Plant Microbe Interaction*, T. Kosurge and E. W. Nestor, eds., Macmillan, New York, pp 381-399.

BIOLOGICAL CONTROL TECHNOLOGY DEVELOPMENT

Status Report on a Survey of the Continental United States for Pathogens of Eurasian Watermilfoil

by
William C. Zattau*

INTRODUCTION

The Waterways Experiment Station (WES) is conducting research on the use of microbial agents for the biological control of problem aquatic plants. Eurasian watermilfoil (*Myriophyllum spicatum* L.) is considered to be one of the most troublesome aquatic plants in waters for which the Corps has primary responsibility. It develops to troublesome population levels in many states.

Microbial agents have only recently been considered for the biological control of troublesome aquatic plants, and investigations of viruses, fungi, bacteria, and nematodes as potential biocontrol agents of Eurasian watermilfoil have been very limited. Other control methods for Eurasian watermilfoil (e.g., herbicides, mechanical harvesting) normally offer short-term relief of the problem, whereas biological control agents, when successfully established, offer long-term control. These shortcomings of currently used control methods support the need for a concerted effort to find effective agents for the biological control of Eurasian watermilfoil.

In recent years, unexplained diebacks of Eurasian watermilfoili have occurred in areas where the plant has been a problem for decades, mainly in the eastern United States. These areas of diebacks may indicate the presence of biological control agents.

In order to exploit these phenomena, WES is conducting a 2-year survey for pathogens of Eurasian watermilfoil. Work to be done in this study consists of a survey for fungi and bacteria in plant tissues. The study also includes the inoculation of healthy Eurasian watermilfoil, grown in controlled optimal conditions, to verify pathogenicity of the microbial agents obtained and their isolation from the diseased tissues.

The objective of this study is to isolate and identify microbial agents that are pathogenic on Eurasian watermilfoil. This study is limited to water bodies within the continental United States and is to include, but is not limited to, those areas which have experienced mysterious diebacks (Figure 1).

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* US Army Engineer Waterways Experiment Station, Vicksburg, Mississippi.

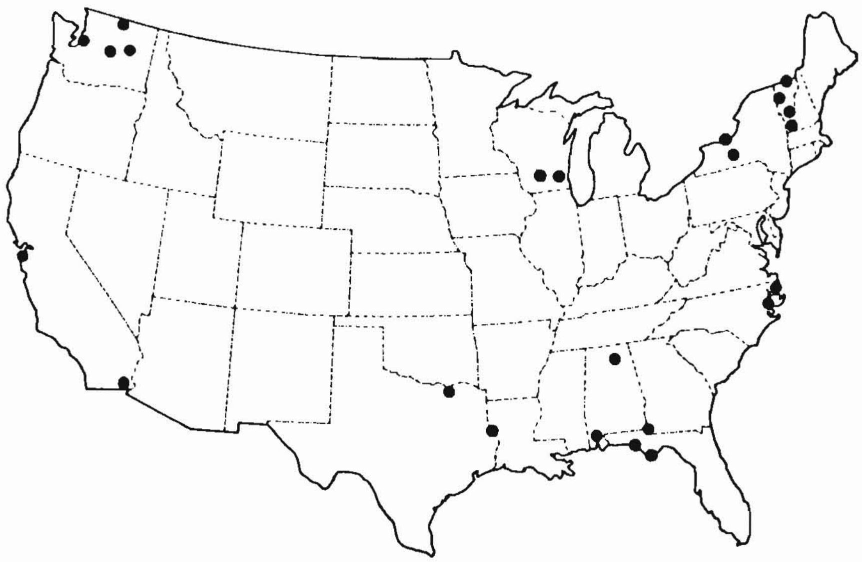

Figure 1. Sampling sites for pathogens of Eurasian watermilfoil

METHODS AND MATERIALS

Samples of Eurasian watermilfoil were collected at sampling sites (Table 1) either from shore or by boat. Sites were initially scanned for diseased vegetation and, if present, 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 and marked with an identifying number and placed in an iced cooler. Upon return to the laboratory, samples were placed in a refrigerator at 7° to 8°C.

Plant material was initially processed by three washings with sterile distilled water to remove debris and placement in a translucent plastic box on a light table where transmitted light passing through the plant highlighted diseased areas. Closer observation of samples was done with a stereo dissecting microscope.

Diseased plant tissue (Figures 2-5) was surface sterilized by dipping into a dilute solution of sodium hypochloride for 60 sec. Small pieces of tissue were aseptically cut from diseased areas with a sterile scalpel and plated on plates of either Potato Dextrose Agar (PDA) or Nutrient Agar (NA). PDA is a selective media for fungi whereas NA is selective for bacteria. The petri plates were incubated at 28°C in total darkness for 3 to 5 days. Fungal or bacterial colonies were subcultured onto fresh plates of the appropriate media until pure cultures were obtained. Isolates were maintained in screw-top PDA or NA test tubes under constant refrigeration.

RESULTS

The first year of the survey has been completed. Forty-three bodies of water, consisting of ponds, lakes, rivers, and canals located in eleven states, have been examined for evidence of disease symptoms associated with the noxious aquatic weed species (Table 1). To date, in excess of 450 fungal and bacterial isolates have

Table 1
Sites Sampled for Pathogens of Eurasian Watermilfoil

Northeast	*Southeast*
Cayuga Lake (N.Y)	Guntersville Reservoir (Ala.)
Sodus Bay, Lake Ontario (N.Y.)	Mobile Bay (Ala.)
Lake Bomoseen (Vt.)	Apalachicola (Fla.)
Lake Carmi (Vt.)	Deer Point Lake (Fla.)
Lake Glen (Vt.)	Lake Seminole (Fla.)
Lake Hortonia (Vt.)	Waukulla River (Fla.)
Lake St. Catherine (Vt.)	
Metcalf Pond (Vt.)	Carrituck Sound (N.C.)
St. Albans Bay, Lake Champlain (Vt.)	Pamlico River (N.C.)
	Toledo Bend Reservoir (La.)
Northwest	*Southwest*
Banks Lake (Wash.)	
Lake Osoyoos (Wash.)	Pat Mayse Reservoir (Tex.)
Lake Washington (Wash.)	
Rock Island Reservoir (Wash.)	Imperial Valley Irrigation District (Calif.)
Rocky Reach Reservoir (Wash.)	Lower Crystal Springs Reservoir (Calif.)
Wells Reservoir (Wash.)	Pelarcitos Reservoir (Calif.)
	San Andreas Reservoir (Calif.)
Midwest	
Lac La Belle (Wis.)	Lake Waubesa (Wis.)
Lake Fowler (Wis.)	Lake Wingra (Wis.)
Lake Kegonsa (Wis.)	Lottes Lane (Wis.)
Lake Lilly (Wis.)	Lower Phantom Lake (Wis.)
Lake Mendota (Wis.)	Ottawa Lake (Wis.)
Lake Oconomowoc (Wis.)	Pine Lake (Wis.)
Lake Pewaukee (Wis.)	Whitewater Lake (Wis.)

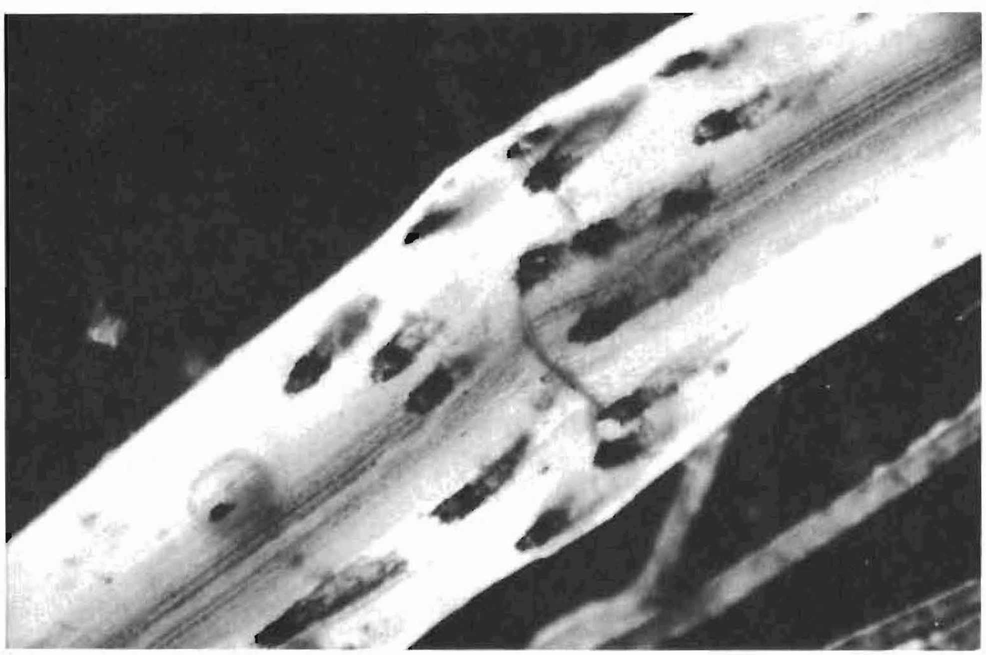

Figure 2. Insect feeding scars provide entry points for invasive microbes into Eurasian watermilfoil stem

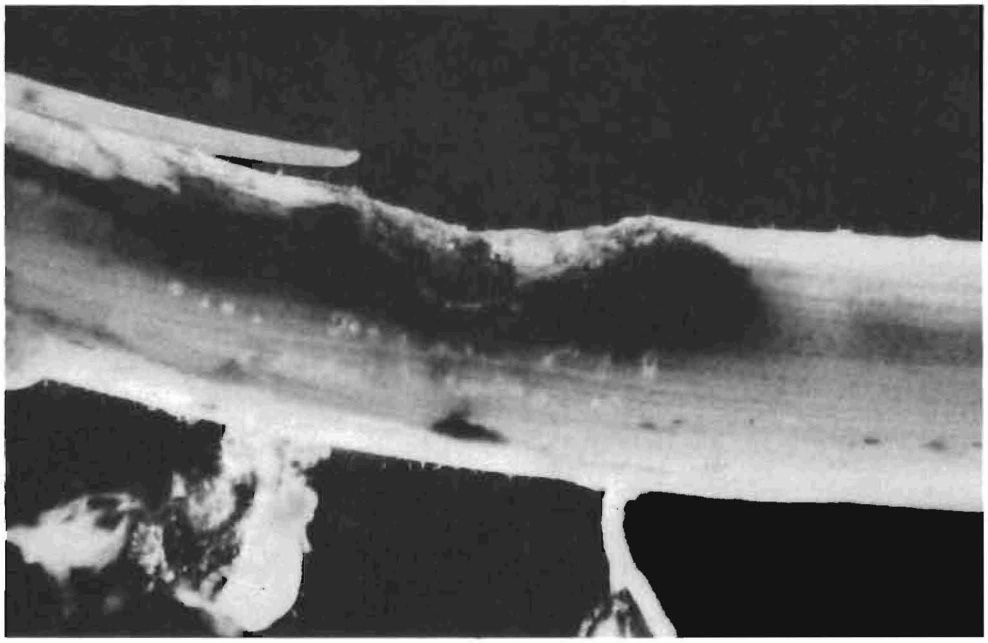

Figure 3. Infected area on Eurasian watermilfoil stem from which both fungi and bacteria were isolated

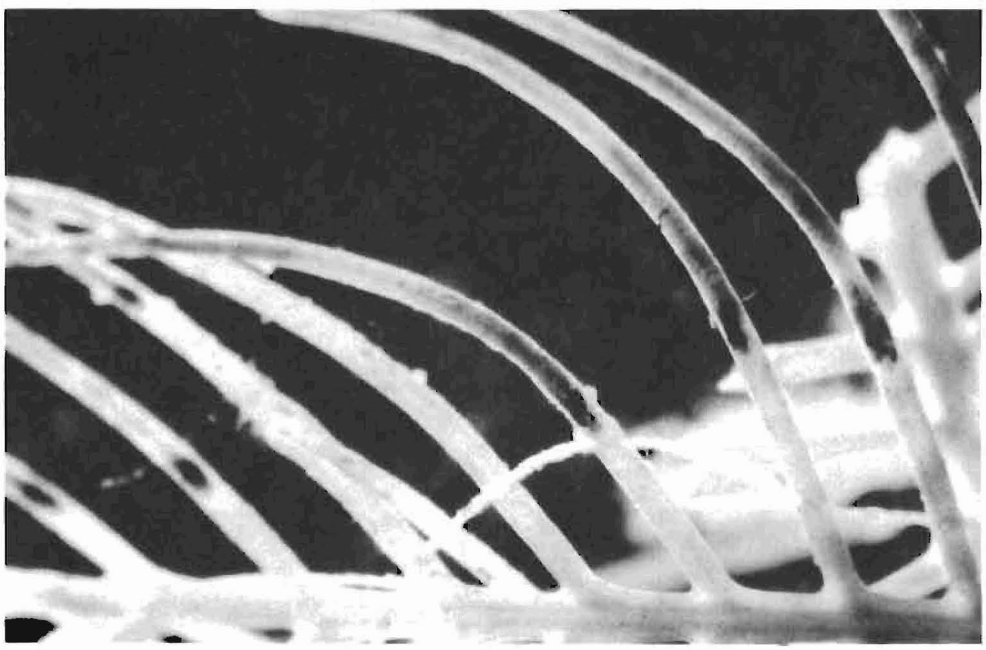

Figure 4. Eurasian watermilfoil leaf divisions showing disease symptoms

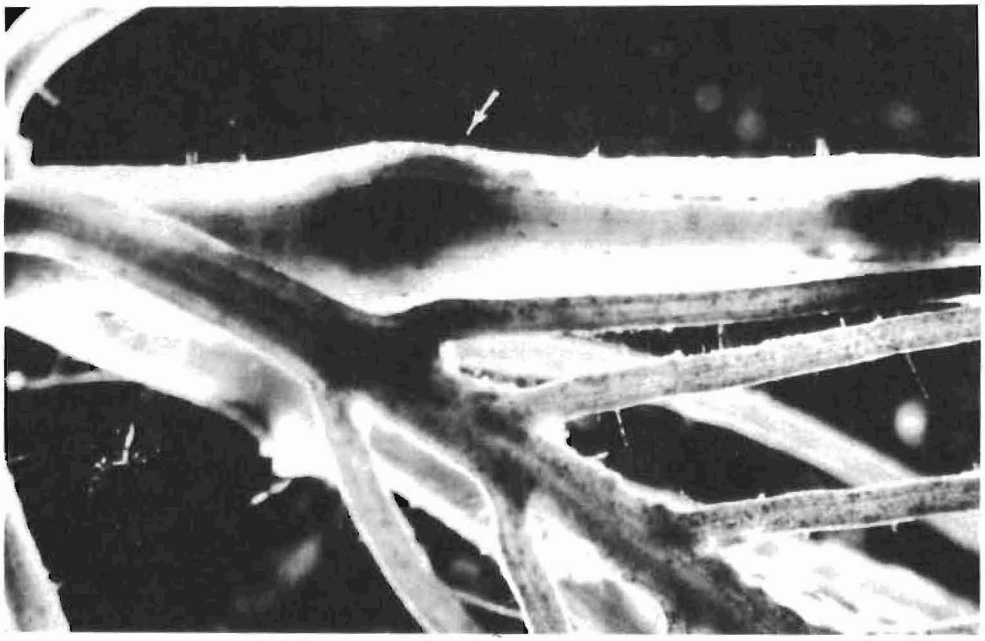

Figure 5. Internal diseased area causing swelling of Eurasian watermilfoil stem (arrow)

been collected in pure culture. Many of these isolates appear to be duplicates and it is likely that some will be proven to be saprophytes.

FY 85 OBJECTIVES

The objectives for FY 85 are as follows:

a. A second round of sampling at all sites currently under study as well as sampling of any additional sites determined to contain diseased plants will be conducted.

b. Isolates will be screened for cellulase and pectinase production on selective media to determine presence of lytic enzyme production.

BIOLOGICAL CONTROL TECHNOLOGY DEVELOPMENT

The Control of Waterhyacinth With *Neochetina:* Status of the Final Report

by
R. Michael Stewart*

A final report is in preparation on the use of *Neochetina eichhorniae* and *Neochetina bruchi* as biological control agents of waterhyacinth. The report will consolidate all relevant information collected under the Aquatic Plant Control Research Program since the introduction of *N. eichhorniae* into the United States in 1972. Summaries of the studies included in the report have recently been given for Florida by Center and Durden (1984), for Louisiana by Sanders (1984), and for Texas and California by Cofrancesco (1984). Additional information for Texas is given by Cofrancesco (1985) and for California by Stewart (1985).

Chapter 1 will present the information on the dates and sites of releases of both species of *Neochetina* in Florida, Louisiana, Texas, and California. Where possible, establishment success will be evaluated and a discussion of natural dispersal from the release site will be provided.

Chapters 2 and 3 will deal chiefly with information regarding control efficacy demonstrated at the field sites within the various states. Though direct, comprehensive comparisons cannot be made between all of the studies due to inconsistencies in data collection among the various researchers involved, general comparisons of similar trends in the data that monitored fluctuations in the *Neochetina* and waterhyacinth populations will be made.

Chapter 4 will present results of miscellaneous studies pertinent to the life history and ecology of *Neochetina*. Topics will include such items as (a) within-shoot dispersion of weevil eggs and larvae, (2) within-shoot dispersion of adult weevil feeding, and (3) a population index based on the number of adult weevil feeding scars.

Chapter 5 will provide a comprehensive discussion of all subject matters relating to the use of *Neochetina* as a biological control agent of waterhyacinth. Results of studies conducted in the United States will be compared to similar studies in other countries where *Neochetina* has been introduced. Recommendations will be made regarding the future role of *Neochetina* in waterhyacinth control programs. In addition, crucial unanswered questions necessary for the maximization of *Neochetina* effectiveness as an operational control of waterhyacinth will be presented.

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* US Army Engineer Waterways Experiment Station, Vicksburg, Mississippi.

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BIOLOGICAL CONTROL TECHNOLOGY DEVELOPMENT

Biological Control of Waterhyacinth and Alligatorweed in Galveston District and at Jean Lafitte Park, Louisiana

by
Al F. Cofrancesco, Jr.*

GALVESTON DISTRICT

Background

Waterhyacinth and alligatorweed have caused numerous problems in the aquatic environment throughout the southern United States (Coulson 1977). The US Army Corps of Engineers has been studying various methods to control these introduced plant species. Studies on the use of biological controls were begun in 1950 and the first agent was released in 1964 on alligatorweed. Over the next 15 years a total of six insect species were approved for introduction in the United States to control these two plant species. The three exotic insects utilized to control alligatorweed are *Agasicles hygrophila, Amynothrips andersoni,* and *Vogtia malloi.* The three insects impacting waterhyacinth are *Neochetina eichhorniae, Neochetina bruchi,* and *Sameodes albiguttalis.*

The biocontrol study being conducted for the US Army Corps of Engineers, Galveston District, for the control of alligatorweed and waterhyacinth was begun in 1979. Prior to the initiation of this project, the use of biocontrol agents on problem aquatic plants in Texas was minimal (Cofrancesco 1984). Initially, a survey of Texas was conducted in 1979 and afterwards six primary study sites were established. These sites were utilized to evaluate the ability of the biocontrol agents to become established and to monitor their impact on the particular plant species in the Texas environment.

Purpose

This study was designed to (a) determine the impact of the biocontrol agents present on alligatorweed and make recommendations on their management and (b) establish the three biocontrol insects on waterhyacinth in nursery areas in Texas and evaluate their potential for waterhyacinth control.

Current status and accomplishments

Alligatorweed. This exotic plant species is currently present in many areas of Texas; however, the biocontrol insects appear to be restricting its growth and distribution in many areas. The greatest impact of the biocontrol agents appears to occur late in the growing season when insect populations are greatest. *Agasicles hygrophila* and *V. malloi,* which mainly attack the aquatic morphotype of the plant, seem to be responsible for most of the impact.

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* US Army Engineer Waterways Experiment Station, Vicksburg, Mississippi.

Terrestrial alligatorweed is generally not impacted by *Agasicles* or *Vogtia*. *Amynothrips andersoni*, which does feed on the terrestrial morphotype, has been difficult to establish because of extreme environmental changes (i.e. water level fluctuation). The only population established for any length of time did produce a significant impact in the terrestrial mat at J. D. Murphree Wildlife Refuge; however, after an extended period of high water, no thrips could be found at the site. *Agasicles* and *Vogtia*, especially at Dam B, appear to be controlling the reintroduction of terrestrial alligatorweed into the aquatic environment.

Waterhyacinth. At the beginning of this project none of the biocontrol agents available for the control of waterhyacinth were present in Texas. The *Neochetina* nursery areas established in 1980 and 1981 show the greatest impact. *Neochetina bruchi* released in 1980 at Wallisville, Tex., underwent a rapid population growth (Figure 1). As the insect population developed, the waterhyacinth mat was reduced. Figure 2 shows the 1980 release site at Wallisville and a 1983 comparison, by which time the waterhyacinth population had been reduced by more than 90 percent. *Neochetina eichhorniae* was not found in Texas during the 1979 survey; however, a small population was noted at the J. D. Murphree Wildlife Refuge when a nursery area was being established in 1981 for *N. eichhorniae*. The population had probably migrated into the area from Louisiana where large migrating populations were noted in 1980. *Neochetina eichhorniae* releases were made at the J. D. Murphree Wildlife Refuge to supplement the small population. It is apparent from data collected at the Wallisville and the J. D. Murphree Wildlife Refuge sites that both species of *Neochetina* are able to reproduce and severely impact the waterhyacinth population in Texas.

The waterhyacinth moth *S. albiguttalis* was found at one location in the J. D. Murphree Wildlife Refuge in 1983; however, no *Sameodes* were found in 1984. The inability to locate *Sameodes* early in the population's buildup has been reported for releases made in Florida (Center, Durden, and Corman 1984) and Louisiana (Sanders and Theriot, In Press).

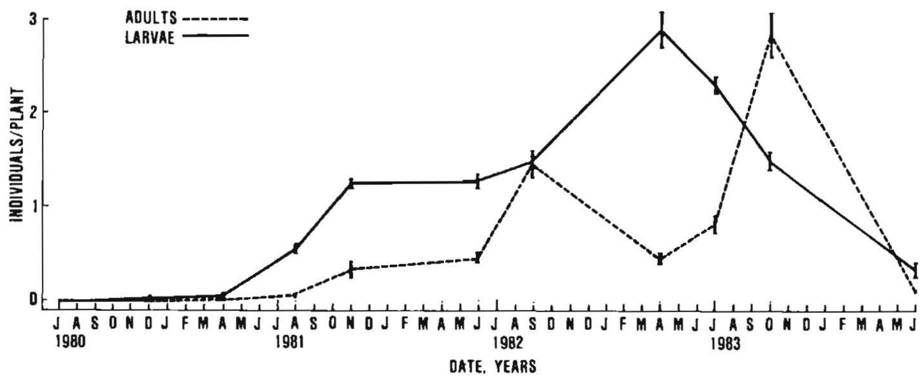
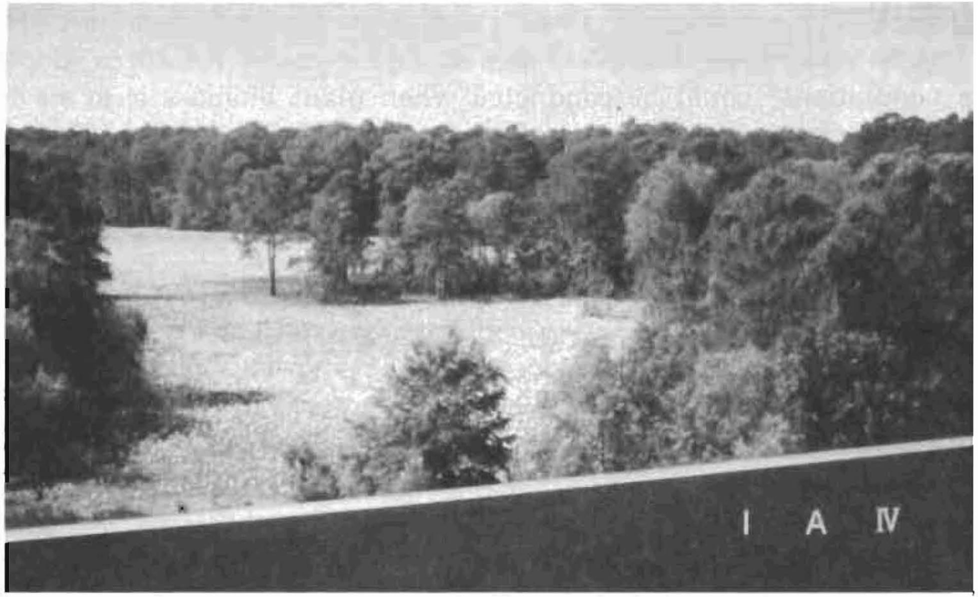


Figure 1. ***Neochetina*** **population at the Wallisville release site**



a. 1980—before weevils were released

b. 1983—after weevil impact

Figure 2. Wallisville release site before and after weevil release

Discussion and recommendations

The alligatorweed problem, especially the aquatic morphotype, can be controlled in Texas by the biocontrol agents. Insect populations need to be monitored so their impact can be maximized. Early releases of the biocontrol insects to supplement existing populations should be conducted when plant biomass is at its lowest. Control of the terrestrial morphotype of alligatorweed will be more difficult and it may require multiple releases each year of the *Amynothrips*. Environmental conditions should also be monitored for their impact on the biocontrol agents and target plants.

Waterhyacinth is still widely distributed in Texas from Brownsville up the coast to the Louisiana state line. *Neochetina* populations need to be dispersed so a rapid population growth will occur statewide. *Sameodes* releases need to be continued because indications from Florida and Louisiana suggest that this insect may play a more important role once the weevils start reducing the waterhyacinth population.

Aquatic plant managers always need to remember that biocontrol agents are tools for the control of aquatic plants and, consequently, need to be managed and maintained just like any other operational tool.

JEAN LAFITTE NATIONAL HISTORICAL PARK

Background

Jean Lafitte National Historical Park was established in 1978 and encompasses many sites throughout southern Louisiana. One site, just south of New Orleans, that is being developed is the Barataria Unit which contains approximately 20,000 acres of swamps and marshes. The major emphasis in this unit will be to canoe or hike through the miles of waterways and trails which allow visitors access to the typical habitats and wildlife of the area.

The canals, swamps, and marshes found within the Barataria Unit are very characteristic of the habitat in south Louisiana. Two exotic plant species, *Alternanthera philoxeriodes* (alligatorweed) and *Eichhornia crassipes* (waterhyacinth), have caused extensive problems in Louisiana. Alligatorweed, which grows in both the aquatic and terrestrial habitat, is extremely troublesome in that it out-competes native vegetation, and as it grows it is able to cover open water areas. Waterhyacinth is a floating plant species which reproduces sexually and vegetatively and has the ability to double its biomass in 4 to 6 weeks (Penfond and Earle 1948). Waterhyacinth is an extreme hindrance to navigation and when left untreated can close waterways.

In developing the Barataria Unit National Park, service personnel were aware of the problems that could arise from these two exotic plant species. Personnel of the Aquatic Plant Control Research Program at the Waterways Experiment Station (WES) were contacted by the National Park Service (NPS) and asked to develop a program for the control of these problem plant species without the use of herbicides. The Department of Interior, of which the NPS is a part, prohibits the use of herbicides in aquatic environments.

An initial survey was conducted in the Barataria Unit in July 1983 by WES personnel. The findings were submitted to the NPS along with recommendations on how to proceed with controlling alligatorweed and waterhyacinth. The WES was requested to submit a proposal on how to accomplish the recommendations made in the initial survey. This proposal was accepted by the NPS in June 1984.

Rationale and approach

Rationale. The initial survey of the aquatic plant problems in Jean Lafitte National Park revealed that a multi-faceted effort, centered on the use of both mechanical and biological control methods, would be required to address the problems. A mechanical control system would initially be needed to open currently blocked portions of the area, and to periodically remove regrowth in critical areas. Such a system would be implemented by NPS and would provide the required short-term problem relief. Biological control methods would be used to produce sufficient long-term stress on the target plants to reduce the frequency of required maintenance operations with the mechanical systems. The combined use of mechanical and biological control methods, together with a methodology for monitoring target plant population levels, would provide the most cost-effective aquatic plant management system available to NPS.

Approach. Initially, data would be collected to quantify the problem levels and growth rates of the target plant species, and to determine the level of control being provided by existing biocontrol agents. At the same time, additional species of biocontrol agents would be established on waterhyacinth and alligatorweed, and several mechanical/biocontrol management strategies would be evaluated. After sufficient quantitative data on population dynamics of the target plants species and biocontrol agents have been obtained, a strawman aquatic plant management plan will be developed, which the NPS can use as a basis for developing a long-term management plan.

Accomplishments and current status

Initial survey. The initial survey found both waterhyacinth and alligatorweed widely distributed throughout the Barataria Unit. Moderate populations of *Neochetina eichhorniae* (mottled waterhyacinth weevil) and small populations of *Sameodes albiguttalis* (waterhyacinth moth) were present on the waterhyacinth; however, no *N. bruchi* (chevroned waterhyacinth weevil) were found at any of the locations examined. Alligatorweed was present in both the aquatic and terrestrial morphotype. *Agasicles hygrophila* (alligatorweed flea beetle) and *Vogtia malloi* (alligatorweed stem borer) were found but their impact was spotty, indicating small populations. The only biocontrol agent which feeds on the terrestrial morphotype of alligatorweed, *Amynothrips andersoni* (alligatorweed thrips), was not found during the survey.

Field studies. Field studies were initiated in August 1984. Nursery areas for *N. bruchi* were established at five locations (Figure 3). Adult weevils were released at sites 1N and 2N and plants infected with all life stages of *Neochetina* were released at sites 1P, 2P, and 3P. Ten survey sites (1-10) were established to monitor the insect populations throughout the Barataria Unit. Nursery areas were established for *A. andersoni* at sites 1A and 2A, and insect monitoring of alligatorweed was also conducted at these sites.

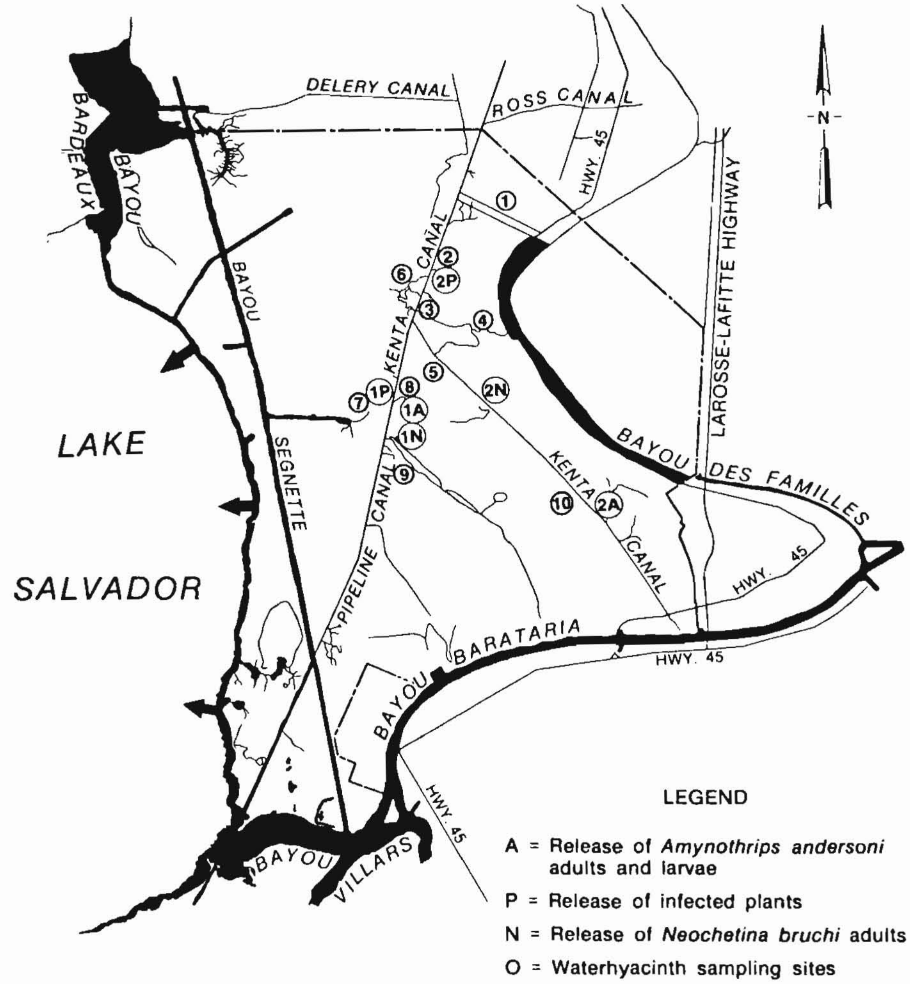


Figure 3. Barataria Unit of Jean Lafitte National Historical Park

Waterhyacinth and alligatorweed plants were monitored in August and October 1984. Reductions in the *Sameodes* populations were observed between the initial survey and the August and October sampling; however, the *N. eichhorniae* populations still remained at a moderate level. Flea beetle and stem borer damage was observed on alligatorweed mats and in many areas damage was severe by October.

In FY 85 additional releases of *N. bruchi* and *A. andersoni* will be conducted at the nursery areas. Early release of *S. albiguttalis* and *A. hygrophila* will also be conducted when the plant biomass is at its lowest. The population density of the biocontrol agents will continue to be monitored along with their impact on alligatorweed and waterhyacinth.

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BIOLOGICAL CONTROL TECHNOLOGY DEVELOPMENT

Biological Control of Waterhyacinth in the California Delta

by
R. Michael Stewart*

INTRODUCTION

The California Delta is an intricate network of rivers, sloughs, and navigation channels located at the confluence of the Sacramento and San Joaquin Rivers. The total acreages of waterhyacinth infestations in this area are not as severe as in the southeastern states. However, persistent invasions of Delta waterways by waterhyacinth from upstream locations in the southern San Joaquin River system have resulted in significant economic burdens to recreational, agricultural, and municipal users of the water resource.

Personnel of the Aquatic Plant Control Research Program (APCRP) at the Waterways Experiment Station (WES) have been involved in biological control activities of waterhyacinth in the California Delta since 1982. This project was initiated at the request of the California Department of Boating and Waterways (CDBW) through the Sacramento District (SPK). At present, all research and operational control efforts are being authorized and funded by the CDBW. Because the legislation giving the CDBW this authority was written to specifically address the needs for waterhyacinth control within the California Delta, waterhyacinth control activities have not been conducted in areas outside the California Delta.

PURPOSE AND OBJECTIVES

The purpose of this project was to apply biological control experimental technology, in conjunction with chemical and mechanical methods, within the California Delta to achieve control of the waterhyacinth infestation. Our objectives were:

- Conduct preliminary assessment of the extent of the waterhyacinth infestation within the Delta.
- Recommend which biological agents should be introduced into the California Delta.
- Petition for the introduction of these agents, and, subsequently, to establish populations of each within the Delta.
- Evaluate the control efficacy of each species and of various species combinations.
- Make recommendations regarding the most effective use of these agents in all areas of California infested with waterhyacinth.

* US Army Engineer Waterways Experiment Station, Vicksburg, Mississippi.

RESULTS AND DISCUSSION

Based on preliminary observations conducted in April 1982, three insect species were recommended for immediate introduction into the California Delta. Permission for these introductions was granted by State and Federal agencies for *Neochetina bruchi* in May 1982, and for *Neochetina eichhorniae* and *Sameodes albiguttalis* in February 1983. Collections of each species were made in southeastern states and were shipped to the US Department of Agriculture (USDA) quarantine facility, Albany, Calif., for species verification and parasite/pathogen examination. After clearing quarantine, collections of the two weevil species (*N. bruchi* and *N. eichhorniae*) were released directly into the field. Collections of the pyralid moth (*S. albiguttalis*), however, were established in a greenhouse facility operated by the California Department of Food and Agriculture (CDFA) in Sacramento, Calif. From this resident colony, field releases of the moth were made as frequently as population size permitted.

Four release sites were chosen within the boundaries of the California Delta. Site selection was based on location within the Delta, size of the waterhyacinth infestation, and quality of the plants. Each characteristic was rated in accordance with the intent of providing a host plant population that would allow ease of establishment of the insects within the release sites, and, subsequently, via natural dispersal, throughout the Delta area. After a given site was selected, the waterhyacinth mat within the site was enclosed with floating booms to prevent the plants from escaping the site during periods of high water flow. This was done to further ensure that the developing biological control agent populations would have sufficient amounts of host plant material for successful establishment.

A summary of the releases of each of the biological agents at each site is given in Table 1. At present, the two weevil species are well established at three sites. *Neochetina bruchi*, the first species released, is effecting noticeable damage to waterhyacinth throughout the Galli site. *Neochetina eichhorniae* is well established at the Whites Slough site, but due to its later initial release date, damage to

Table 1
Summary of Biocontrol Agent Releases in the California Delta

Release Site	*Agent*	*First Release*	*Total to Date**
Galli	*Neochetina bruchi*	Jul 1982	1,324
	Sameodes albiguttalis	Aug 1983	3,527
Trapper Slough	*Neochetina bruchi*	Oct 1982	850
	Neochetina eichhorniae	Aug 1983	200
	Sameodes albiguttalis	Aug 1983	2,000
Whites Slough	*Neochetina eichhorniae*	Apr 1983	3,969
	Sameodes albiguttalis	Aug 1984	3,532
Veale Tract	*Neochetina bruchi*	Oct 1983	559
	Neochetina eichhorniae	Oct 1983	3,331
	Sameodes albiguttalis	Nov 1983	3,511

* Total releases of each species at each site as of November 1984.

waterhyacinth at Whites Slough is not apparent throughout the entire site. Similarly, both weevil species are well established at Veale Tract but are still in the dispersal phase within the site. Efforts to establish *S. albiguttalis* at these three sites have been less successful than anticipated. Though we have (on occasion) documented the presence of the moth at the Galli and Veale Tract sites, no significant buildup has been observed within the Delta. It appears that spring releases of *S. albiguttalis* are necessary for establishment. Spring releases were not made in 1983 and 1984 because of reduced viability of the greenhouse population of *S. albiguttalis* during winter and spring months. Improvements in rearing techniques will hopefully alleviate this problem in 1985.

During the same time that biological control efforts have been limited to the three release sites, the CDBW has been conducting a chemical spray program to control waterhyacinth within all major waterways of the California Delta. This chemical control effort has been highly successful and has prevented nuisance levels of waterhyacinth from developing in the majority of waterways within the Delta boundaries. However, herbicide application has not been possible in certain areas of the Delta. Due to the rapid growth and dispersal rates of waterhyacinth, the plants which remain untreated in these inaccessible areas can potentially reinfest previously treated waterways.

For these reasons, efforts should be initiated to disperse the biological agents into all Delta areas infested with waterhyacinth where herbicide usage is not applicable. Though natural dispersal from the release sites would accomplish this need in time, widespread dispersal can be more expeditiously accomplished by transplanting infested waterhyacinth from the release sites into these areas. After the biological agents become well established in these areas, the productivity of the waterhyacinth will be decreased and result in a reduced potential for waterhyacinth outbreaks in Delta waterways.

As explained above, the concurrent usage of chemical and biological control technologies offers a practical and efficient maintenance program for waterhyacinth within the California Delta. We, therefore, recommend that necessary action be initiated to achieve the implementation of such a program. Additionally, there is a need to expand this type of waterhyacinth control program into areas outside the California Delta. Of particular importance are those waterhyacinths located in the upstream portions of the San Joaquin River system that provide potential infestations of waterhyacinth to the California Delta waterways.

CHEMICAL CONTROL TECHNOLOGY DEVELOPMENT

An Overview

by
Howard E. Westerdahl*

Research during this past year has been focused on identifying and understanding the effects of conventional herbicides on specific target aquatic macrophytes. Results of this research will form a framework upon which herbicide selection and application techniques for flowing-water environments may be based. Based on previous research and continued US Army Engineer Waterways Experiment Station (WES) interest in registering new herbicides for aquatic use, Duphar, Inc., of The Netherlands announced their intention to obtain a potable water tolerance for dichlobenil (CASORON).

APPROACH

For FY 1985, four on-going research work units will address specific needs of the District Offices:

I—Herbicide/Adjuvant Evaluation in Flowing Water.

II—Herbicide Concentration/Exposure Time Relationships for Aquatic Plant Control.

III—Controlled-Release Poly GMA/2,4-D Development and Evaluation.

IV—Herbicide Application Techniques for Flowing Water.

One additional work unit to assess efficacy of selected herbicides on physiologically stressed plants was postponed based on recommendations by Corps of Engineer District representatives at the Aquatic Plant Control Research Program (APCRP) review immediately following the 19th annual APCRP meeting. A brief review of the objectives and planned activities of each of the four aforementioned work units will be followed by an in-depth discussion by Principal Investigators on each work unit.

The objectives of Work Unit I are to determine which adjuvants are effective in keeping conventional herbicide in contact with submersed aquatic plants (Eurasian watermilfoil) to sufficiently control the plants. During FY 1985, Endothal and selected adjuvants will be evaluated as was 2,4-D/adjuvant mixtures in FY 1984. These two herbicides represent nonpolar and polar-type herbicides (referring to chemical bonding characteristics). Analysis of test results will allow decisions to be made concerning whether all herbicides may require similar evaluations. If adjuvants release both herbicides in a similar time period, then further evaluations with other herbicides may not be necessary. Dr. Kurt Getsinger, WES, will discuss results to date and planned activities for FY 1985 and 1986, respectively.

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* US Army Engineer Waterways Experiment Station, Vicksburg, Mississippi.

Interpretation of research results from Work Unit I, relative to efficiency, is dependent on results from Work Unit II. The objective is to identify effective ranges of herbicide concentration and exposure times necessary to control aquatic plants (Eurasian watermilfoil). Mr. Jerry Hall, WES, will summarize research results in which the technical formulation was used and Dr. Thai Van, US Department of Agriculture-Aquatic Plant Management Laboratory (USDA-APML), will summarize similar research using the commercial formulation. Similar results are expected, thus providing cross-checks and verification of the herbicides' activity. In FY 1985, both 2,4-D and diquat will be evaluated, followed by Endothal in FY 1986.

Work Unit II was reactivated following 1 year in which analyses of available data from several previous years' research were reviewed and scale-up problems identified. The objective of Work Unit II is to develop scale-up procedures in FY 1985 for the controlled release Poly GMA/2,4-D formulation and production of 500-lb a.i. for field testing in FY 1986. Preliminary evaluations at the USDA-APML showed this to be a very promising controlled-release formulation.

Successful management of submersed aquatic plants in flowing water using conventional herbicide will require development of innovative application techniques. The objective is to identify application techniques which maximize herbicide contact time. During FY 1985, available flow-velocity data in submersed aquatic plant beds in flowing water environments from around the United States will be reviewed. Multiple herbicide applications, various herbicide injection techniques, and granular herbicide application are some of the techniques which will be evaluated. In FY 1986, the most promising techniques will be tested and evaluated under various flow conditions in the flume facility and at selected field sites.

Finally, Dr. Richard Dunn of Southern Research Institute, Birmingham, Alabama, will describe on-going research concerning production of controlled-release fluridone pellets. This pelletized formulation will be field tested in FY 1985 and 1986, respectively, in lake and flowing-water environments.

CHEMICAL CONTROL TECHNOLOGY DEVELOPMENT

Evaluation of Herbicide/Adjuvant Mixtures in Flowing Water

by

Kurt D. Getsinger*

INTRODUCTION

The herbicide/adjuvant evaluation study is designed to determine which herbicide/adjuvant mixtures show potential in controlling submersed weeds in flowing water and to compare those mixtures with conventional granular or liquid herbicide formulations. Various 2,4-D DMA/adjuvant mixtures and conventional 2,4-D formulations were tested on the submersed species Eurasian watermilfoil (*Myriophyllum spicatum* L.) under different flow velocities. Adjuvants used in this phase of the study included the inverting oils/emulsions Asgrow 403 and I'vod and the polymers Nalquatic and Poly Control. These 2,4-D DMA/adjuvant mixtures were compared with liquid 2,4-D DMA and granular 2,4-D BEE formulations.

MATERIALS AND METHODS

Herbicides/adjuvant evaluations were conducted in a hydraulic flume at the Waterways Experiment Station (WES), previously described by Getsinger (1984).** Duplicate stands (0.7 m tall by 0.8 m wide by 3 m long) of rooted Eurasian watermilfoil were established in the flume prior to treatment.

Conventional herbicide formulations used in the study were liquid 2,4-D DMA (Weedar 64) and granular 2,4-D BEE (Aqua-Kleen). Liquid 2,4-D DMA (Weedar 64) was also used as the herbicide component in the 2,4-D/adjuvant mixtures. Herbicide/adjuvant formulations consisted of the following mixtures: 2,4-D DMA/Asgrow 403 (invert), 2,4-D DMA/I'vod (invert), 2,4-D DMA/Nalquatic (polymer), and 2,4-D DMA/Poly Control (polymer). Invert formulations were blended with water and 2,4-D DMA to form a thick, mayonnaise-like material using a 7:1 water to inverting oil ratio. Polymer formulations were blended with water and 2,4-D DMA, using 2.5-percent polymer, to form a thick, mucous-like material.

Herbicide/adjuvant and liquid herbicide formulations were injected below the surface, on and into the plant beds, with a hand-held wand (fan-jet nozzle) connected to a paint pot pressurized at 1.36 atm (20 psi). The granular 2,4-D BEE formulation was broadcast evenly over the surface of the plant beds. All formulations were prepared to give a 2,4-D treatment of 45 kg ae†/ha(40 lb ae/acre).

* US Army Engineer Waterways Experiment Station, Vicksburg, Mississippi.

** K. D. Getsinger. 1984. "Herbicide/Adjuvant Evaluation in Flowing Water," Miscellaneous Paper A-84-4, US Army Engineer Waterways Experiment Station, Vicksburg, Mississippi.

† ae = acid equivalent.

Water depth in the flume was set at 70 cm. Duplicate treatments of all formulations were evaluated at flow velocities of 1.5 and 3.0 cm/sec (0.05 and 0.1 fps). Water samples were collected in the center of each flume channel, covering a water column depth of 10 to 60 cm, 2 m downstream from the plant beds. Samples were taken at 2-min posttreatment intervals for 180 min, composited to represent 12-min periods and analyzed for 2,4-D residue. Water samples collected 5 m upstream from each plant bed during experimental runs showed no herbicide contamination.

RESULTS AND DISCUSSION

Herbicide release rates from the liquid 2,4-D DMA formulation and the granular 2,4-D BEE formulation at 1.5 cm/sec and 3.0 cm/sec flow velocities are compared in Figures 1 and 2. The DMA treatments showed a large, initial release of herbicide with 2,4-D concentrations falling below detection (< 0.01 mg/ℓ) by 60 min posttreatment at the low velocity and 36 min posttreatment at the high velocity. In contrast, 2,4-D was released from the BEE formulation in smaller increments, and 2,4-D concentrations did not fall below detection until 168 min posttreatment at the low velocity and 84 min posttreatment at the high velocity.

Herbicide release rates from the liquid 2,4-D DMA formulation are compared with the herbicide release rates from the 2,4-D DMA/invert formulations (Asgrow 403 and I'vod) in Figures 3 and 4. Both of the invert formulations released 2,4-D more slowly than did the DMA formulation. I'vod maintained 2,4-D residues for the entire 180-min run at both velocities, whereas 2,4-D residues from Asgrow 403 fell below detection by 156 min posttreatment at the low velocity and 72 min posttreatment at the high velocity.

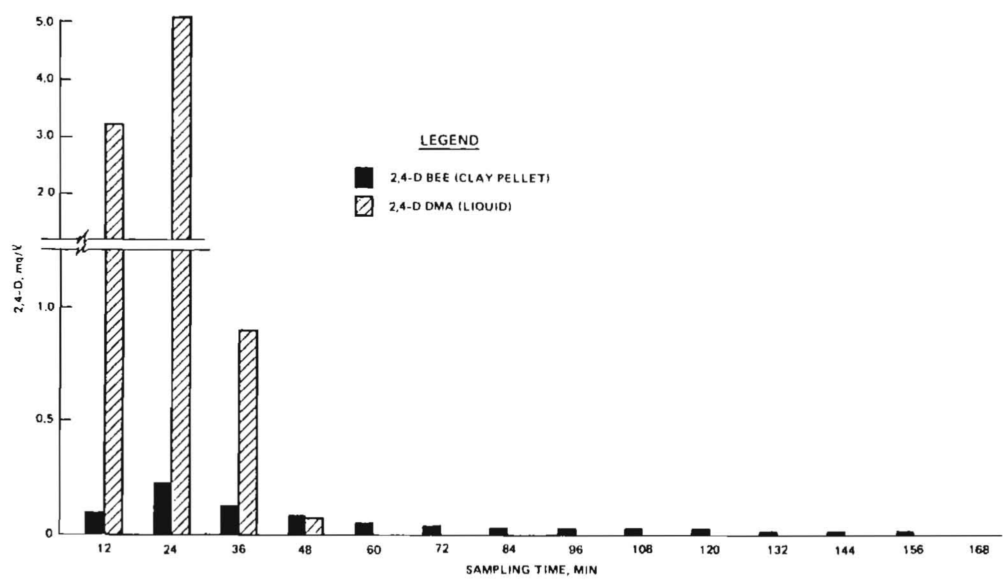

Figure 1. Effect of time on 2,4-D residues monitored 2 m downstream of plant beds treated with 2,4-D DMA and 2,4-D BEE at 1.5-cm/sec flow velocity. Data represent the mean of two treatments

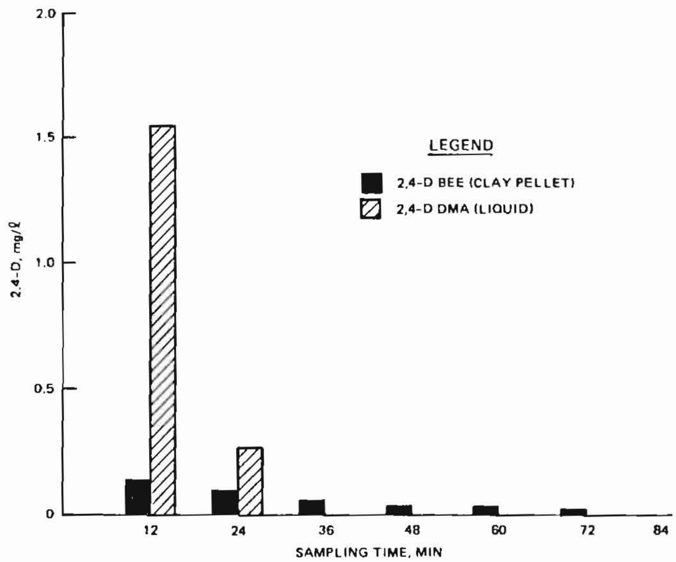

Figure 2. Effect of time on 2,4-D residues monitored 2 m downstream of plant beds treated with 2,4-D DMA and 2,4-D BEE at 3.0-cm/sec flow velocity. Data represent the mean of two treatments

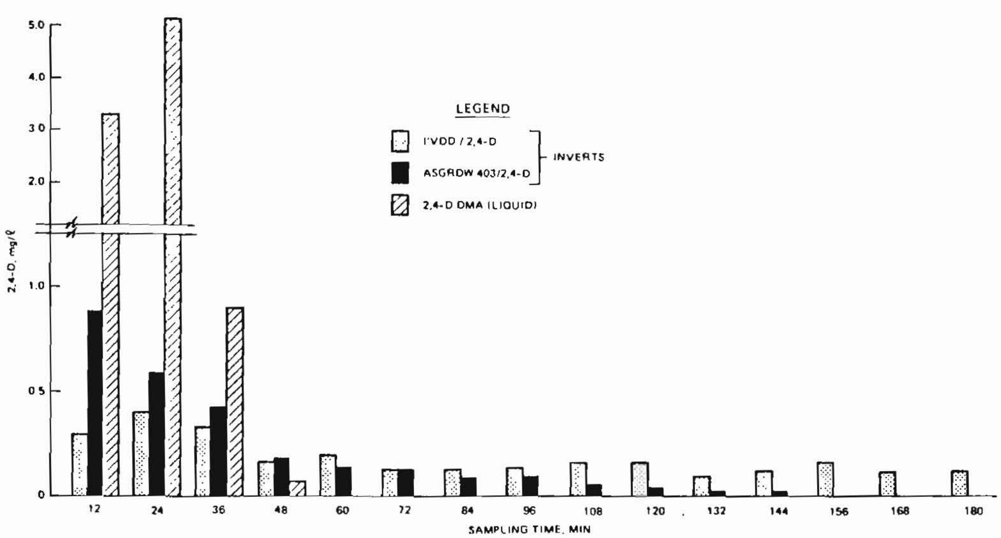

Figure 3. Effect of time on 2,4-D residues monitored 2 m downstream of plant beds treated with 2,4-D DMA, 2,4-D DMA/I'vod, and 2,4-D DMA/Asgrow 403 at 1.5-cm/sec flow velocity. Data represent the mean of two treatments

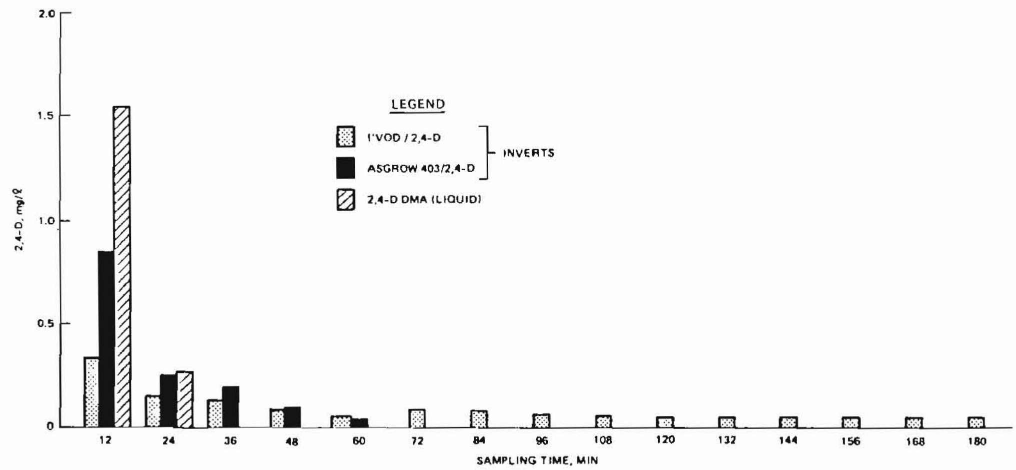

Figure 4. Effect of time on 2,4-D residues monitored 2 m downstream of plant beds treated with 2,4-D DMA, 2,4-D DMA/I'vod, and 2,4-D DMA/Asgrow 403 at 3.0-cm/sec flow velocity. Data represent the mean of two treatments

A comparison of herbicide release rates for 2,4-D DMA and the 2,4-D DMA/polymer formulations, Poly Control and Nalquatic, at 1.5-cm/sec and 3.0-cm/sec flow velocities are shown in Figures 5 and 6. Herbicide residues were present for 180 min posttreatment with Poly Control at 1.5 cm/sec, but below detection with Nalquatic by 84 min posttreatment. At the 3.0-cm/sec flow velocity, 2,4-D residues were below detection at 84 min posttreatment with Poly Control and 60 min posttreatment with Nalquatic.

All of the herbicide/adjuvant formulations tested released 2,4-D for longer periods of time than did the conventional, liquid 2,4-D DMA formulation, when applied at 1.5- and 3.0-cm/sec flow velocities. However, when herbicide release rates from the 2,4-D DMA/adjuvant formulations were compared with herbicide release rates from the conventional, granular 2,4-D BEE formulation, only I'vod and Poly Control released 2,4-D for longer periods than BEE at the 1.5-cm/sec flow velocity; and only I'vod released 2,4-D longer than BEE at the 3.0-cm/sec flow velocity.

Comparative rankings, based on exposure-time ratios of the 2,4-D formulations used in this study versus the conventional, liquid 2,4-D DMA formulation and the conventional, granular 2,4-D BEE formulation, are shown in Tables 1 and 2.

Although many of the herbicide formulations released 2,4-D for greater than 60 min posttreatment (most notably Poly Control and I'vod, which released 2,4-D for 180 min), the concentrations of 2,4-D were very low and exposure times relatively short ($<$ 0.5 mg/ℓ at 60 min posttreatment and $<$ 0.1 mg/ℓ at 120 min posttreatment). Herbicide concentration/exposure-time studies are currently under way to determine if the low 2,4-D doses and short exposure times demonstrated in the flowing-water evaluations can give effective control for Eurasian watermilfoil.

CONCLUSIONS AND RECOMMENDATIONS

These data suggest that at flow velocities within the plant beds of $\leq$ 3.0 cm/sec,

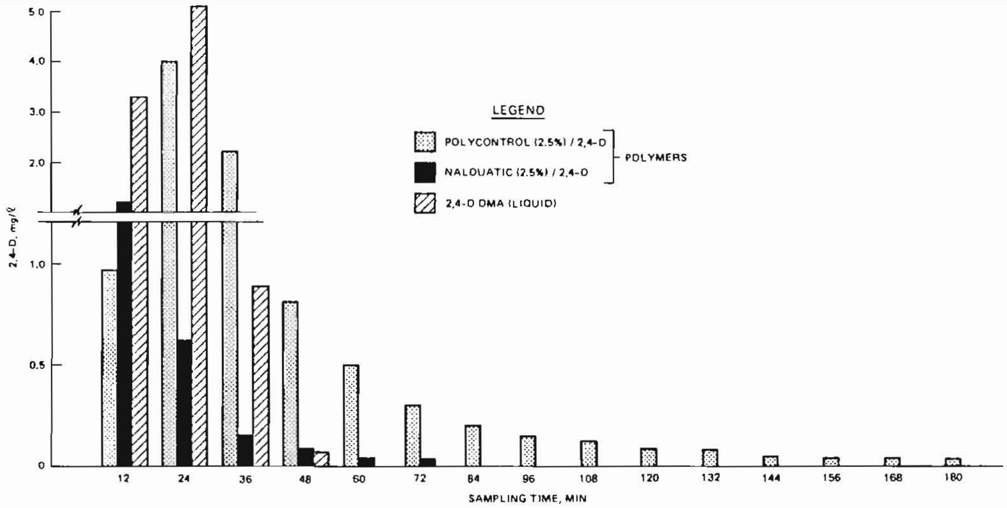

Figure 5. Effect of time on 2,4-D residues monitored 2 m downstream of plant beds treated with 2,4-D DMA, 2,4-D DMA/Poly Control, and 2,4-D DMA/Nalquatic at 1.5-cm/sec flow velocity. Data represent the mean of two treatments

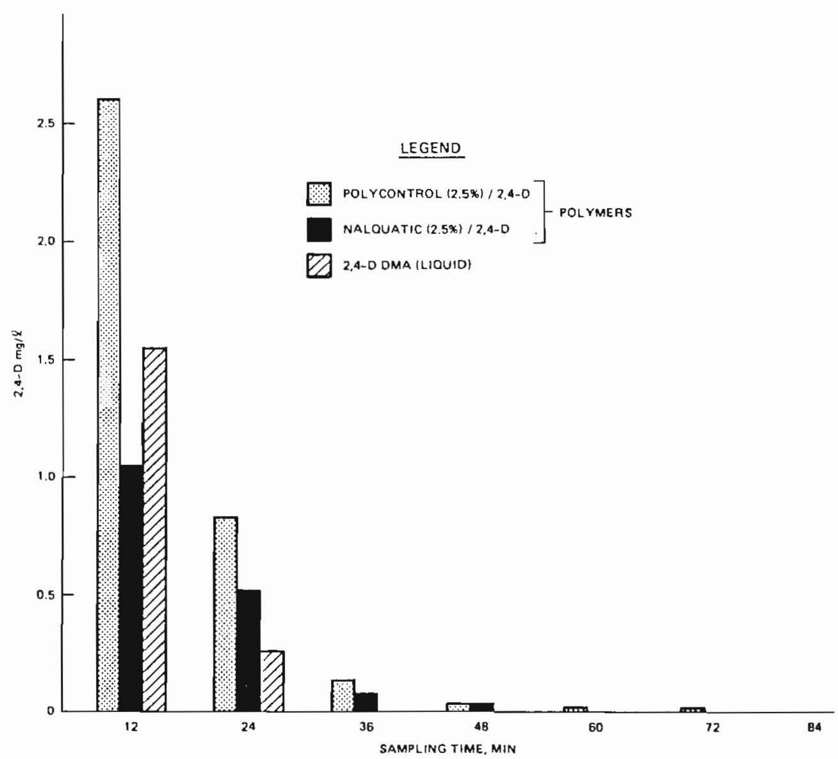

Figure 6. Effect of time on 2,4-D residues monitored 2 m downstream of plant beds treated with 2,4-D DMA, 2,4-D DMA/Poly Control, and 2,4-D DMA/Nalquatic at flow velocity of 3.0 cm/sec. Data represent the mean of two treatments

Table 1
Comparative Rankings of 2,4-D/Adjuvant Formulations and 2,4-D BEE Formulation Versus 2,4-D DMA Formulation

Formulation	*Ratios** 1.5 cm/sec*	*Ratios** 3.0 cm/sec*
I'vod (invert)	3.00	5.00
Poly Control 2.5% (polymer)	3.00	2.33
2,4-D BEE (clay granule)	2.80	2.33
Asgrow 403 (invert)	2.60	2.00
Nalquatic 2.5% (polymer)	1.40	1.67
2,4-D DMA (liquid)	1.00	1.00

$$\text{*Ratio} = \frac{\text{2,4-D exposure time with 2,4-D/adjuvant, BEE or DMA formulation}}{\text{2,4-D exposure time with 2,4-D DMA formulation}}$$

Table 2
Comparative Rankings of 2,4-D/Adjuvant Formulations and 2,4-D DMA Formulation Versus 2,4-D BEE Formulation

Formulation	*Ratios** 1.5 cm/sec*	*Ratios** 3.0 cm/sec*
I'vod (invert)	1.07	2.14
Poly Control 2.5% (polymer)	1.07	1.00
2,4-D BEE (clay granule)	1.00	1.00
Asgrow 403 (invert)	0.93	0.83
Nalquatic 2.5% (polymer)	0.50	0.71
2,4-D DMA (liquid)	0.36	0.43

$$\text{*Ratio} = \frac{\text{2,4-D exposure time with 2,4-D/adjuvant, DMA or BEE formulation}}{\text{2,4-D exposure time with 2,4-D BEE formulation}}$$

the use of a properly prepared 2,4-D DMA/adjuvant formulation will extend herbicide exposure time compared with a conventional, liquid 2,4-D DMA formulation, but will not extend herbicide exposure time (with the exception of I'vod) when compared with a conventional, granular 2,4-D BEE formulation. Data from an ongoing herbicide concentration/exposure-time study will determine whether the 2,4-D concentrations and exposure times identified in the flowing-water evaluations will give desired efficacy on Eurasian watermilfoil.

The recommendations suggested by this study include the following:

a. Combinations of the herbicide endothall with the adjuvants Asgrow 403, I'vod, Nalquatic, and Poly Control, along with conventional endothall formulations, should be analyzed with respect to release rates in flowing water and compared with the 2,4-D flowing-water evaluations.

b. Herbicide concentration/exposure-time studies should be continued to evaluate the effectiveness of various release rates in flowing water.

ACKNOWLEDGMENTS

The author would like to acknowledge Dr. Troy Stewart, Mr. Jerry Hall, Ms. Dawn Meeks, and Ms. Nancy Craft of WES for assistance in this study; Mr. Joe Zolczynski, Alabama Department of Conservation and Natural Resources, for field assistance; and the Laboratory Branch, Tennessee Valley Authority, for herbicide-residue analysis. The author also wishes to thank Asgrow Florida Company, JLB International Chemical, Nalco Chemical Company, and Union Carbide Agricultural for providing the chemicals used in this study.

CHEMICAL CONTROL TECHNOLOGY DEVELOPMENT

Determination of the Fluridone Concentration/Exposure Time Relationship for the Control of *Myriophyllum spicatum* and *Hydrilla verticillata*

by
Jerry F. Hall*

INTRODUCTION

The relationship between herbicide concentration and exposure time is a concept that has received very little attention regarding herbicides registered for use in the aquatic environment. Information concerning this relationship would be beneficial for static water applications and is essential for flowing water treatments. The developers of conventional and controlled-release herbicide formulations would benefit by having much clearer guidance for identifying treatment and release rates. The objective of this study was to determine the effective fluridone concentration and exposure time required to control *Myriophyllum spicatum* (Eurasian watermilfoil) and *Hydrilla verticillata* (hydrilla).

MATERIALS AND METHODS

A diluter system (Westerdahl and Hall 1983) was modified to permit delivery of two constant-rate concentrations of fluridone for five different exposure periods. A previous study (Hall 1984) showed that fluridone concentrations of 4 and 9 $\mu g/l$ at exposure times of 0.5, 1, 12, 20, and 40 days had no effect on watermilfoil or hydrilla. The fluridone concentrations and exposure times selected for this study were 15 and 30 $\mu g/l$ for 1, 6, 12, 20, and 40 days. These concentrations and exposure times were selected so that treatment and release rates of an experimental controlled-release formulation could be evaluated. Following exposure of an aquarium to a given fluridone concentration for a designated time period, only reconstituted natural hard water (US Environmental Protection Agency 1975) was permitted to flow through the aquarium for the remainder of the study. One set of reference aquaria containing the target macrophytes received only reconstituted hard water throughout the study period.

Approximately 4 weeks prior to testing, four 15-cm meristematic cuttings of watermilfoil were planted in each 250-ml glass beaker by burying the cut end of the plant 5 cm in the substrate. Sand was placed over the substrate to an approximate depth of 2 cm to prevent the substrate from mixing with the overlying water during handling. The substrate was a natural fine-textured sediment obtained from Brown's Lake at the US Army Engineer Waterways Experiment Station. The same procedure was followed using meristematic cuttings of hydrilla. Six beakers

* US Army Engineer Waterways Experiment Station, Vicksburg, Mississippi.

containing watermilfoil and six beakers containing hydrilla were placed in each aquaria. During the 4 weeks prior to testing, only reconstituted natural hard water flowed through the aquaria to allow root development from the plant cuttings.

Water samples for fluridone analysis were obtained at 4 and 8 hr following initiation of herbicide flow through those aquaria receiving the 1-day fluridone exposure. Water samples from the remaining aquaria were collected 1, 3, 7, 13, 28, and 36 days during and following exposure to fluridone.

At the end of 13 weeks, the plants were removed from each beaker by washing the substrate with deionized water. Shoots and leaves of watermilfoil and hydrilla, representing each fluridone concentration and exposure time, were subsampled from each aquarium. Plant tissues were extracted to clarity in vials containing 20 ml of dimethylsulphoxide (DMSO) using the procedures of Hiscox and Israelstam (1980). All chlorophyll measurements were made on a Beckman Model 26 spectrophometer and chlorophyll *a* and *b* concentrations were calculated as described in the literature (Arnon 1949). The remaining plants within each beaker were separated into roots and shoots, dried at 80° C for 24 hr, and weighed (accuracy ±0.5 mg).

The effects of two fluridone concentrations and five exposure times on the growth of watermilfoil and hydrilla were determined using percent plant injury (0 = no control, 100 = total kill) and a set of qualitative factors currently in use at the Aquatic Plant Management Laboratory in Fort Lauderdale, Florida (Hoeppel and Westerdahl 1981). The qualitative factors include: heavy algal cover, roots evident; absence of meristems on stems and branches; leaf loss; evidence of solarization; stem flaccidity; degree of node or internode decomposition; stem and branch tip decomposition; general decomposition of plants; advanced decomposition; complete disintegration of plant material; and subsequent regrowth. Duncan's Multiple Range Test was used to make comparisons among shoot and root biomass means, total chlorophyll, and chlorophyll *a* and *b* means at each fluridone concentration and exposure time and the reference. All statements of significance refer to the 5-percent level of statistical significance.

RESULTS AND DISCUSSION

Periodic fluridone analysis of the inflow water to each aquarium permitted evaluation of the fluridone concentration passing into the aquaria. The mean fluridone concentrations and ± standard error (n = 48) flowing into the aquaria were 15.0 ± 0.4 $\mu g/\ell$ or 30.0 ± 0.5 $\mu g/\ell$.

Eurasian watermilfoil

The effects of two fluridone concentrations and five exposure times on shoot and root biomass of watermilfoil are seen in Table 1. At the 15-$\mu g/\ell$ floridone concentration, statistically significant reductions in shoot biomass occurred after 12 to 20 days exposure. Shoot biomass was reduced by 31 percent after a 12-day exposure and by 55 percent after a 20-day exposure. Shoot biomass was reduced by 59 percent after the 40-day exposure. No statistically significant reductions in root biomass occurred following exposure to 15 $\mu g/\ell$ fluridone for 1-40 days. At the

Table 1

Shoot and Root Biomass of Watermilfoil and Hydrilla Exposed to Two Fluridone Concentrations and Five Exposure Times

Fluridone Concentration $\mu g/\ell$	*Exposure Time, days*	*Watermilfoil*	*Hydrilla*
		Shoots	
Reference	-	344 a	725 a
15	1	244 bc	689 a
15	6	271 ab	646 ab
15	12	239 abc	421 bc
15	20	154 bc	420 bc
15	40	142 c	340 c
Reference	-	344 a	725 a
30	1	272 ab	622 ab
30	6	230 abc	582 ab
30	12	197 bc	490 bc
30	20	187 bc	468 bc
30	40	186 bc	359 c
		Roots	
Reference	-	359 a	192 a
15	1	246 a	216 a
15	6	306 a	190 a
15	12	285 a	218 a
15	20	268 a	171 a
15	40	256 a	177 a
Reference	-	359 a	192 a
30	1	294 a	217 a
30	6	304 a	197 a
30	12	238 a	189 a
30	20	218 a	180 a
30	40	235 a	178 a

Note: Values in a column followed by the same letter are not statistically different at the 5-percent level as determined by Duncan's Multiple Range Test (n = 2).

30-$\mu g/\ell$ fluridone concentration, statistically significant reductions in shoot biomass occurred after a 12-day exposure. At this exposure time, shoot biomass was reduced by 43 percent. Shoot biomass was reduced by approximately 46 percent following 20- and 40-day exposures. No statistically significant reductions in root biomass occurred following exposure to 30 $\mu g/\ell$ fluridone for 1-40 days. No attempt was made to differentiate between live and dead root biomass.

The effects of fluridone on the chlorophyll content of watermilfoil are seen in Table 2. Total chlorophyll content in watermilfoil was reduced significantly after a 12-day exposure to 15 $\mu g/\ell$ fluridone. A reduction of 30 percent occurred at this concentration and exposure time. Reductions in total chlorophyll content of 39 and 43 percent occurred after 20- and 40-day exposures, respectively. Chlorophyll *a* content in watermilfoil was significantly reduced following exposure to 15 $\mu g/\ell$ for 1 day. Chlorophyll *a* was reduced by 19 percent at this concentration and exposure time. A 6-day exposure time also reduced chlorophyll *a* by 19 percent. Chlorophyll *a* reductions of 37, 48, and 46 percent occurred after exposures of 12, 20, and 40 days, respectively. Exposure of waermilfoil to 15 $\mu g/\ell$ for 1-40 days had no significant effect on chlorophyll *b* content.

At the 30-$\mu g/\ell$ fluridone concentration, significant reductions in total chlorophyll

Table 2
Total Chlorophyll, Chlorophyll *a*, and Chlorophyll *b* Content for Watermilfoil and Hydrilla Exposed to Two Fluridone Concentrations and Five Exposure Times

Fluridone Concentration μg/ℓ	*Exposure Time, days*	*Mean Chlorophyll Content, μg/ℓ Fresh Tissue* — *Watermilfoil*	*Hydrilla*
		Total Chlorophyll	
Reference	-	1080 a	745 a
15	1	885 ab	610 a
15	6	900 ab	585 ab
15	12	760 bc	620 a
15	20	620 c	290 b
15	40	660 c	255 b
Reference	-	1080 a	745 a
30	1	980 ab	705 a
30	6	765 b	580 ab
30	12	775 b	490 bc
30	20	420 c	360 c
30	40	395 c	110 d
		Chlorophyll *a*	
Reference	-	830 a	530 a
15	1	670 b	430 ab
15	6	670 b	405 b
15	12	520 c	420 ab
15	20	430 c	170 c
15	40	450 c	155 c
Reference	-	830 a	530 a
30	1	670 ab	490 ab
30	6	485 bc	380 bc
30	12	529 cd	300 cd
30	20	290 d	210 d
30	40	250 e	60 e
		Chlorophyll *b*	
Reference	-	250 a	215 a
15	1	215 a	180 ab
15	6	230 a	180 ab
15	12	240 a	200 a
15	20	190 a	100 c
15	40	210 a	120 c
Reference	-	250 a	215 a
30	1	310 a	215 a
30	6	280 a	200 a
30	12	250 ab	190 a
30	20	130 c	150 a
30	40	140 bc	50 b

NOTE: Values in a column followed by the same letter are not statistically different at the 5-percent level as determined by Duncan's Multiple Range Test (n = 2).

occurred after a 6-day exposure. Total chlorophyll was reduced by 29 percent at this concentration and exposure time. A 12-day exposure time reduced total chlorophyll by 28 percent. Exposure times of 20 and 40 days reduced total chlorophyll by 61 and 63 percent, respectively. Chlorophyll *a* content in watermilfoil was significantly reduced by 42 percent following exposure to 30 μg/ℓ for 6 days. Exposures of 12, 20, and 40 days resulted in chlorophyll *a* reductions of 37, 65, and 70 percent, respectively. Chlorophyll *b* content in watermilfoil was significantly reduced by 58 percent following exposure to 30 μg/ℓ for 20 days. Chlorophyll *b* content was significantly reduced by 53 percent following a 40-day exposure.

Table 3 presents the response of watermilfoil to various concentrations and

Table 3
The Response of Watermilfoil and Hydrilla to Two Fluridone Concentrations and Five Exposure Times Over a 13-Week Study Period

Fluridone Concentration µg/ℓ	*Exposure Time days*	*Percent Injury* - Weeks Posttreatment*							
		1	*2*	*4*	*6*	*8*	*10*	*12*	*13*
		Watermilfoil							
15	1	0	0	0	0	5	15	10	10
	6	10	15	20	20	20	20	15	15
	12	20	20	20	30	30	35	35	30
	20	20	20	25	35	40	40	45	65
	40	20	25	30	45	55	60	70	75
30	1	0	0	0	5	10	15	10	10
	6	20	20	20	25	25	30	30	20
	12	20	20	20	25	25	35	50	60
	20	20	20	25	30	30	40	55	60
	40	20	25	35	40	50	60	75	80
Reference	-	0	0	0	0	0	5	5	5
		Hydrilla							
15	1	0	0	0	5	5	10	10	5
	6	0	0	0	10	15	15	15	10
	12	0	5	10	15	20	20	30	20
	20	0	10	15	20	30	45	55	60
	40	0	20	30	35	40	50	65	75
30	1	0	0	0	0	5	10	15	15
	6	0	5	5	10	20	15	20	20
	12	0	15	20	20	25	40	55	65
	20	0	15	20	30	25	40	65	75
	40	0	20	30	30	50	60	70	80
Reference	-	0	0	0	5	0	5	5	5

* Injury ratings were made on two replicate aquaria each containing 24 plants of watermilfoil and 24 plants of hydrilla (n = 2). Hence, ratings are composite evaluations of each plant species.

exposure periods of fluridone. A 20-day exposure to 15 µg/ℓ fluridone resulted in 65-percent injury to watermilfoil after 13 weeks. A 40-day exposure to 15 µg/ℓ fluridone resulted in 75-percent injury to watermilfoil after 13 weeks. A 12-day exposure to 30 µg/ℓ fluridone resulted in 60-percent injury while 20- and 40-day exposure periods resulted in 60- and 80-percent injury, respectively.

Hydrilla

The effects of two fluridone concentrations and five exposure times on shoot and root biomass of hydrilla are seen in Table 1. At the 15-µg/ℓ fluridone concentration, statistically significant reductions in shoot biomass occurred after 12 to 20 days exposure. Shoot biomass was reduced by 42 percent following exposure for both 12 and 20 days. A 40-day exposure resulted in shoot biomass reductions of 53 percent. No statistically significant reductions in hydrilla root biomass occurred following exposure to 15 µg/ℓ for 1-40 days. At the 30-µg/ℓ fluridone concentration, statistically significant reductions in hydrilla shoot biomass occurred following a 12-day exposure. At this concentration and exposure time shoot biomass was reduced by 32 percent. Exposure times of 20 and 40 days resulted in biomass reductions of 35 and 50 percent, respectively. No statistically significant reductions in hydrilla root biomass occurred following exposure to 30 µg/ℓ for 1-40 days.

The effects of fluridone on the chlorophyll content of hydrilla are seen in Table 2. Total chlorophyll in hydrilla was reduced significantly after a 20-day exposure to 15 $\mu g/\ell$ fluridone. A reduction of 61 percent occurred at this concentration and exposure time. A 40-day exposure time resulted in a 66-percent reduction in total chlorophyll content of hydrilla. Chlorophyll *a* content of hydrilla was significantly reduced following exposure to 15 $\mu g/\ell$ fluridone for 20 days. Chlorophyll *a* was reduced by 68 percent at this concentration and exposure time. A reduction of 71 percent occurred following a 40-day exposure. Chlorophyll *b* was significantly reduced by 53 and 44 percent following exposure to 15 $\mu g/\ell$ for 20 and 40 days, respectively.

At the 30-$\mu g/\ell$ fluridone concentration, significant reductions in total chlorophyll content of hydrilla occurred after a 12-day exposure. Total chlorophyll was reduced by 34 percent at this concentration and exposure time. A 20-day exposure time reduced total chlorophyll by 52 percent. A reduction of 85 percent occurred after a 40-day exposure. Chlorophyll *a* content in hydrilla was significantly reduced by 28 percent following a 6-day exposure to 30 $\mu g/\ell$ fluridone. Chlorophyll *a* content was reduced by 43 percent following a 12-day exposure. Exposure time of 20 and 40 days resulted in reductions of 60 and 89 percent, respectively. Chlorophyll *b* content in hydrilla was not significantly affected by exposure to 30 $\mu g/\ell$ fluridone for 1, 6, 12, or 20 days. Chlorophyll *b* was significantly reduced by 75 percent following a 40-day exposure.

The results of hydrilla response to fluridone treatments are presented in Table 3. A 20-day exposure to 15 $\mu g/\ell$ fluridone resulted in 60-percent injury to hydrilla. A 40-day exposure resulted in 75-percent injury after 13 weeks. A 6-day exposure to 30 $\mu g/\ell$ fluridone resulted in 20-percent injury while 12- and 20-day exposures resulted in 65 and 75 percent injury, respectively. An 80-percent injury rating resulted from a 40-day exposure to 30 $\mu g/\ell$ fluridone.

CONCLUSIONS

Because of the long contact time required for effective watermilfoil and hydrilla control with fluridone, those plants exposed for short periods of time, i.e. 1, 6, and 12 days, exhibited some degree of regrowth during the last 4 weeks of the study.

The response of shoot and root biomass and chlorophyll content to the two fluridone concentrations and five exposure periods was used along with the injury ratings to determine the relationship between concentration and exposure time. A fluridone concentration of 15 $\mu g/\ell$ for 12-20 days controlled growth of watermilfoil; whereas, a fluridone concentration of 30 $\mu g/\ell$ for 12 days also controlled growth of watermilfoil. Hydrilla growth was controlled following a 20- to 40-day exposure to 15 $\mu g/\ell$ fluridone and a 12-day exposure to 30 $\mu g/\ell$ fluridone.

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CHEMICAL CONTROL TECHNOLOGY DEVELOPMENT

Herbicide Evaluation Program

by
Thai K. Van*

This report presents some preliminary results for FY 1984 of our program to evaluate new chemicals or chemical formulations for their potential application in the management and control of aquatic vegetation.

Imazapyr [2-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl)nicotinic acid] is American Cyanamid's newest herbicide for use in noncropland areas. Previous studies indicated that the herbicide is effective at 0.56 to 1.12 kg a.i./ha on numerous economically important aquatic weed species. During FY 1984, a study was conducted on a 0.07-ha pond infested with waterhyacinth [*Eichhornia crassipes* (Mart.) Solms] in Fort Lauderdale, Florida, to determine residue levels and persistence of imazapyr in the aquatic environment after chemical treatment. The herbicide rate used was 1.12 kg a.i./ha which provided complete control of the waterhyacinth in the pond 2 months after treatment. Chemical residues in the pond water are presented in Figure 1. Regression analysis of the data indicated a half-life of imazapyr in water of about 90 days. Sediment samples were collected before treatment and at different times after treatment (Table 1). None of the sediment samples contained detectable imazapyr concentrations (0.05 mg/ℓ).

Biological control of waterhyacinth with the weevils *Neochetina* has been effectively demonstrated in states such as Louisiana where the insects have become established. The time required between introduction of insects and expansion of populations to the point where plant growth is suppressed and adequate control is attained can be 24 months or longer. The problem is that plant growth rate can exceed growth rate of insect populations in several instances. As a consequence, there is a considerable lag before plant growth is stressed by the insects. A study was conducted in outdoor aquaria to determine the potential for control of waterhyacinth by combining the insect biocontrol agents with the growth retardant PP-333 [1-(4-chlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-1-yl)pentan-3-ol]. A single application of 1.12 kg a.i./ha of PP-333 at the beginning of the experiment was sufficient to sustain growth-retarding effects over the 6-month study period. Weevils alone effected a slight decline in waterhyacinth growth; however, the effect was not rapid enough for practical control purposes. The combination of weevils and the growth retardant resulted in near elimination of plants after 6 months (Table 2). The result suggests a synergistic effect from integrating the two methods of control.

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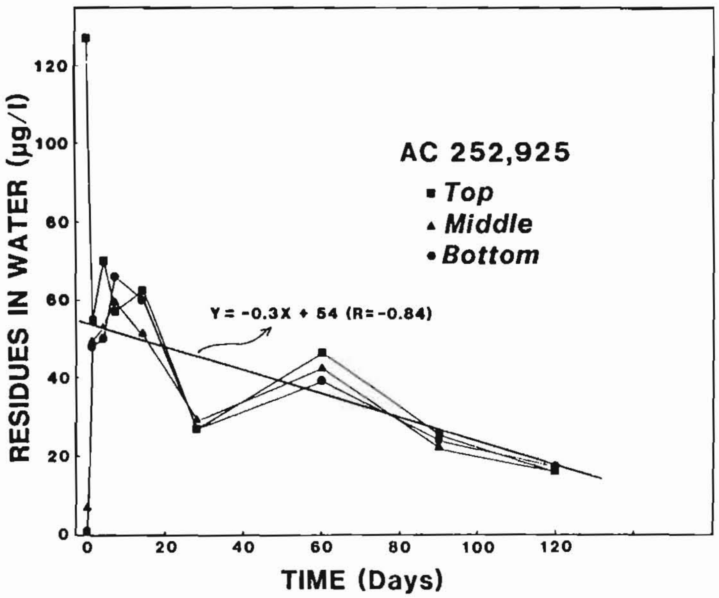

Figure 1. Imazapyr residue in pond water after the application of AC 252,925 (Arsenal®)

Table 1

Imazapyr Residue in Sediment after the Application of AC 252,925 (Arsenal®)*

Sampling Date	*Days After Treatment*	*Residues ppm*
13 Oct 83	Pretreatment	0.01
14 Oct 83	1	<0.05
17 Oct 83	4	<0.05
20 Oct 83	7	<0.05
27 Oct 83	14	<0.05
10 Nov 83	28	<0.05
12 Dec 83	60	<0.05
11 Jan 84	90	<0.05
10 Feb 84	120	<0.05

* Treatment was made on 13 Oct 1983 at the rate of 1.12 kg a.i./ha to a 0.07-ha pond infested with waterhyacinth.

Table 2

Biomass (kg dry weight/m²) of Waterhyacinth as Affected by Weevils and Growth Retardant

	Retardant (kg/ha PP-333)		
Weevils	*0*	*1*	*Difference*
Without	2.35	1.20	1.14*
With	2.16	0.26	1.90**
Difference	0.19 NS	0.94*	

* Significant at 5-percent level.
** Significant at 1-percent level.

Several formulations of diquat/alginate were evaluated for control of hydrilla [*Hydrilla verticillata* (L.f.) Royle] in flowing water. Laboratory studies indicated that the diquat/alginate formulations released all of their diquat within 4 hr (Table 3) after the formulations were immersed in reconstituted water (pH 8). Similarly, a 4- to 6-hr release time was observed in a dynamic test using natural pond water with the inflow adjusted to provide one complete water volume change every 24 hr (Figure 2). The short release time of the diquat/alginate formulations was responsible for the poor hydrilla control observed in the dynamic test (Table 4). Also, a commercial formulation of diquat/alginate (Midstream®) provided no control of hydrilla when treated at 6.7 kg/ha diquat to a drainage canal in south Florida. The canal was about 3 m deep with a surface water velocity of 0.25 m/sec at the time of the treatment. Further studies indicated that a minimum contact time of 24 hr was required to control hydrilla at the rate of 1.0 mg/ℓ diquat (Figure 3). At the same treatment rate, control of Eurasian watermilfoil (*Myriophyllum spicatum* L.) was achieved with a contact time of 1 hr.

Table 3
Cumulative Percent Release of Diquat from Different Alginate Formulations into Reconstituted Water

	Percent Diquat Released, Hours After Treatment*						
Formulation	*1*	*2*	*4*	*6*	*8*	*12*	*24*
Diquat liquid	10 ± 6	10 ± 6	98 ± 6	96 ± 3	103 ± 6	104 ± 7	98 ± 6
Diquat/alginate	58 ± 10	74 ± 5	82 ± 3	94 ± 3	90 ± 1	97 ± 1	101 ± 4
Diquat + Cu/alginate	52 ± 8	75 ± 3	84 ± 2	85 ± 3	93 ± 4	102 ± 7	106 ± 7
Midstream®	69 ± 15	79 ± 14	87 ± 12	92 ± 6	102 ± 4	106 ± 3	99 ± 2

* Means of six replicates ± standard error.

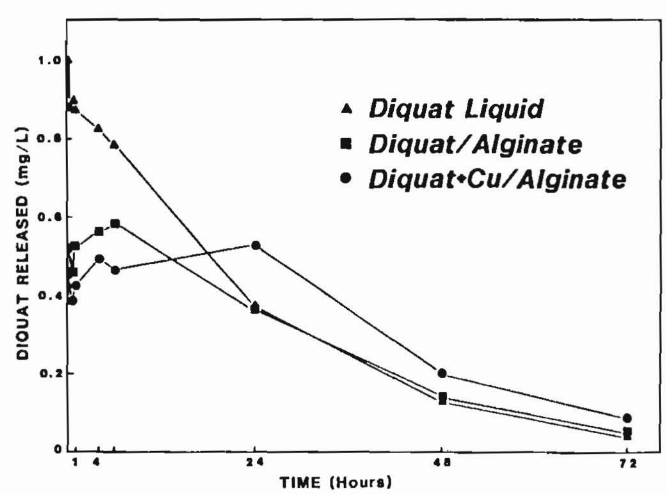

Figure 2. Release of diquat from various formulations in a dynamic test using pond water with inflow adjusted to provide one water volume change every 24 hr

Table 4

Hydrilla Control by Various Formulations of Diquat in 20-ℓ Glass Vessels with Flowing Water to Provide One Water Volume Change Every 24 hr

	Rate mg/ℓ	*Percent Injury* Weeks Posttreatment*					*Dry Weight, g***	
		1	*2*	*4*	*6*	*8*	*Shoots*	*Roots*
Diquat liquid	0.5	28	55	62	50	38	2.37[b]	0.18[b]
	1.0	72	92	87	82	52	0.86[c]	0.08[b]
	2.0	77	92	88	82	55	0.66[c]	0.05[b]
Diquat/alginate	0.5	38	62	58	45	27	2.44[b]	0.18[b]
	1.0	60	93	87	82	45	1.01[c]	0.09[b]
	2.0	77	95	90	87	73	0.55[c]	0.04[b]
Diquat + Cu/alginate	1.0 + 1.0	77	92	90	88	80	0.47[c]	0.05[b]
Control	-	10	13	20	20	20	8.45[a]	0.49[a]

* Average of three replicates.

** Harvested 8 weeks after treatment. Values in a column followed by the same letter are not significantly different at the 5-percent level as determined by the Waller-Duncan test. Each value is the mean of three replicates.

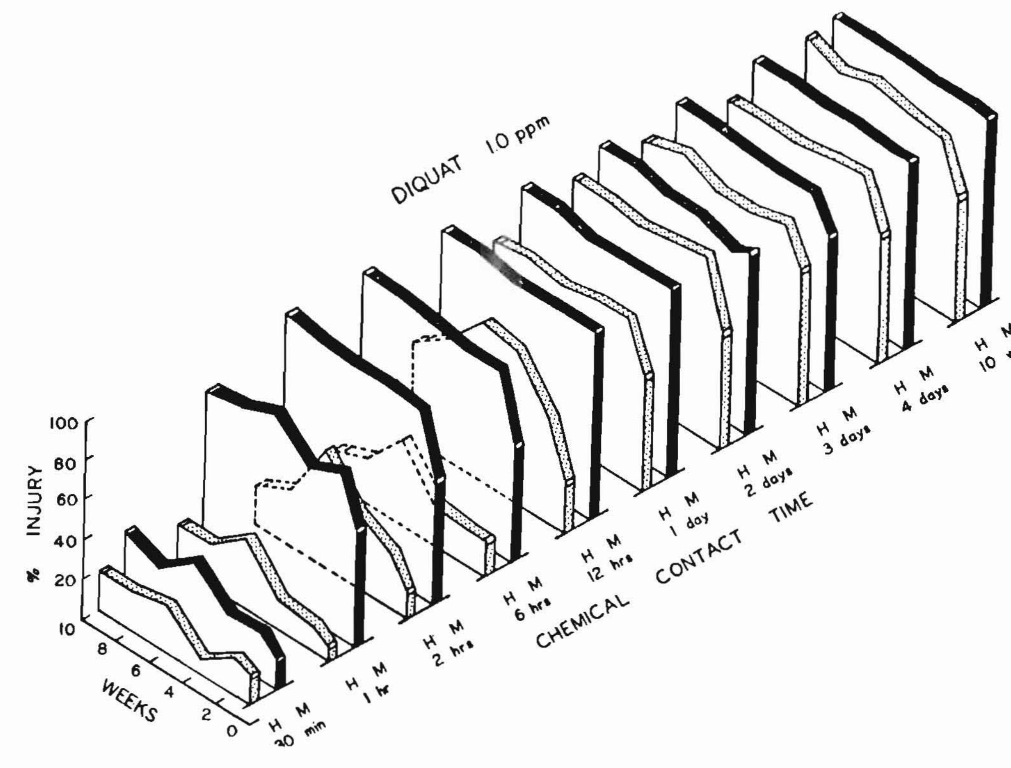

Figure 3. Control of hydrilla (H) and Eurasian watermilfoil (M) during a 10-week period following treatments of 1.0 mg/ℓ diquat with different chemical contact time

CHEMICAL CONTROL TECHNOLOGY DEVELOPMENT

Polymeric Pellet Delivery Systems for the Controlled Release of Fluridone

by

Richard L. Dunn,* John W. Gibson,* Janna D. Stevens,* and Michael W. Price*

INTRODUCTION

Fibrous delivery systems have been developed for various aquatic herbicides including diquat and fluridone. It was expected that the fibers would entangle with the aquatic weeds and provide a long-term and local delivery of the herbicide. We prepared these systems by incorporating the herbicide into polycaprolactone (PCL), a biodegradable polymer, and extruding the blend into fibers. Because the fluridone/PCL controlled-release fibers proved to be the most effective on a laboratory scale, they were produced in pilot-scale quantities for three field trials in 1983. As expected, the fibers gave a prolonged and local release of fluridone that was effective in weed control in waters with low flow rates. Two problems, however, arose with the fibers during these field trials. The fibers could not be applied in the field using conventional application equipment, and the fibers remained on the surface of the water too long before they sank and became entangled in the aquatic weeds. For these reasons, we decided to convert the fibrous delivery system to a pellet system. Pellets for controlled release of fluridone offer several advantages. They can be applied with conventional equipment, they sink quickly even in flowing water, and they tend to limit the migration of herbicide released.

To develop the desired pellet system, we used three approaches. First, we coated a bundle of the fluridone controlled-release fibers with a water-soluble polymer and cut the resulting rod into sections. Second, we produced monolithic pellets from the same PCL/fluridone blend used to prepare the fibers but with the addition of water-soluble materials into the matrix. When exposed to water, these additives leached out of the pellets, creating a porous polymeric matrix and increasing the rate of fluridone release. Third, we used the same pellet formulation but manipulated the geometry to optimize release characteristics.

EXPERIMENTAL

Materials

Fluridone Lot 827EG2 (Eli Lilly & Co., Indianapolis, Ind.) was used for all of the pellet delivery systems. All experiments were performed with PCL Lot 6361 (Union Carbide, Danbury, Conn.) cryogenically ground to <20 mesh at Wedco, Inc. (Bloomsburg, N. J.).

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Four water-soluble additives were evaluated for their effect on fluridone release: hydroxypropylcellulose (Type GF, Hercules, Inc., Wilmington, Del.), polyethylene glycol 8000 (Carbowax 8000, Union Carbide, Danbury, Conn.), sodium chloride, and triethyl citrate (Pfaltz and Bauer, Inc., Stamford, Conn.).

A number of polyethylene glycols of different molecular weights were examined as possible coatings for fluridone/PCL fibers. They included polyethylene glycol 8000, 3250, and 600. All were purchased from Union Carbide (Danbury, Conn.). Various combinations of these polyethylene glycols were also tested.

Preparation of coated fibers

To prepare coated fluridone/PCL fibers, the coating material was first melted completely in a U-shaped glass tube approximately 1 in. in diameter. A bundle containing five to seven strands of fiber sample was then pulled through the melted coating material and air cooled. This procedure was repeated until rods of the required diameter were obtained. The coated fibers were then cut into 1-cm lengths for in vitro evaluation of their fluridone-release characteristics.

Preparation of monolithic pellets

Materials used to make monolithic pellets were sieved, if necessary, to obtain particle sizes of 60 to 80 mesh and then dry blended in a twin-shell blender. Pellets were prepared with a Tinius Olsen extrusion plastometer. Each dry blend was charged into the preheated chamber of the plastometer and equilibrated at the extrusion temperature. Weights were then added to the ram of the extruder to force the molten blend through a die mounted in the bottom of the chamber. Rod diameters were altered by melt-chamber temperatures and the weights applied to the ram. The extruded rod was air cooled and cut into appropriate lengths to form pellets. Holes were drilled in some pellets to increase the ratio of surface area to volume. Trilobal pellets were also prepared with the aid of a drill.

Herbicide release rates

Pellets were evaluated for fluridone-release characteristics by placing triplicate samples, each containing four to six pellets, in separate vessels containing reconstituted hard water as the receiving fluid. The sealed vessels were agitated in an Eberbach shaker bath set at 23° C. Periodically, the receiving fluid was exchanged for fresh fluid and assayed for fluridone by measuring UV absorption at 236 nm on a Perkin-Elmer Lambda 3B spectrophotometer. Fluridone was quantified by using a Beer's Law plot of fluridone concentration versus absorbance.

RESULTS AND DISCUSSION

Coated fiber bundle

Our initial approach to the development of fluridone pellets was simply to modify the fibrous fluridone system we had prepared earlier. First, a method of coating fibers with a water-soluble excipient was developed and then fiber bundles (containing five to seven short strands of fiber) were coated with different water-soluble excipients. We found that a 95/5 wt percent blend of PEG 3350/PEG 600 coated the fibers well and resulted in pellets that were supple and did not flake or adhere to each other at room temperature. The release profiles of these pellets and the fibers from which they were prepared are shown in Figure 1. Fluridone release from the pellets closely paralleled the release from the original fibers. The slight differences in release that were observed may be due to fluridone crystallization in the fiber with time, exposure of the fibers to the heated coating bath, or changes in the in vitro conditions (e.g., temperature, agitation) between studies.

A fluridone-releasing system of coated fibers has two advantages. The coating prevents the fibers from floating on the water, and the coating dissolves within 20 min after exposure to water, releasing the fiber segments. The PEG used to coat the fibers, however, may not be the best polymer because it has a low softening temperature. Under storage at high temperatures, it could become soft, and the pellets could possibly adhere to one another. A water-soluble polymer with a higher melting point should be evaluated.

Pellets with water-soluble additives

Our previous studies with fibrous fluridone/PCL systems indicated that fluridone is released by a solution-diffusion mechanism. When this mechanism is in operation, decreasing the loading of active agent decreases the release duration. Therefore, to compensate for the larger diameter of pellet systems and subsequent larger reservoir of fluridone which would normally increase the duration of release, we began our studies with the lowest practical loading of fluridone, 10 wt percent.

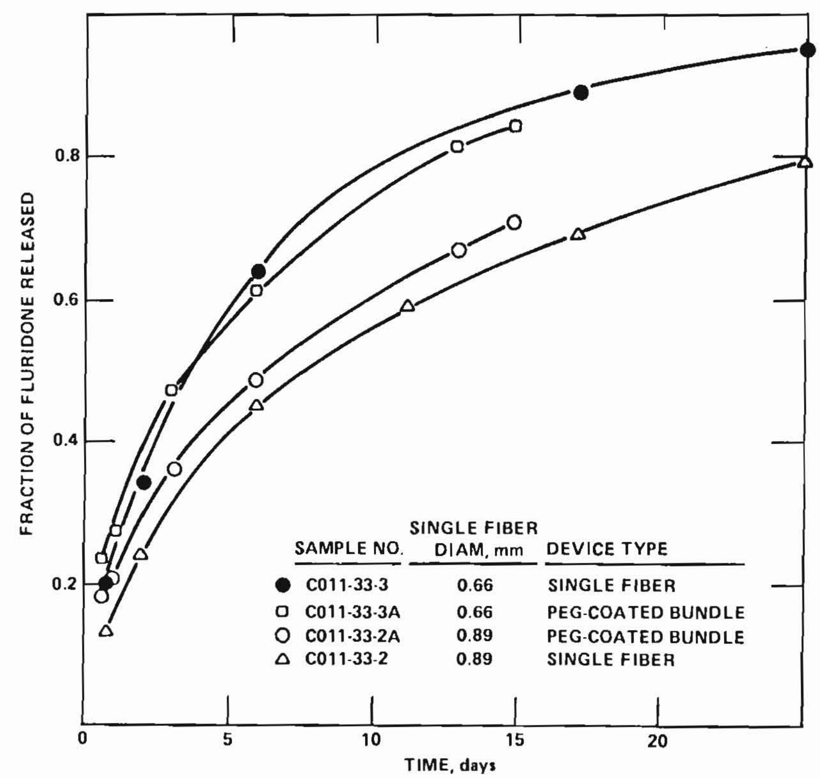


Figure 1. Fluridone release from fluridone/PCL (25/75) fibers coated with a mixture of polyethylene glycol 3350 and 600 (95/5)

We examined first the effect of different water-soluble additives on fluridone release from pellets. Upon exposure to water, the additives should leach from the pellets and increase the porosity of the polymeric matrix. This should, in turn, increase the amount of fluridone released. The release profiles of pellets with 20 to 90 wt percent loadings of hydroxypropylcellulose (HPC) are shown in Figure 2. Fluridone release was maximal in samples containing 50 wt percent HPC. Except for the samples containing 90 wt percent HPC and no PCL (which disintegrated within 1 week), HPC loadings higher and lower than 50 wt percent resulted in less fluridone release. We suspect that this may be due to differences in pore structure at different HPC loadings or to the solubility of fluridone in the two excipients. However, we did not investigate this phenomenon further.

The effect of polyethylene glycol 8000 (PEG 8000) on fluridone release is shown in Figure 3. With this additive, fluridone release increased as the loading of the additive was increased from 10 to 70 wt percent. Polyethylene glycol 8000, however, was not as effective as HPC in promoting fluridone release. Moreover, because these pellets are waxy at PEG loadings greater than 50 wt percent, they may adhere to one another during storage.

Sodium chloride was also incorporated into pellets containing 10 wt percent fluridone. It had little effect on fluridone release, as shown in Figure 4. We used scanning electron microscopy (SEM) to examine the effects of NaCl and HPC, the most promising additive, on the structure of the polymeric matrix (Figure 5). The SEM photographs indicate that discontinuous pores are formed upon dissolution of the NaCl. These pores may act like pockets that trap and immobilize dissolved fluridone. Because fluridone must still diffuse through the bulk PCL phase, release is not increased as a result of the pockets created. Hydroxypropylcellulose, on the

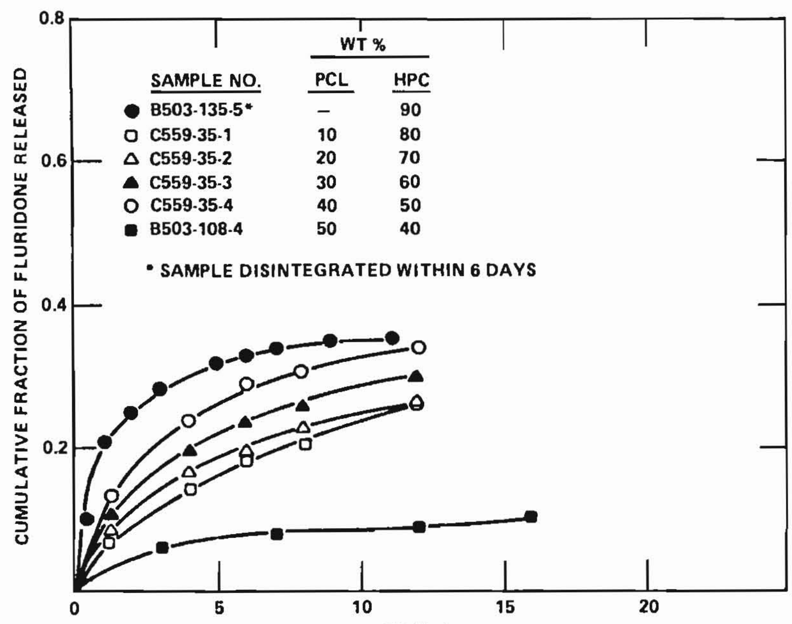


Figure 2. Effect of hydroxypropylcellulose on fluridone release from monolithic pellets containing 10 wt percent fluridone

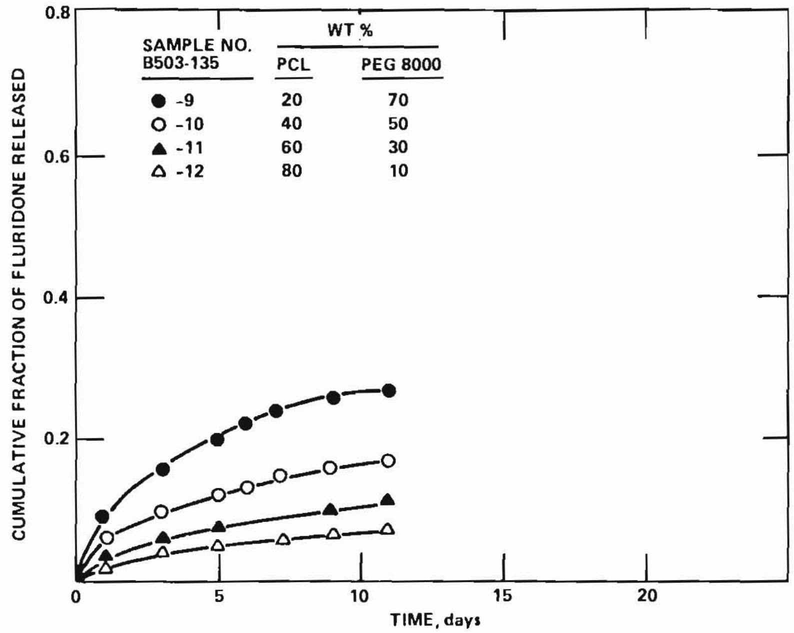


Figure 3. Effect of polyethylene glycol 8000 on the release of fluridone from monolithic pellets containing 10 wt percent fluridone

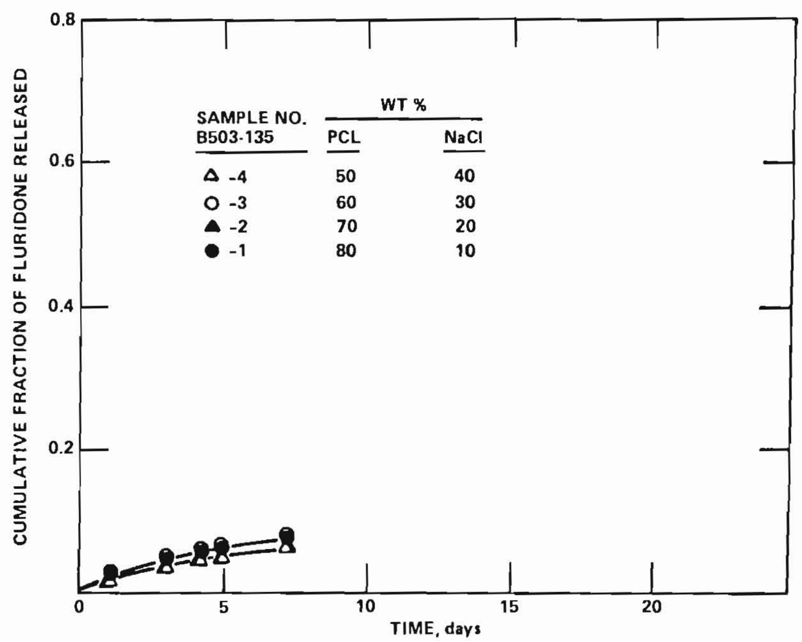
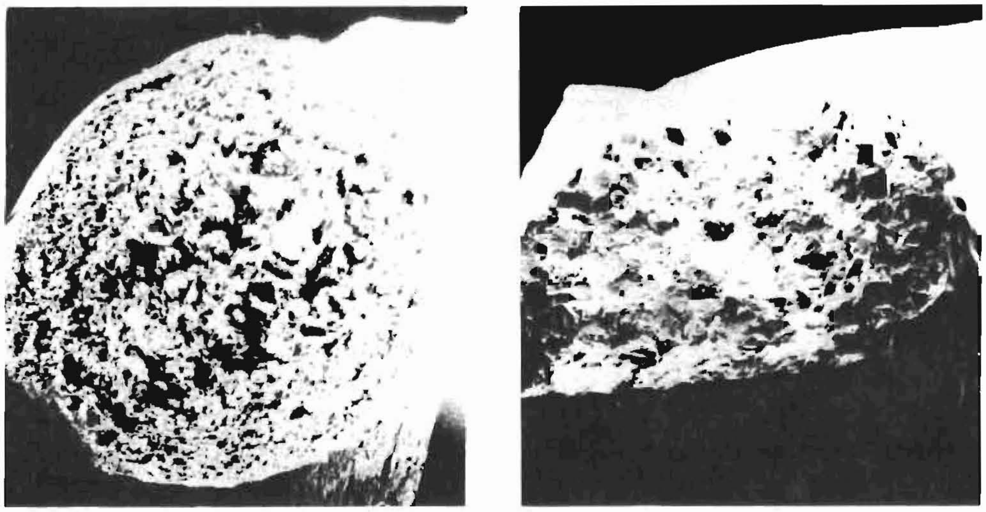


Figure 4. Effect of sodium chloride on fluridone release from monolithic pellets containing 10 wt percent fluridone



a. 10/10/80 pellet   b. 10/50/40 pellet

Figure 5. Scanning electron micrographs of a fluridone/PCL/HPC (10/10/80) pellet and a fluridone/PCL/NaCl (10/50/40) pellet

other hand, appears to form a continuous pore structure or channels in the PCL matrix. These channels can increase the rate of release by providing an alternative diffusion pathway for fluridone.

The effect of one other water-soluble additive, triethyl citrate (TEC), on fluridone release was examined. In these experiments, fibrous devices containing TEC were compared to previously prepared fluridone/PCL fibers with 20- and 40-day durations of release. Unlike the other additives tested, TEC decreased fluridone release, as shown in Figure 6. We believe this may be due to low fluridone solubility in TEC and partitioning of the fluridone into the PCL phase. This would, in effect, increase the loading of fluridone in PCL and, consequently, decrease release.

Overall, HPC appeared to be the most promising water-soluble additive for obtaining the required durations of fluridone release, and we selected the fluridone/PCL/HPC composition of 10/40/50 wt percent for optimization of release characteristics.

Pellet geometry

In designing fibrous systems for the controlled release of fluridone, the required 20- and 40-day release durations could be achieved simply by varying the diameter of fibers once an optimal composition had been selected. Therefore, we examined pellet geometry to determine its effect on fluridone release. To accomplish this, we prepared pellets with various ratios of surface area to volume (SA/VOL). Holes were drilled in some pellets to obtain high values for SA/VOL. The effect of SA/VOL on fluridone release from fluridone/PCL/HPC (10/40/50) pellets is shown in Figure 7. It is evident that fluridone release increases with increasing SA/VOL.

The time required for 90- and 100-percent fluridone release from each sample represented in Figure 7 was calculated using the following expression of Fick's Law of diffusion for devices with cylindrical geometry:

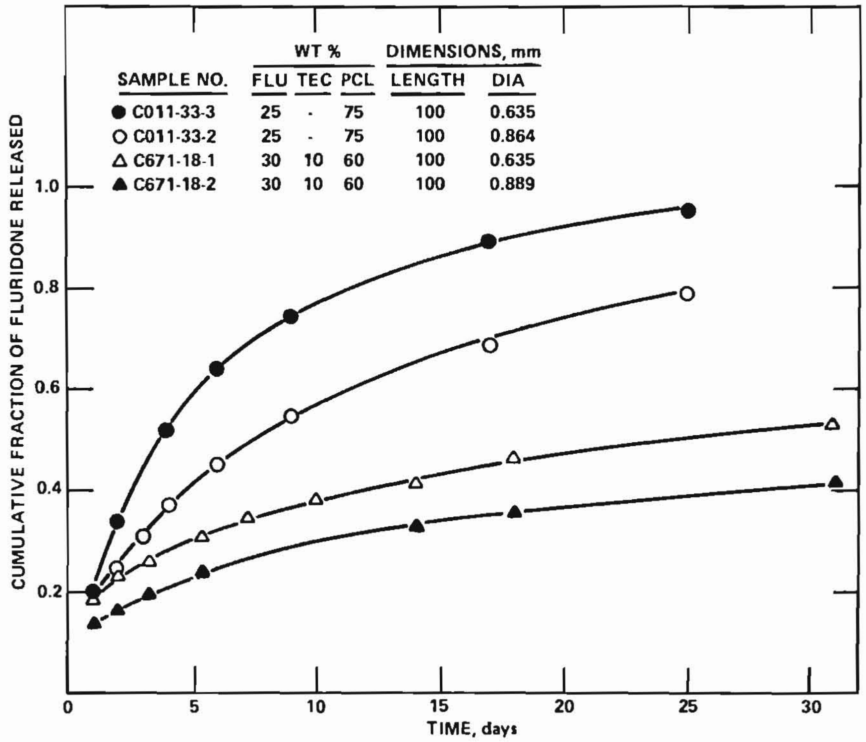


Figure 6. Effect of triethyl citrate on fluridone release from large-diameter monolithic fibers

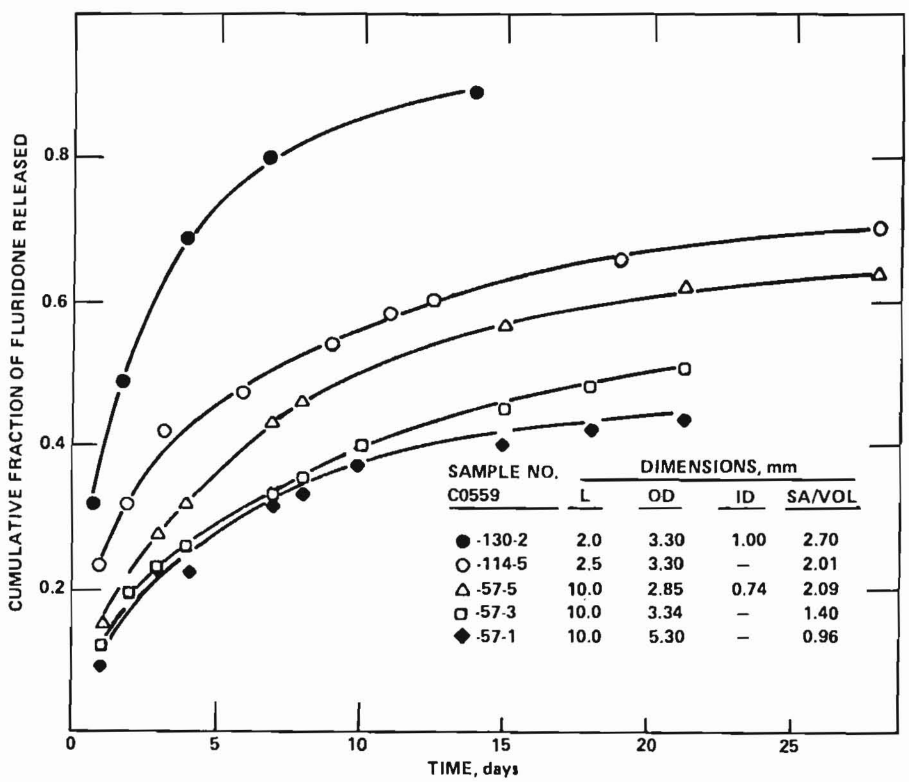


Figure 7. Effect of pellet geometry on fluridone release from monolithic fluridone/PCL/HPC (10/40/50) systems

$$\left(\frac{C_o r_o^2}{4}\right)\left(\left[1-\frac{M_t}{M_\infty}\right]\left[\ln\left(1-\frac{M_t}{M_\infty}\right)\right]+\frac{M_t}{M_\infty}\right)=DC_s t$$

where

C_o = fluridone loading, $g \cdot cm^{-3}$

r_o = radius of cylinder, cm

M_t/M_∞ = fraction of initial mass released at time t

D = diffusion coefficient of fluridone in polymer, $cm^2 \cdot sec^{-1}$

C_s = saturation solubility of fluridone in polymer, $g \cdot cm^{-3}$

t = time, sec

Figure 8 shows the durations of release that can be obtained with fluridone/PCL/HPC (10/40/50) pellets having different SA/VOL. Data from this figure also allow us to design pellets with specific durations of release. For example, pellets with a 20-day duration of release will require a SA/VOL of 2.5 to 2.75, while pellets with a 40-day duration of release will require a SA/VOL of 2.1 to 2.5.

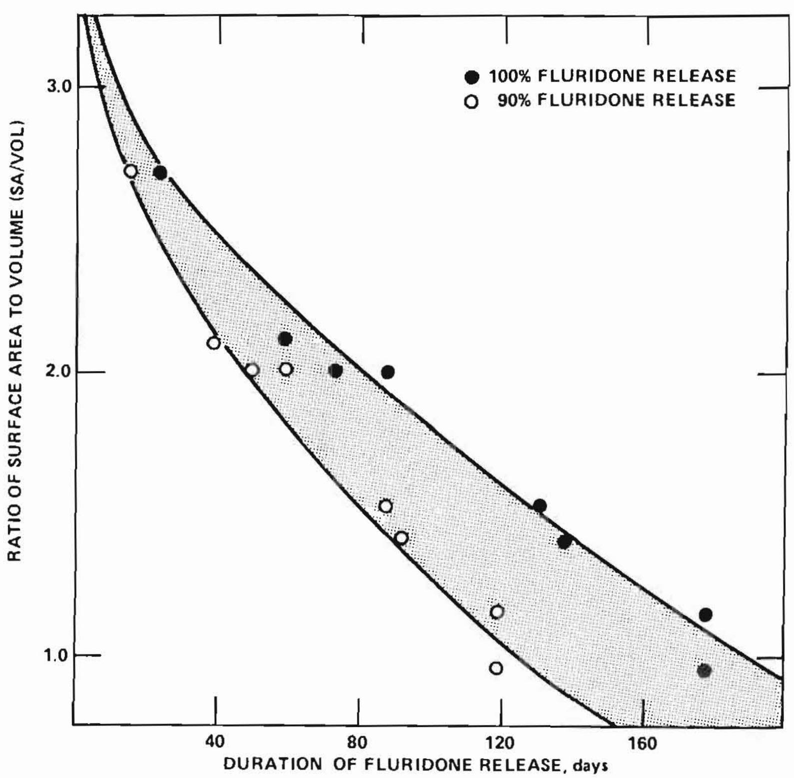


Figure 8. Duration of fluridone release from monolithic fluridone/PCL/HPC (10/40/50) pellets as a function of the ratio of surface area to volume

CONCLUSIONS AND RECOMMENDATIONS

Two different controlled-release systems for fluridone in pellet form were developed. Both systems will release fluridone for 20 and 40 days. The first system consists of pellets of the composition 10/40/50 wt percent fluridone/PCL/HPC. The ratio of surface area to volume of the pellets is varied to obtain the required durations of release. A ratio of approximately 2.7 provides a 20-day release duration, and a ratio of approximately 2.4 provides a 40-day release duration. Further, durations of release ranging from 15 to 180 days are possible with pellets of this composition. The second controlled-release system for fluridone consists of a bundle of fluridone/PCL fibers that is coated with a water-soluble polyethylene glycol mixture. Durations of release of 20 and 40 days are obtained by employing the two fiber formulations that were prepared previously.

We recommend that both pellet systems be evaluated in field trials.

ACKNOWLEDGMENTS

The assistance of Dr. David P. Vanderbilt, Research Polymer Chemist, and Mr. J. David Rotenberry, Chemical Technician, in preparing the pellets and conducting the in vitro release studies is most appreciated. We also wish to thank Dr. Howard E. Westerdahl and Dr. Thomas L. Hart of the Corps for their suggestions during the course of the program. Financial support for the project was provided by the US Army Corps of Engineers Aquatic Plant Control Research Program.

MIMOSA PIGRA - A POTENTIAL WETLAND WEED PROBLEM IN FLORIDA

by
Joseph C. Joyce* and Deborah White*

Mimosa pigra is a thicket-forming leguminous shrub that rapidly spreads in agricultural, wetland, and riverine communities. A native of Central America, in the past 10 years this aggressive weed has spread to all tropical regions of the world. It is especially troublesome in southeast Asia where in 1947 it had been introduced into Thailand as a cover crop and for erosion control. The spread of this species into subtropical Florida is now documented from several localities. Based on these reports and its worldwide reputation, *Mimosa pigra* var. *pigra* was included on the Federal noxious weed list (7CF, Part 360) in the *Federal Register* (1984).

Mimosa, commonly called the giant sensitive plant, stands 1 to 4 m tall. Distinctive field characteristics include: (1) sensitive (folding upon touch) leaves, (2) recurved prickles and spines on the stems and leaves and at the junctions of leaflets, (3) flowers arranged in a head with the pink stamens of each flower extended, (4) clusters of flat brown pods each with transverse sections held by the suture, and (5) single seeded sections of the pod with golden hairs scattered on the surface which break from the suture leaving an empty frame.

A complex of morphological and physiological characteristics enables this species of mimosa to quickly colonize new areas. It often produces seeds year-round in the tropics and thus a mature plant may produce an average of 42,000 or more seeds a year (Chanarong Duangsaad et al., no date). The light bouyant seed is covered with hooked hairs and is ideal for dispersal via wind, water, or moving objects. The seeds remain viable for many years and may germinate in a wide range of environmental conditions (Hidejiro Shibayama 1982). Once established, this plant can withstand almost total submergence by readily forming adventitious roots on aerial and submerged stems. It can tolerate upland soils and moisture regimes such as occur along roadways, in secondary forests, and even in highly saline marine habitats in Australia (Miller 1982). The thickets formed by mimosa cause problems by increasing sedimentation along rivers and irrigation systems. This then augments flooding and nutrient levels by obstructing water flow. These shrub barricades block access to habited areas as well as those used for recreation and tourism, agriculture, and fisheries. As the thickets spread they replace native plant species in natural communities and compete with cultivated species on agricultural lands.

Although problems caused by mimosa are reported from the New World Tropics, Mexico, and Africa, it has had the most impact in the West Indies. The intense use of land in these areas has probably caused its rapid spread and also compounds the implications of infestations. Local farmers utilize every open space as part of an integrated plan for the support of livestock, crops, and family. For instance, if

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mimosa dominates roadsides, the cattle that usually graze these areas are forced onto adjacent agricultural fields. Native people have also developed uses for the plant in medicines and as firewood. All of these cultural pressures must be considered in developing management schemes for the control of this shrub.

However, as the patterns of reproduction and establishment of mimosa populations are studied, the natural limits to its growth are being discovered. Germinating seeds and subsequent stages of the young mimosa seedlings are vulnerable to water inundation and their establishment success is possibly restricted to areas of fluctuating or low water levels (Hidejiro Shibayama 1982). As is typical of many legume species, seed germination is induced by ground fires. This affords mimosa an advantage in establishing in nutrient-rich exposed areas. If, however, induced germination is combined with subsequent water inundation, seedling success could be reduced. Its occurrence is also associated with environmental disturbance or newly exposed land. Since ruderal habitats are rapidly increasing everywhere, both of these establishment characteristics can be helpful in predicting where mimosa will spread. Also, a study of *M. pigra* var. *berlandieri* (L) B. L. Turner, which occurs in Mexico and southern Texas, indicates it is a successional species and thus is eventually replaced in areas where succession is allowed to proceed (Farrald and Lonard 1984). Although it is unlikely that *Mimosa pigra* can be eradicated in areas where extensive populations have become established, the characteristics of mimosa's growth ecology or a combination are being used to develop strategies in limiting its spread.

Several methods of control are currently in use. Manual cutting and burning or mechanical operations temporarily control the spread of the mimosa but rapid regrowth reduces the effectiveness of these methods. Chemical management is often the most feasible strategy although repeated treatments are usually necessary. Among the herbicides shown to be effective are glyphosate, hexazinone, and fosamine (Miller, no date). Biological controls such as plant pathogens and insects are being researched. One Mexican beetle species (*Acanthoscelides* spp.) that feeds on mimosa seeds may be effective in reducing the number of viable seeds produced (Miller, no date). Management schemes likely will involve several types of controls to reduce cost and environmental impact.

The extensive open wetlands and waterways in Florida are similar to the habitats mimosa has invaded elsewhere. Locating populations of this pest is essential in assessing its biological potential in Florida. As managers and users of our waters become aware of the threat posed by *Mimosa pigra*, it is very important that they report any populations, even "suspect" ones, to the appropriate authorities.

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THE WHITE AMUR

Latest Developments of the Triploid White Amur

by
J. M. Malone*

The "dream and dreamer have met," to quote an "ole" friend, might best describe the triploid white amur (grass carp) as a candidate for biological weed control in the United States. As such, there are three points, today, that I wish to cover:

a. Is the triploid white amur "for real"?
b. What are the economics of the triploid white amur?
c. What will the supply be of triploid white amur?

TRIPLOID WHITE AMUR ARE FOR REAL

Considering the short and errant commercial life of the F_1 hybrid grass carp (female *Ctenopharyngoden idella* × male *Hypophthalmichthys nobilis*), which was sterile but suffered from a lack of efficacy (35 percent of white amur), the question of the reality of the triploid white amur becomes the keystone on which all commercial possibilities must rely.

Efficacy

Independent research conducted co-jointly by the Coachella and Imperial Valley Water Districts (personal communication with Paul R. Beatty, Director, Aquatic Research) essentially agrees with the aquatic biology technical reports (September 1984), "Biological Control of Aquatic Macrophytes by Herbivorous Carp," of the Illinois Natural History Survey, which finds that the efficacy of the triploid white amur is 90 percent of that of the diploid. The disparity in efficacy, it has been concluded, is not sufficient to alter stocking rates currently assigned to the diploid.

Sterility

Given a working equivalence with diploids in efficacy, sterility then asserts its correct importance in providing a professionally acceptable, innocuous introduction into the environment. As of July 1984, no man-made triploid *fish* of any species had been found capable of reproduction (personal communication of John G. Stanley, Director, US Fish and Wildlife Service Cooperative Units). Preliminary investigations by Drs. Serge and Julia Doroshov, genetists at the University of California at Davis, indicate that the triploid white amur is "predictably functionally sterile." The Doroshovs declared the F_1 hybrid grass carp functionally sterile in all forms (diploid and triploid). The work of this team will be a determining factor in the decisionmaking process of California Game & Fish Commission in considering an Environmental Impact Report on triploid grass carp, to be submitted in the spring of 1985.

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Availability of 100-percent triploidy

Assuming that triploidy ensures sterility, many responsible agencies will be justifiably concerned with whether or not a program involving supply of triploid white amur is manageable. By providing the industry with 100-percent triploid white amur in commercial quantities, through individual blood analysis, this firm has met the prime concern of professional environmental management. The other elements of management, regarding permitting, inspection, and verification of 100-percent certification of triploidy, have been erected and are being employed in Florida. Five other states have already initiated similar programs.

The economics of triploid white amur

The economics is comparable only to other methods offered in control of aquatic growth — chemical and mechanical harvesting. In 1972, the wholesale price for an 8- to 11-in. size diploid white amur (minimum size to stock in the presence of predators) was $1.50 each, FOB hatchery. At the successful stocking rate of 12 fish per acre, the price was well under the cost of one chemical application per acre. At the time stocking diploid white amur into Lake Conroe was accomplished in 1980, the price was $2.00 per fish and today (1984) this price is unchanged. The cost of raw materials for the chemical industry is up from 4 to 12 times 1972 costs and current prices of chemicals reflect this.

How does this translate with a 100-percent triploid white amur? The 1984 price is $2.50 each, plus a $1.00 fee for blood work, totaling $3.50 per fish for an 8- to 11-in size certified 100-percent triploid white amur. The cost of the fish can be amortized over at least 5 years (personal communication with H. von Zon of The Netherlands). Chemical costs, at best, are an annual expense *plus* the labor costs for application. Further, stocking rates of the triploid fish are more sophisticated today with utilization of computer models for achievement of increased accuracy and desired results in control of aquatic vegetation.

A supply of triploid white amur is available

All current orders have been supplied. The foreseeable future demands for 100-percent triploid white amur can be met. We are prepared to offer high percentage (90 percent plus) triploid fingerlings, where applicable, for grow-out prior to doing blood analysis and to provide the techniques and protocol for certification of a 100-percent triploid. Since anyone can make triploids by induction methods of thermal shock, chemical shock, atmospheric pressure shock, and physical shock, the overall supply will ultimately be enhanced, but not the price, as other levels of triploidy produced, to date, between the public and private sector range from a low of 6 percent to a high of 57 percent.

SUMMARY

In summary, the triploid white amur (grass carp) is for real, is affordable, and is available. WHAT IS THE MANDATE?!

THE WHITE AMUR

An Overview of the Use of Triploid Grass Carp (*Ctenopharyngodon idella*) as a Biological Control of Aquatic Macrophytes in Washington State

by
Gilbert B. Pauley,* Gary L. Thomas,* Scott A. Bonar,* and Amy Unthank*

DESCRIPTION OF THE PROBLEM

Washington State has banned the use of the white amur or grass carp (*Ctenopharyngodon idella*) as a potentially risky management tool because of its reproductive potential and possible impact on the various sport fish and wildlife in the state. The potential influence of the white amur, following reproduction and survival of these fish, is the principal issue surrounding the controversy of whether or not to use it as a biological control agent for aquatic macrophytes in various parts of the world as well as Washington State (Stanley, Miley, and Sutton 1978). The grass carp offers a very attractive alternative aquatic weed control mechanism compared to the currently used mechanical removal and chemical elimination methods that are employed in Washington State waters.

The Washington State Department of Ecology (WDOE) and the Washington State Department of Game (WDG) are the primary State agencies involved in controlling the excessive growth of aquatic macrophytes and managing the game fish and wildlife in Washington State lakes. This past year a joint venture was initiated through the Washington Cooperative Fishery Research Unit to study the use of the newly developed triploid grass carp strain.** It is thought that this triploid strain of white amur is probably sterile. Therefore, the use of a potentially sterile grass carp led these State agencies (WDOE and WDG) to give serious consideration to it as an aquatic macrophyte control agent.

In the Pacific Northwest, there is always considerable concern by the various State management agencies that introduced species of fish may interfere in some way with either (a) the various anadromous salmon and trout species, or (b) the various resident warmwater species found in the inland waters such as bass, sunfish, and perch, all of which have enormous recreational and economical importance in Washington State. The ability to introduce a sterile grass carp would greatly reduce the potential for causing irreversible damage to existing native fish

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** The funding for this joint venture research project was accomplished through the Washington Cooperative Fishery Research Unit by the Washington State Department of Ecology, The Washington State Department of Game, the US Fish and Wildlife Service, the US Army Corps of Engineers, and the University of Washington. The triploid strain was obtained from J. M. Malone and Son, Lonoke, Arkansas.

and wildlife populations and lake ecosystems. This triploid fish rekindles the hope of using a sterile fish as a biological control agent that was initially brought about by the sterile hybrid cross between the white amur and bighead carp (Sutton, Stanley, and Miley 1981), which has since proven less successful as an aquatic weed control agent than the normal diploid white amur.

The ultimate goal of this work is to understand how to control, not eliminate, aquatic macrophytes in Washington State by using the white amur in small controlled lake environments of approximately 20 acres. All study lakes have been carefully chosen geographically on private land so that they can be effectively quarantined (Figure 1). They lack both inlet and outlet streams. They possess surface macrophytes that cover at least 40 percent of the lakes' surface area, in addition to submerged macrophytes. They have established reproducing populations of largemouth bass (*Micropterus salmoides*) in addition to at least one other species of spiny-rayed fish — either yellow perch (*Perca flavescens*), bluegill sunfish (*Lepomis macrochirus*), or pumpkinseed sunfish (*Lepomis gibbosus*).

Ultimately, it is hoped that the information obtained from these small test lakes can be used to predict what will happen if grass carp are moved into larger test lakes subsequent to the completion of the initial 5-year study. However, before the actual introduction of any grass carp will be allowed as a means of controlling excessive aquatic macrophyte growth, several phases of the current research must be completed. This research includes a series of laboratory experiments and 2 years

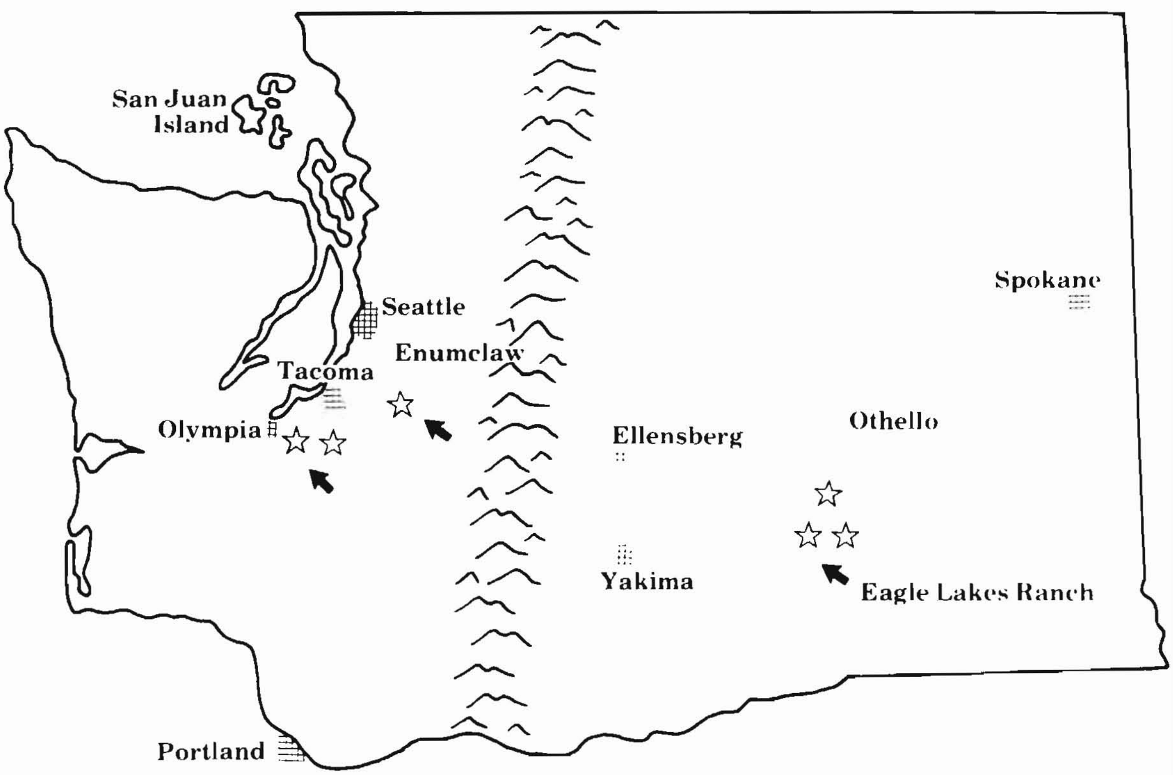

Figure 1. Map of Washington State showing the location of the six study lakes that will be used to evaluate the efficacy and ecological interactions of the triploid white amur. Since climate and geography are considerably different in eastern and western Washington, three study lakes are located on each side of the Cascade Mountains

of field baseline studies in experimental ponds to document the pre-existing limnetic conditions with 2 years of poststocking evaluation (Figure 2).

All fish used in laboratory experimental studies will be quarantined at the US Fish and Wildlife Service National Fisheries Research Center laboratory in Seattle, Wash.

Years 1 & 2 (1985-86)	**Baseline lake studies (East & West)** **Laboratory Studies**
→	**GRASS CARP INTRODUCTION INTO LAKES**
Years 3-5 (1987-89)	**Lake efficacy and interaction studies** **Project evaluation and recommendations**

Figure 2. Task and stocking sequence for triploid white amur in Washington State lakes

STUDY OBJECTIVES AND METHODS

The following paragraphs list the major tasks to be performed in this study, with objectives of each item of work:

Task 1. Comparisons of diploid and triploid grass carp

Morphometric comparisons of the external characteristics of the diploid and triploid grass carp will be made to evaluate the feasibility of a rapid field separation technique. The cost and accuracy of using flow cytometry, coulter-counter, or light microscopy techniques to separate triploid (TGC) and diploid (DGC) fish are being evaluated. This task is explained in detail in our accompanying paper (Bonar et al. 1985).

Task 2. Verification of sterility in the triploid condition

Triploid grass carp will be held in the laboratory through at least one breeding cycle to evaluate gonad development and reproductive behavior. Since maturity is reported to be about 3 to 4 years in the United States (Shireman and Smith 1983), fish in this experiment will be held for 5 years. Systematic dissection of selected individuals will be performed each year to evaluate gross and microscopical gonad maturation. Artificial inducement of gonad maturation and reproduction will be performed using horomone injections (Haddock 1971; Hickling 1967; Konradt 1968). Quarantined diploid grass carp will serve as controls for the test.

Task 3. Evaluate efficacy of plant control by the triploid strain

Both preference and efficiency experiments will be conducted in the laboratory using plants that are a common nuisance in Washington State lakes and ponds.

Plants commonly found as problems in small lakes in Washington include duckweed (*Lemna* sp.), coontail (*Ceratophylum* sp.), waterweeds (*Elodea* sp.), pondweeds (*Potamogeton* sp.), and Eurasian watermilfoil (*Myriophyllum spicatum*). The preference experiments will evaluate the selection of food by the white amur when offered a variety of common aquatic plants in various combinations. The efficiency experiments will evaluate daily consumption rates of specific aquatic plant species offered as food. These laboratory feeding experiments will be designed to estimate parameters of the US Army Corps of Engineers simulation Stocking Rate Model (Miller and Decell 1984) as modified for the triploid grass carp, pondweed, and Eurasian watermilfoil. The white amur Stocking Rate Model was developed for diploid grass carp and *Hydrilla* sp. (Miller and Decell 1984). Three different experimental stocking densities of TGC will be evaluated for controlling *Potamogeton* sp. and *M. spicatum* through the laboratory feeding preference and efficiency experiments and modification of the Stocking Rate Model. These simulated stocking rates will then be used for actual introduction of triploid grass carp into the experimental lakes. The experiment will be conducted with a BACI (before-after/control-treatment) design. Each experimental pond will be divided into four experimental sections with barrier nets that prevent the grass carp from escaping from one section into another. These four sections will be used for each of the three treatment densities developed from the Stocking Rate Model and one for the internal control containing no stocked fish. Two such experimental lakes and one untreated control lake (Figure 3) will be used in both western Washington and eastern Washington for a total of six test lakes. Approximately 250 lakes were screened to locate the six test lakes that will be used. Baseline and efficacy measurements of aquatic plants will be made using standard visual methods for surface plants and hydroacoustics for submerged plants (Maceina and Shireman 1980; Maceina et al. 1984; Thomas et al. 1984).

Task 4. Evaluate triploid grass carp effect on the biota and ecosystem

Since it is possible that the grass carp may interact with the existing fish populations either directly or indirectly, estimates of the existing fish species size, growth rates, age distribution, and length-weight relationship will be needed. Catch per unit of effort (CPUE) mesurements and stomach samples will be taken, along with length, weight, and scale samples. Live capture of the fish will allow us to use a mark-recapture procedure on any of the various species present. Procedures for

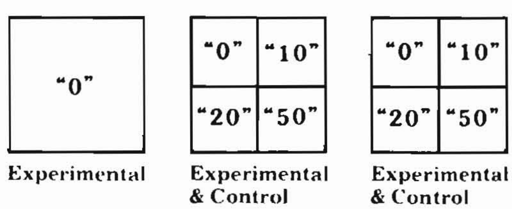

Figure 3. BACI design that will be used in eastern and western Washington experimental and control lakes. Actual numbers will be derived from simulation Stocking Rate Model

gathering and evaluating fish feeding habits, life history data, and mark-recapture data will be those employed by Pflug and Pauley (1983, 1984). Fyke netting and electroshocking techniques will be the primary sampling methods used, along with sports angling. Major invertebrates, such as crayfish, as well as phytoplankton and zooplankton will be monitored before and after the introduction of grass carp to estimate possible predatory and competitive interactions of the white amur for food resources.

Evaluation of the grass carp effect on primary production in the experimental ponds will be measured by monitoring carbon, nitrogen, and phosphorus throughout the study. This part of the study, subsequent to harvest of the aquatic macrophytes by the white amur, should demonstrate the possible relocation of the plants' component carbon, nitrogen, and phosphorus. The WDOE is extremely interested in the impact that the grass carp may have on nutrient cycling and the carbon, nitrogen, and phosphorus budgets in the ecosystem of these ponds. Major ecological items that will be sampled to demonstrate the carbon, nitrogen, and phosphorus budgets and cycling distribution are fish, zooplankton, benthic invertebrates, aquatic plants, phytoplankton, microbes, water column, sediments, input and output (Figure 4).

Task 5. Evaluate effect of grass carp on migratory waterfowl

Large numbers of Pacific flyway waterfowl and other waterfowl spend some part of the year in Washington State. Since food availability may determine the site of overwintering of migratory waterfowl, it is logical to assume that resident waterfowl may be affected to an even greater extent by changes in forage availability. Visual counts of waterfowl by species will be made and evaluated with respect to both diel and seasonal variability. Interaction of white amur on nesting, breeding, habitat selection, and feeding habits of various waterfowl will be

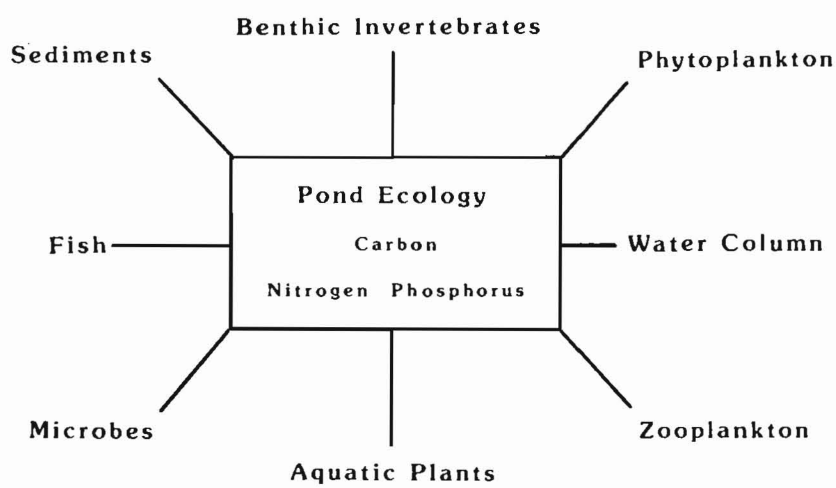

Figure 4. Ecosystem items that will be sampled to determine carbon, nitrogen, and phosphorus nutrient budgets and cycling following white amur introduction

evaluated. Nest spacing and social interactions of various waterfowl species will be evaluated in a manner similar to the work of Lokemoen, Duebbert, and Sharp (1984).

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THE WHITE AMUR

An Evaluation of Fisheries Sonar Techniques as a Tool for Measuring Aquatic Macrophyte Biomass

by
G. L. Thomas,* D. A. Marino,* R. E. Thorne,* and G. B. Pauley*

INTRODUCTION

Excessive growth of aquatic macrophytes interferes with the navigation and recreational use of many of our inland waters (Rottman 1977; Mitzner 1978). These situations have created the need for water-use management agencies to select aquatic plant harvesting and control techniques from the variety of chemical, mechanical, and biological methods that are presently available. The initial strategy of many agencies was to remove all plants which made the evaluation of suitable control techniques relatively easy since presence or absence of aquatic plants before and after the application was all that was needed to verify success. However, today many agencies are concerned with selecting aquatic plant control techniques that can "maintain" a healthy aquatic plant community below nuisance levels. The development and evaluation of controlling techniques of this nature require the ability to measure plant biomass with a much higher degree of accuracy than presence or absence.

The traditional discrete sampling methods for assessing the distribution and density of aquatic macrophytes are laborious, costly, and subject to error. The development of more cost-effective sampling methods is needed by agencies in order to increase their management capabilities (Sabol 1984). Maceina and Shireman (1980) and Maceina et al. (1984) pioneered the use of commercial recording fathometers to determine the distribution and to estimate biomass of aquatic plants. Their results suggest that considerable savings in time and manpower can be achieved with sonar techniques over the traditional direct sampling techniques. The purpose of this research is to investigate the feasibility of applying scientific quality sonar equipment and acoustic propagational theory to improve present aquatic plant measurement methodology.

The specific objective of the proposed research is to evaluate the capability of fisheries sonar equipment to measure Eurasian watermilfoil (*Myriophyllum spicatum*) density in Lake Washington, Seattle. Measurement precision was compared for a variety of combinations of the available acoustic system parameters, such as beam pattern, transmit power, receiver sensitivity, pulse length, frequency, and other pertinent parameters.

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METHODS

The central area of Union Bay, Lake Washington, Seattle, was chosen for study because it was infested exclusively with Eurasian watermilfoil. Three areas with different plant density (high, medium, low) were located visually by estimating the percentage of canopy cover on a 30-m line between two buoys. Bottom depth along the 30-m transect lines ranged between 2.0 and 2.3 m. Plant density ranged from less than 5-percent plant cover along the low density transect, 50-percent plant cover in the medium density area, to 100-percent plant cover in the high density area. Plants in all areas extended from the bottom to within 25 cm (average) of the surface, which required a V-fin carrying the transducer to be towed as close to the surface as possible. The transducer face depth in tow was estimated at 20 cm for the 10-deg transducer and 25 cm for the 6-deg transducer.

A Biosonics 101 transceiver with a carrier frequency of 420 KHz was employed as the echosounder system by triggering internally at a pulse rate of 10 pulses/sec. This system had a time varied gain (TVG) circuit that began at a range of 2.5 m. Consequently, over the range of interest, the TVG was not functional.

The initial selection of system parameters was based upon theory. The aquatic macrophytes occupied depths to 2.5 m, which indicated that the highest frequencies and shortest pulse lengths available in our fisheries sonar systems were desirable (420 KHz and 0.1 msec). Given these characteristics, the empirical evaluation of the equipment involved the selection of power and beam pattern characteristics. The power requirement was discovered to be lower than what was available in the equipment, primarily due to the large target strength of the plants and the absence of the need to sample large volumes to "find" the plants (a characteristic critical to fisheries assessment because of the mobility and relatively low target strength of the targets). Thus, the lowest power setting available was utilized to evaluate a variety of beam patterns. All calibration was performed at the 0-dB calibrator level. The settings generated a constant calibration (cal.) tone of 0.32 volt peak to peak (vpp) to a range of 3.3 msec or 2.5 m.

The 8-KHz signal was digitized on the right video channel of a Sony PCM-F1 Digital Audio Processor, and recorded on a Sony SL-2005 Beta format video cassette recorder. The record level on the digital audio processor was adjusted such that a 5-volt peak (vp) signal (generated by the echosounder calibrator) would not saturate the tape. Calibration signals and echosounder monitoring were done on a Tektronix 305 DMM oscilloscope.

Six- to ten-degree transducers were mounted in a 2-ft Braincon V-fin and deployed from a boom mounted forward the bow of a 6-m flat-bottom boat. The 115-volt AC equipment was powered using a 500-watt, 12-volt static inverter. Mean boat speed was measured at 1.4 m/sec. One hundred and thirty-two acoustic transects were conducted which included twenty-two replications each for six combinations of three plant densities and two transducer beam widths.

The same data recording system was used for playback of the recorded echo signal. Echo analysis was conducted with the aid of a Tektronix 7313 oscilloscope with storage capability which allowed each pulse echo to be evaluated. Since the pulse rate of 10 pulses/sec, or 600 pulses/min, was too fast to evaluate for the presence of bottom echo, the data were subsampled at a rate of 56 pulses/min or approximately 1 pulse/sec. The percentage of pulses with bottom echo, the major variable, was calculated for each replicate. The mean percentage of pulses with bottom detection was then determined for each transect and transducer.

RESULTS AND DISCUSSION

The Eurasian watermilfoil bed in Union Bay formed an intermittent to solid subsurface canopy. This area of solid canopy had extremely high target strength which consequently caused the plant signals to saturate at very short ranges. We concluded that this saturation problem would have to be resolved before plant biomass could be estimated since the saturation resulted in loss of the bottom signal (Figure 1), and indicated divergence from acoustical propagation assumptions. Thus, our preliminary efforts were to configure a hydroacoustic system that could allow continuous bottom detection in the highest milfoil density.

The two system parameters that we chose for attempting to increase bottom detection were the reduction of transmission power and tranducer beam widths. First, the reduction of transmission power to minimum settings (-13 dB) provided only slight increases in bottom signal detection. Second, two transducer beam patterns which had 10-deg and 6-deg full angles were empirically evaluated. The 10-deg transducer performed only 53 percent as efficiently as the 6-deg transducer at sampling a solid canopy (100-percent cover) of Eurasian watermilfoil (Figure 2) (where efficiency is defined by the proportion of bottom echo detections). In an intermittent canopy (30-percent to 50-percent cover), the 10-deg transducer was 93 percent as efficient as the 6-deg transducer. Finally, in an area of less than 5-percent plant canopy, the 10-deg transducer was 99 percent as efficient as a 6-deg transducer.

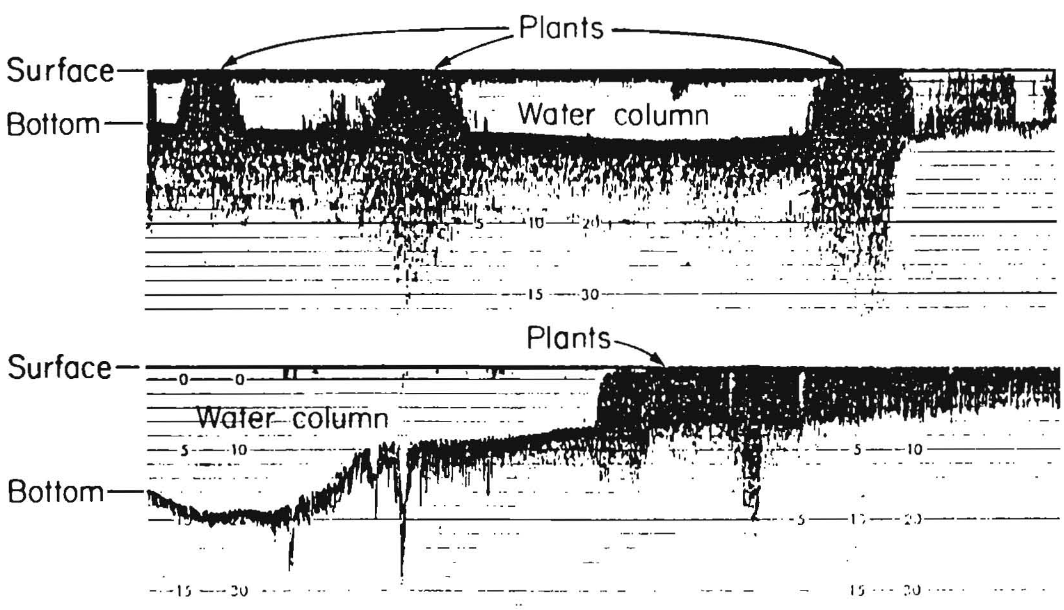

Figure 1. Two echograms illustrating the excessive shading of lake bottom and signal saturation

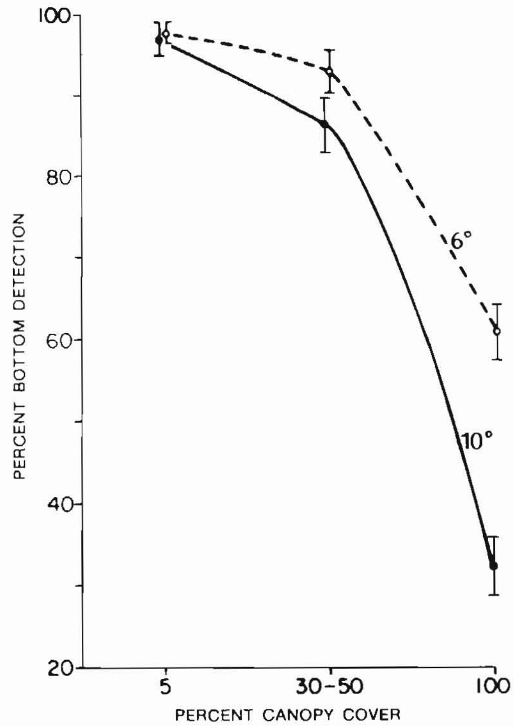

Figure 2. Percent bottom detection with 6- and 10-deg transducers in three different densities of Eurasian watermilfoil

The Eurasian watermilfoil in Union Bay of Lake Washington, Seattle, grows to the surface in late August. This presented another problem with respect to the use of fisheries sonar equipment. The 420-KHz transducer near-fields extended to a 40-cm depth for the 10-deg transducer and 60 cm for the 6-deg transducer. Acoustic propagation and scattering characteristics are not predictable in the near-field ranges. Therefore, a system is needed that allows not only penetration of the plant layer, but also has a short near-field range to allow data processing at close range. A higher system frequency of 1 MHz or greater would permit use of a narrow beam transducer for bottom detection while at the same time have a short near-field range (Figure 3). We see that development of this equipment is the next logical step in the evaluation of the use of sonar techniques for aquatic plant biomass assessment.

CONCLUSIONS

Fisheries sonar equipment is not suited for aquatic plant biomass estimation because of design characteristics that maximize the range and volume sampled by use of higher transmit power, lower frequencies, and wider beam patterns than are suitable for aquatic plant measurement. However, our analyses suggest that new acoustic equipment can be developed that would enable researchers to examine the application of acoustic propagational theory to plant biomass estimation.

ACKNOWLEDGMENTS

This work was supported by the US Fish and Wildlife Service, University of

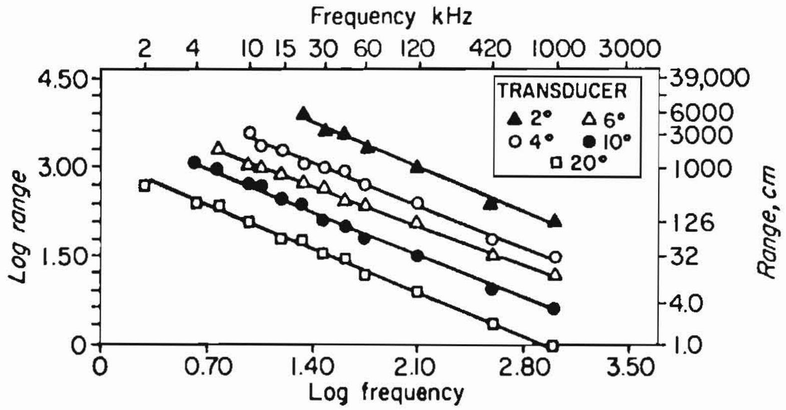

Figure 3. Relationship showing decrease in near-field range with increasing frequency and/or beam angle

Washington, Washington State Departments of Game and Ecology, Biosonics, Inc. (Seattle), and the US Army Corps of Engineers (Seattle District).

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THE WHITE AMUR

An Evaluation of Ploidy Separation Techniques for Grass Carp (*Ctenopharyngodon idella*), A Potential Biological Control of Aquatic Macrophytes in Washington State

by
Scott A. Bonar,* Gary L. Thomas,* Gilbert B. Pauley,* and Amy Unthank*

INTRODUCTION

Induced triploidy has been researched as a method to provide sterile populations of herbivorous grass carp *(Ctenopharyngodon idella)* (Stanley 1979). The production of triploid grass carp involves batch treatment of diploid zygotes with temperature, chemical, and/or pressure shocks with the intention of producing meiotic blockage and retention of a second polar body. Whatever the treatment, the production of triploids is variable, usually 60 to 90 percent, but never 100 percent (Allen and Stanley 1979; Gervai et al. 1980; Chourrout 1980; Thorgaard, Jazwin, and Stier 1981; Allen, Gagon, and Hidu 1982). Therefore, cost-efficient techniques for separation of mixed triploid and diploid fish are necessary before Washington State management agencies will consider field introductions of grass carp to control aquatic plants. The objective of this research is to compare the speed, accuracy, and cost of a variety of techniques for separating triploid and diploid fish.

The methods of rapid separation being investigated are:

- The comparison of external morphology and meristics.
- The comparison of internal morphology by ultrasound.
- Flow cytometry, a technique which measures the actual DNA of blood cells (Thorgaard et al. 1982; Allen 1983; Allen and Stanley 1983).
- The use of the Coulter Counter to measure erythrocyte volume (Wattendorf 1984).
- The direct measurement of erythrocyte dimensions by the use of light microscopy (Allen and Stanley 1979; Thorgaard and Gall 1979; Wolters, Chrisman, and Likey 1982; Wattendorf 1984).

Presently, the most accepted techniques are those methods which measure blood cell parameters. Flow cytometry measures the relative DNA content of fish erythrocytes. The DNA of erythrocytes is treated with a florescent dye and passed through a laser beam, where flourescence measured by the machine corresponds to the relative amount of DNA within the cells. The measured flourescence is commonly standardized with the flourescence of chick erythrocyte DNA.

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The Coulter Counter is commonly used to estimate particle size by measuring the increase in resistance experienced by a continuous current passing through a small orifice (70 μ) whenever a particle passes through displacing the electrolyte (Wattendorf 1984). The measurements of resistance are processed by a channelizer and displayed as a probability distribution which provides an indication of particle size. Size differences of diploid and triploid erythrocytes make this method practical.

A slower method, but one which requires less elaborate equipment (i.e. less initial capital expenditure), is the actual measurement of erythrocyte nuclear volume by light microscopy. The triploid nuclei is more elongated, making separation of triploid and diploid erythrocytes possible by the calculation of volume from measurements of nucleus major axis length (Allen and Stanley 1979; Wattendorf 1984).

The use of internal morphological measurements of diploid and triploid fish has previously been given little attention because of the impracticality of having to sacrifice the fish examined. However, if differences in internal structure are present, ultrasound may constitute a feasible method to separate fish of different ploidy. Ultrasound produces a picture of internal anatomy in much the same way as an echo sounder on a ship produces an image of a segment of the ocean floor and its associated water column. This method was previously used to separate fish according to sex (Martin et al. 1983).

The first phase of this research, the comparison of the external morphology of the diploid and triploid grass carp, has been completed. The benefits of discovering a successful visual sorting technique are that expensive sorting equipment and highly skilled technicians are not required. This paper summarizes the results of our investigation of differences in diploid and triploid external morphology and their feasibility for use in rapid separation.

METHODS

Twenty-five diploid and twenty-five triploid grass carp (standard length 178.26-245.03 mm) were obtained from J. M. Malone and Son, Lonoke, Arkansas.* Two diploid fish were excluded from examination because of deformities. Figures 1 and 2 and Table 1 show measurements, scale counts, and fin formulas that were recorded for comparative purposes. These parameters were noted according to standard methods used for freshwater fishes (Sterba 1962; Hubbs and Lagler 1947). All measurements were divided by the standard length of the fish, except scale counts and fin formulas, which were left in original form. The Shapiro-Wilk test was then used to check the normality of the data (Zar 1974). Once normality was determined for data of each measurement, diploid and triploid measurements were compared with either the parametric one-tailed t-test or the nonparametric Mann-Whitney test (Zar 1974). The null hypothesis tested was that the mean value for each

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* Fish were separated using the Coulter Counter technique. Subsequent meristics and morphological characters will also be obtained from fish separated by the Coulter Counter in combination with flow cytometry and separation by light microscopy.

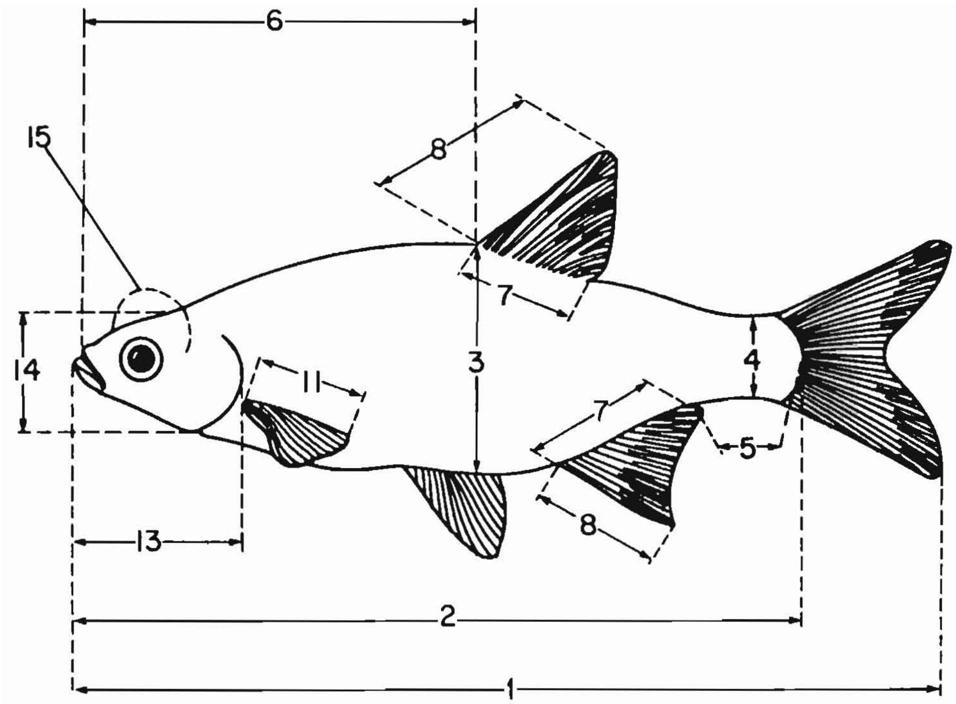

Figure 1. Schematic of body measurements made to compare triploid and diploid grass carp (code interpretations are presented in Table 1). Measurement codes 9, 10, 12, 20, 22, and 27 left off for clarity

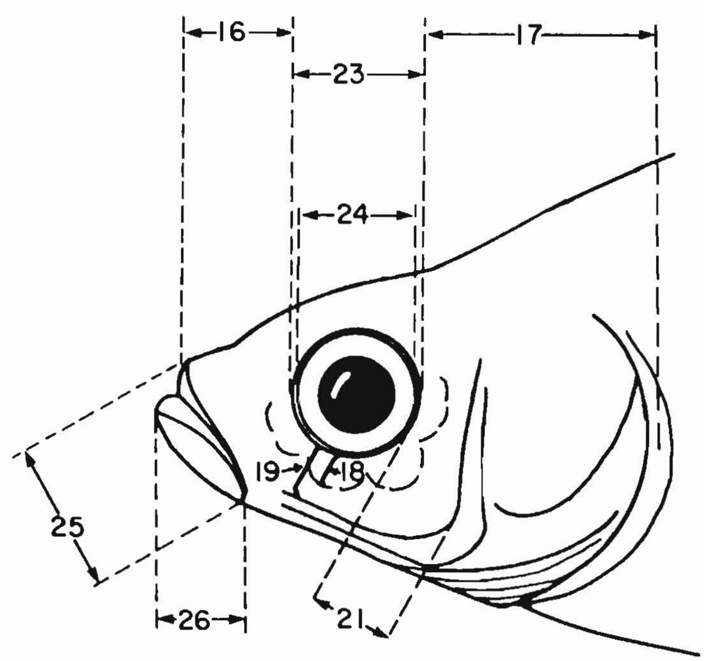

Figure 2. Schematic of body measurements taken from head region (code interpretations are presented in Table 1). Measurement No. 17 taken from orbit edge to both fleshy and bony margin of opercle

Table 1
Measurements, Scale Counts, and Fin Formulas Recorded. Number of Characteristic Corresponds to Those on Figures 1 and 2

Measurements	
(1) Total length	(13) Head length
(2) Standard length	(14) Depth of head
(3) Body depth	(15) Head width
(4) Depth of caudal peduncle	(16) Snout length
(5) Length of caudal peduncle	(17) Postorbital length of head
(6) Predorsal length	(18) Suborbital width
(7) Length of dorsal and anal fin base	(19) Height of cheek
(8) Height of dorsal and anal fin	(20) Length of cheek
(9) Length of depressed dorsal and anal fin	(21) Orbit to angle of preopercle
(10) Length of longest dorsal and anal fin ray	(22) Interorbital width
(11) Length of pectoral and pelvic fin	(23) Length of orbit
(12) Length of longest pectoral and pelvic ray	(24) Length of eye
	(25) Length of upper jaw
	(26) Length of mandible
	(27) Width of gape
Scale Counts and Fin Formulas Taken	
(1) Lateral line scale count	(5) Circumferential scale count
(2) Count of scales above lateral line	(6) Caudal peduncle scale count
(3) Count of scales below lateral line	(7) Pectoral, pelvic, dorsal, anal, and caudal fin formulas
(4) Count of scales before the dorsal fin	

triploid measurement was equal to the mean value of each diploid measurement. The a=0.05 level of significance was used as a criterion for the rejection or acceptance of the null hypothesis.

RESULTS

The comparisons that exhibited significant differences between diploid and triploid fish are shown in Table 2. The data show that no single measurement was sufficient to separate diploid and triploid fish with 100-percent accuracy. The triploid fish had more body weight per unit of length than the diploid, as is shown by the triploid's larger mean ratios of body depth and caudal peduncle depth divided by standard length. Corresponding mean circumferential scale counts and mean caudal peduncle scale counts were also larger than those for the diploid. The characteristic exhibiting the largest significant difference between diploid and triploid fish was the mean circumferential scale count. Other significant differences were noted in the bones of the cheek region. Mean suborbital width and mean height of cheek measurements were larger for the diploid than the triploid. Mean gape width was slightly larger for the triploids. The lateral line count, which has shown

Table 2
Morphological Characteristics of Triploid and Diploid Grass Carp That Exhibited Significant Differences Upon Comparison. All Measurements Were Made to the Nearest 0.01 mm

Measurement or Count	*Ploidy*	$\bar{X}$ *	*SE*	*Test*	*Test Statistic*	*p*	*df*
Body depth	Triploid	0.22515	0.0025	t-test	t=3.75	0.0003	36.5
	Diploid	0.21090	0.0032				
Caudal peduncle width	Triploid	0.11898		Mann-W.	w=720.0	0.0272	
	Diploid	0.11158					
Suborbital width	Triploid	0.03504	0.00044	t-test	t=-3.13	0.0018	33.7
	Diploid	0.03795	0.00082				
Cheek height	Triploid	0.04742	0.00036	t-test	t=-3.19	0.0002	39.6
	Diploid	0.04990	0.00052				
Circumferential scale count	Triploid	31.52	0.15	t-test	t=5.90	0.0000	36.9
	Diploid	29.78	0.25				
Caudal peduncle scale count	Triploid	17.72	0.17	t-test	t=3.29	0.0012	35.4
	Diploid	16.61	0.29				

* For body measurements, $\bar{X}$= mean of measurements divided by standard length; for scale counts, $\bar{X}$= mean count. For caudal peduncle width, which had a nonnormal data distribution, medians were recorded in $\bar{X}$column instead of means.

promise in other examinations of diploid and triploid grass carp as a method of separation,* did not show significant differences.**

DISCUSSION

Although several differences in external characteristics were noted between triploid and diploid fish in this particular study, none could be used for a determination of ploidy with 100-percent accuracy. The variable exhibiting the largest difference between diploid and triploid measurements was the circumferential scale count, which was difficult and time-consuming to obtain because of the very small and poorly defined scales on the ventral surface of the fish. Significant differences in four "thickness" variables (mean body depth, mean caudal peduncle depth, mean circumferential scale count, and mean caudal peduncle scale count), which were larger for triploid than diploid fish, could indicate differences in growth rates and length/weight relationships which could potentially be used to separate diploid and triploid fish. More work needs to be conducted on these measurements to determine if large populations of triploid and diploid fish actually exhibit these apparent differences or if they are just characteristic of this particular sample. Triploid fish from other sources need to be obtained and studied with their significant characteristics noted. Other tests are presently being conducted on this sample to determine: (1) if combinations of variables could be used to significantly

* Personal Communication. Jeff Underwood, Biological Scientist II, Fisheries Research Lab, 601 W. Woodward Avenue, P.O. Box 1903, Eustis, Fla.

** Lateral line statistics for this sample were as follows: triploid mean scale count, 42.36; diploid mean scale count, 41.70; t=1.17, p=0.25, df=44.0.

increase the accuracy of separation over that of single variables, and (2) if other nonstandard measurements, such as the volume of individual fish, could be used as a method of separation. Based on our results, separating triploid and diploid grass carp with 100-percent accuracy according to external morphology does not appear to be practical at this time. However, use of external morphology to maximize numbers of triploid grass carp in a sample to be examined with another method such as flow cytometry, Coulter Counter, or light microscopy examination of erythrocytes is definitely feasible.

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THE WHITE AMUR

A Method for Inducing Triploidy in Grass Carp and Growth Variations of Diploid and Triploid Grass Carp

by
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INTRODUCTION

The triploid or sterile grass carp (*Ctenopharyngodon idella* Val.) is rapidly gaining acceptance as an aquatic weed control agent in areas where diploid or fertile grass carp were once banned. In Florida, a permit system has been established by the Florida Game and Fresh Water Fish Commission enabling the use of sterile grass carp in many situations. Methods for inducing triploidy in other species (Thorgaard 1983) are expected to be similar to those necessary for producing triploidy in grass carp. However, methods presently being used by commercial producers have not been made available. This report provides a general overview of the results obtained from two studies conducted at the Lee County Hyacinth Control District during 1984 dealing with methods for inducing triploidy in grass carp and subsequent growth patterns of triploid grass carp grown together with diploid fish and grown separately. Detailed reports of both studies will be available from the senior author.

TRIPLOID INDUCTION

Thermal shocks of fertilized eggs have been used to induce triploidy in several species of fish (Gervai et al. 1980; Hoornbeck and Burke 1981; Wolters, Libey, and Chrisman 1981). However, the timing, temperature, and duration of the thermal shock that produces the best results varies between species and to some extent among individuals. Other methods involving chemical inducers such as cytochalasin B and colchicine have also resulted in polyploid offspring (Refstie, Vassvik, and Gjedrem 1977; Allen and Stanley 1979).

Hypophysation of broodfish can be accomplished by a variety of hormone injection techniques and is reviewed by Smith and Shireman (1983). Our best results were obtained by two injections of human chorionic gonadotropin (HCG) (440 IU/kg first injection and 1870 IU/kg second injection) 24 hr apart and a third injection of carp pituitary (9.9 mg/kg) 24 hr after the second HCG injection.

Hot and cold water baths were maintained by means of a thermostatically controlled water heater-circulator or chiller-circulator. In situations where eggs were acclimated to the cold shock (temperatures preceded by ~, i.e. ~7°C), ice was used to gradually lower the temperature to the target temperature as close to 5 min after egg-sperm activation as possible. A total of 45 different combinations of shock

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temperature, timing, and duration were conducted on eggs from a total of nine females. The best combinations are listed in Table 1. The reasons for inter-female/egg variations from the same or similar inducements is unknown to us but may be related to egg ripeness, and the timing of ovulation and stripping, among other factors. The relatively low survival is disappointing, although a large female weighing 10 to 12 kg could produce upwards of one million eggs in which 5 percent survival and 30 percent triploid could still yield as many as 10 to 15 thousand triploid fry. This level was achieved on at least five occasions.

Further research dealing with egg ripeness and its relationship to thermal shock susceptibility (induced triploidy) will be required for better survival and consistency of results.

GROWTH COMPARISONS

Growth of fry and fingerling diploid and triploid grass carp was evaluated under a variety of situations involving high and low stocking density in conjunction with various levels of food availability (duckweed, *Lemna* sp.). Diploid grass carp grew faster and had significantly ($p < 0.05$) higher condition factors when grown together with triploid grass carp in every situation. When diploid and triploid grass carp were grown separately and fed to satiation with duckweed, there were no significant differences ($p < 0.05$) in growth rate, condition (k), conversion, or rate of consumption. The combined effect of a slightly more efficient feed conversion rate and higher consumption for diploid fish is probably responsible for their faster growth when grown together with triploid fish competing for the same resources. The physiological and or behavioral reasons for this phenomenon are unknown.

The differences between the rate of growth between diploid and triploid grass carp can be utilized to presegregate diploid from triploid fish, especially at the fry and fingerling stage, so as to reduce the time spent certifying ploidy by means of a

Table 1
Thermal Shock Treatments Resulting in the Highest Percentage of Survivors and Triploid Offspring

Treatment °C	*Ambient Temp., °C*	*Minutes After Activation*	*Duration*	*Percent Viable**	*Percent Triploid***	*Date*†	*Percent Viable* Control*
5	23.8	4:45	6:00	78	18	15 Apr	82
5	23.0	4:30	30:00	2	67	24 May	99
5	23.0	4:00	25:00	16	33	02 Jun	87
5	23.0	4:00	27:00	3	100††	02 Jun	87
~7‡	23.0	2:00	25:00	12	67	20 Jun	53
~7	23.0	2:00	27:00	4	33	20 Jun	53
~7	23.0	2:00	30:00	7	83	20 Jun	53
~7	23.0	2:00	25:00	13	50	25 Jun	94

* Survival at 6 to 8 hr after egg-sperm activation (blastula stage).

** Determined from chromosome preparations for 12 larvae from each treatment.

† Different dates indicate results from different females.

†† Eggs from second stripping, 51 min after first stripping.

‡ Acclimated to the target temperature ± 1° C before 5 min after egg-sperm activation.

Coulter Counter. Visual segregation of triploid fish which are less plump can be accomplished with larger fish (≥300 mm total length) with accuracy exceeding 95 percent if the fish have not been overcrowded or underfed. This is more time-consuming since each individual must be examined and the accuracy may vary between individuals making the inspection.

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THE WHITE AMUR

Biological Control of Vegetation and its Ecological Effects in Lake Conroe, Texas

by
R. K. Betsill,* R. L. Noble,* and R. D. Martyn*

Lake Conroe is an 8,000-ha reservoir impounded in 1973. *Hydrilla verticillata* was first observed in the reservoir in 1975 and by 1979 the submersed weed infested approximately 30 percent of the reservoir's surface area. Aerial color infrared photography was used to monitor vegetation levels. By 1981, approximately 46 percent of the reservoir was infested with surface mats of vegetation extending to the 6-m contour. In 1981-1982, grass carp (*Ctenopharyngodon idella*) were introduced at a rate of 75 fish (200-mm minimum) per vegetated hectare. Vegetation was significantly reduced in 1982 and, by late 1983, virtually all submersed vegetation had been removed. Sampling of ecological conditions, initiated in 1980, included bi-weekly or monthly samples of physical-chemical limnology, plankton, and fish populations at eight stations. In addition, annual cove rotenone samples of fish were conducted at six sites.

Limnological conditions varied little among years until 1983. Reduction in macrophytes was accompanied by a decrease in water clarity, as measured by Secchi disk, of about 40 percent. Chlorophyll *a* concentrations approximately doubled in 1983, indicating that the decrease in water clarity was for the most part a result of increased phytoplankton densities. Phytoplankton density in 1983 peaked at approximately twice the density of 1980-1982. Summer phytoplankton density in 1984 was intermediate between 1983 and previous years. Summer and fall 1984 chlorophyll levels were slightly reduced from 1983, and water clarity was slightly increased. Zooplankton peaked in early 1984 at a density approximately twice that of previous years. Potassium concentrations increased in 1983 and declined to intermediate levels in 1984.

Fish populations responded rapidly to the vegetation control. Planktivorous gizzard and threadfin shad increased during late 1982 and early 1983 and remained high through May 1984 as indicated by cove rotenone samples. Largemouth bass, the principal game species, had year classes approximately two thirds smaller in 1983 and 1984, as indicated by summer seine samples and May 1984 cove rotenone data. Growth rate of young bass and survival from age one to two increased in 1983. Cove rotenone samples showed a marked reduction in diversity and biomass of sunfishes (*Lepomis* spp.). Most pronounced declines occurred for small, vegetation-dependent species. Further changes in the fish community are anticipated and research is scheduled to continue through 1986.

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THE POTOMAC RIVER HYDRILLA PROJECT

A Brief Introduction

by
William N. Rushing*

The submersed aquatic plant species *Hydrilla verticillata* (hydrilla) was positively identified at Dyke Marsh on the Virginia side of the Potomac River in 1982. Dyke Marsh is located just south of the Woodrow Wilson Memorial Bridge in sight of the Washington, D. C., metropolitan area. Since its initial discovery, the plant has spread generally within the reach of the Potomac from Washington, D. C., south to Quantico, Virginia, and beyond. In 1984 it was estimated to cover some 500 acres.

Hydrilla exists as both dioecious (male and female flowers on separate plants) and monoecious (both male and female flowers on the same plant) forms. The plants in the Potomac have been identified as the monoecious biotype. Heretofore, only the dioecious biotype had been known in the continental United States.

Because of serious aquatic plant management problems posed by the growth and spread of hydrilla in the southern portions of the United States since 1960, considerable concern has developed about the future growth of the plant in the Potomac River. This concern has evolved throughout the private and public sector, including the boating public; fishermen; environmental groups; local, State, and Federal agencies; the scientific community; and political entities at every level.

The US Army Corps of Engineers through its District Office at Baltimore has the responsibility for development of an operational program for management of aquatic vegetation in the Potomac River. This process involves the preparation of an Environmental Impact Statement and a General (State) Design Memorandum and the subsequent implementation of an operational program. The Baltimore District has secured the assistance of the US Army Engineer Waterways Experiment Station through its Aquatic Plant Control Research Program to provide technical input to aid in the development of the program. This input involves assistance in conducting demonstration trials and in directing short-term and longer range research necessary to answer questions pertinent to the overall knowledge and management of monoecious hydrilla.

The session of the annual meeting on which these proceedings are based was organized to provide a forum for reporting the initial demonstration and research efforts being conducted in support of the Potomac River Hydrilla Project.

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THE POTOMAC RIVER HYDRILLA PROJECT

Hydrilla verticillata in the Tidal Potomac River, 1983 and 1984

by
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INTRODUCTION

From 1978 through 1981, the US Geological Survey conducted a survey of the submersed aquatic vegetation of the tidal Potomac River and Estuary (Paschal et al. 1982; Haramis and Carter 1983; Carter, Gammon, and Bartow 1983; Carter, Paschal, and Bartow 1983) (Figure 1). This survey was part of an interdisciplinary study of the hydrodynamic, chemical, and biological processes in the tidal Potomac River and Estuary (Callender et al. 1984). The 1978-81 survey showed that the tidal river was nearly devoid of submersed aquatic plants, and that the greatest abundance and diversity was found in the transition zone (upper end of the estuary between Quantico, Virginia, and the US Highway 301 bridge).

In 1983, numerous species of submersed aquatic plants were found in the tidal river, giving scientists reason to believe that environmental conditions and water quality had improved. One of the new submersed aquatic plants was *Hydrilla verticillata* (hydrilla),** an exotic species from Southeast Asia which has become a nuisance species in California, Florida, and other states. Hydrilla was positively identified in Dyke Marsh, Virginia, in 1982 (Figure 1). A shoreline survey in 1983 showed that hydrilla was most abundant within 1 to 2 miles of Dyke Marsh on the Virginia side of the river, and south of Quantico, Virginia, in a small tributary of Chicamuxen Creek, Maryland. Hydrilla is believed to be a relative newcomer to the Washington, D.C., area (Steward et al. 1984); it grows and reproduces rapidly and has the potential to outcompete other species.

METHODS

Shoreline surveys for hydrilla and all other submersed aquatic macrophytes were made by boat at low tide in July 1983 and July 1984. Rakes were used to gather samples and to ascertain whether plants were rooted or floating. In 1983, cover was classified as dense or patchy. In 1984, percent cover by submersed aquatic vegetation and the proportion of each species were estimated and referenced to 1-km grids shown on US Geological Survey 7½-min quadrangles, with bathymetric data added. The ranges of percent cover used (<10, 10 to 40, 40 to 70, and 70 to 100)

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* US Geological Survey, Reston, Virginia.

** Data on the distribution and abundance of other species of submersed aquatic vegetation in the tidal Potomac River and transition zone are available in Carter et al. (1985). Substrate types and water quality characteristics are also included.

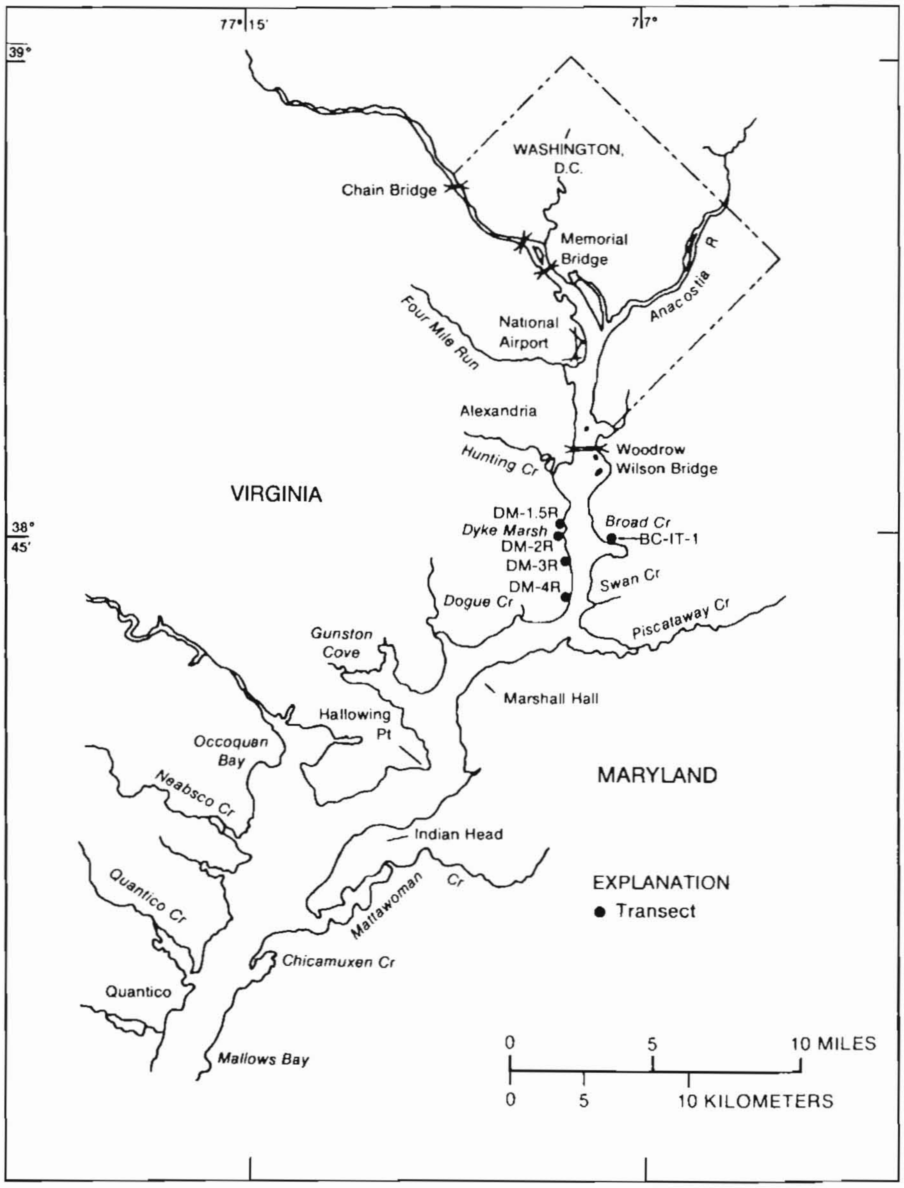

Figure 1. The tidal Potomac River showing transect locations for the *Hydrilla verticillata* study

follow those of Orth, Moore, and Gordon (1979). Spot checks at selected locations also were made during 1984. Based on these data, maps were made of hydrilla distribution and relative abundance in 1983 and 1984 (Figures 2 and 3, respectively).

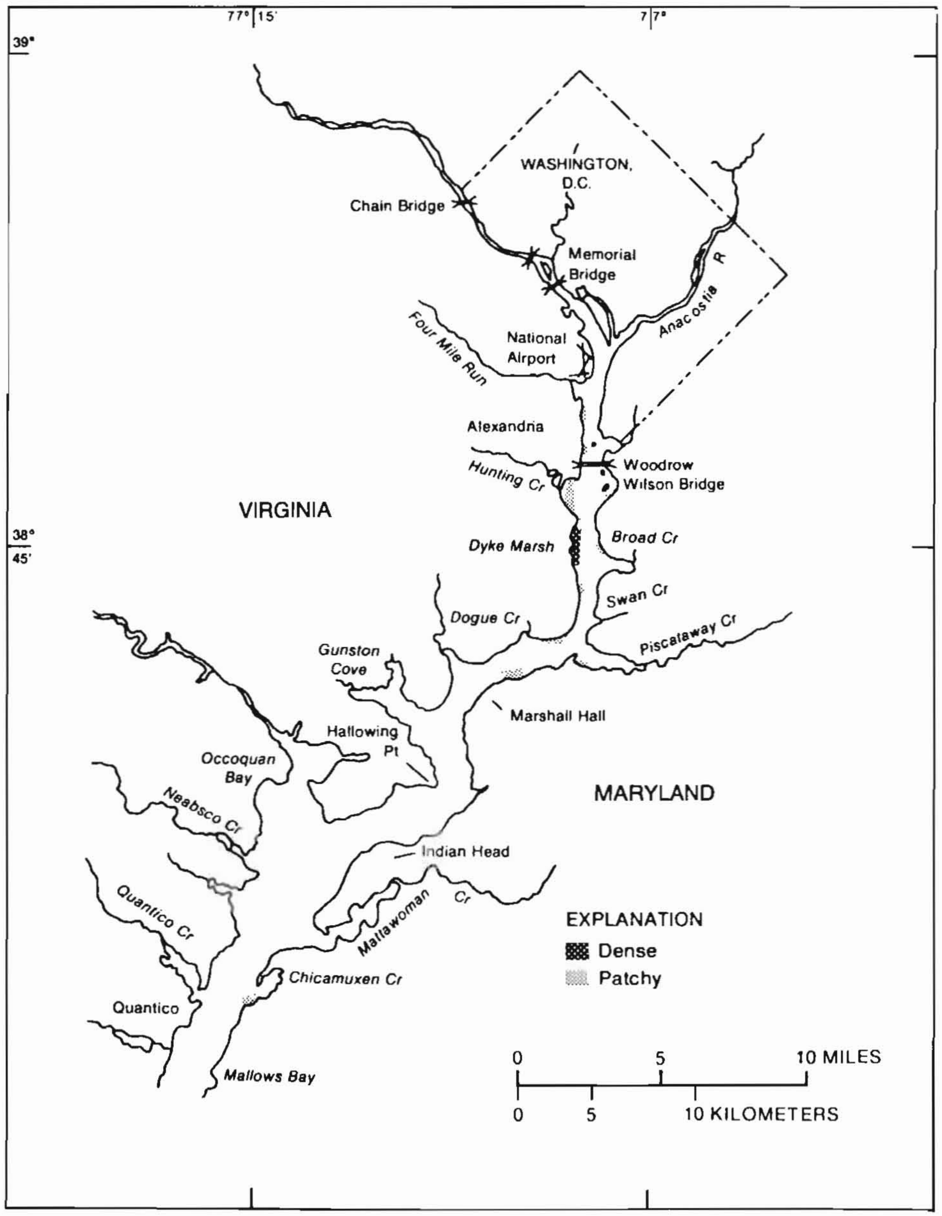

Figure 2. Distribution of *Hydrilla verticillata* in the tidal Potomac River in 1983

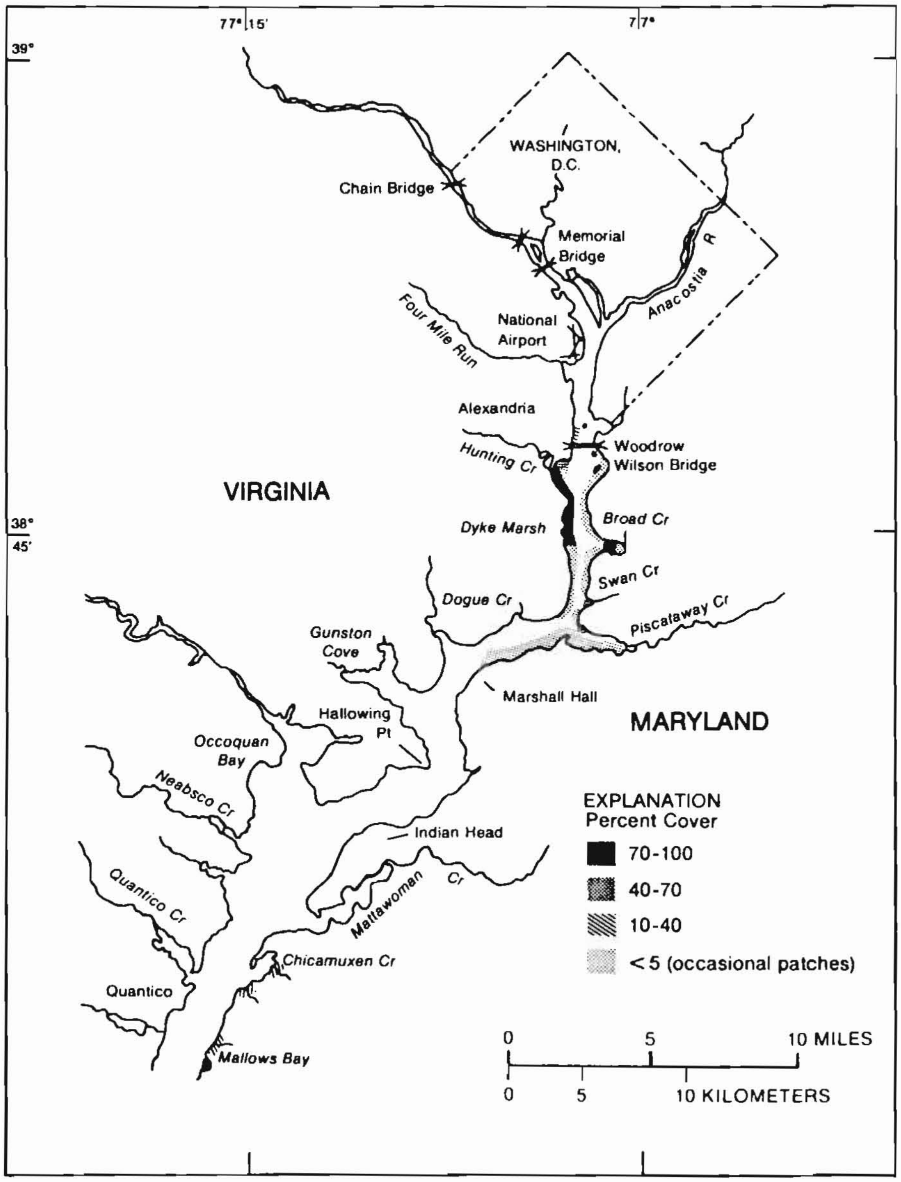

Figure 3. Distribution of *Hydrilla verticillata* in the tidal Potomac River in 1984

ACKNOWLEDGMENTS

This work was partially supported by the US Army Engineer Waterways Experiment Station, Vicksburg, Mississippi. We thank all our colleagues in the National Park Service, the Government of the District of Columbia, and the Northern Virginia Community College for their assistance. We also appreciate the assistance of associates from the Audubon Naturalist Society who helped with the fieldwork on several occasions.

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THE POTOMAC RIVER HYDRILLA PROJECT

Shoreline Survey of Submersed Aquatic Vegetation Including *Hydrilla* in the Transition Zone of the Potomac River During the Summer and Fall of 1984

by
Ruth Ann Allaire,* Kennard W. Potts,* Thomas P. Sheehan,* and Norman R. Sinclair*

INTRODUCTION

Because of the potential environmental hazards of rapid spread of *Hydrilla verticillata* Royle (hydrilla) into the Potomac River, a survey of the current distribution with potential areas for spread was investigated during the summer and fall of 1984 (Figure 1). A comparison with the distribution of other submersed aquatic vegetation was also made.

Objectives included:

- Mapping the southward migration of hydrilla.
- Surveying the shoreline of submerged aquatic vegetation and providing data on present species, percentage, and density of each species.
- Providing data on dissolved oxygen, salinity, temperature, light penetration, and soil type at each site.
- Analyzing data.
- Reporting results with recommendations.

Because the transition zone may act as a natural barrier to the downriver spread of *Hydrilla* colonizations, physical parameters of the area were also included in the study. The southernmost record of hydrilla during the summer of 1983 was the Chicamuxen Creek at the upper reaches of the transition zone. Since *Hydrilla* reportedly can live in salinities of 13 ppt, with very low light intensities and poor aeration, many parts of the transition zone appeared to be suitable for colonization.

The study area consisted of approximately 150 miles of shoreline on the Potomac River from the Quantico Creek and Chicamuxen Creek in the north to the Route 301 bridge in the south (Figure 1). This is called the transition zone because the salinity varies from 0 in the estuaries and northern part of the zone and in the spring of the year, to 8-14 ppt in the southern areas depending upon the season and the relative abundance of fresh water in the river (Lippson 1977).**

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** A. J. Lippson, 1977. *The Chesapeake Bay in Maryland*, The Johns Hopkins University Press.

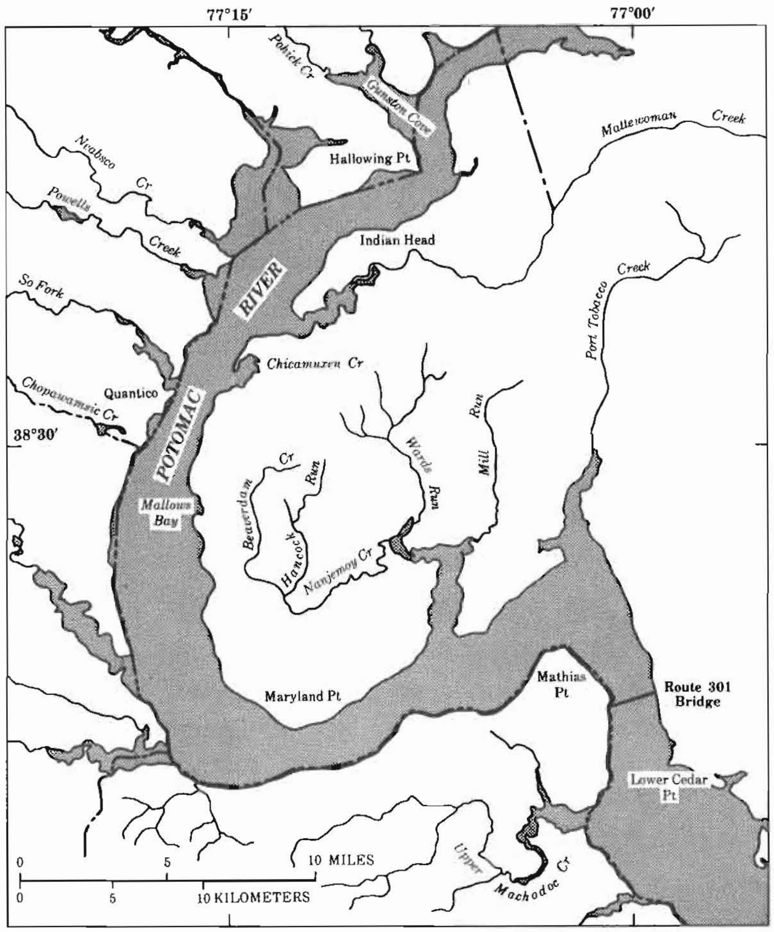

Figure 1. Potomac River study area

DISCUSSION

The limited time frame within which this survey was conducted, coupled with the lack of data from other years, provided a very narrow picture of the submersed aquatic vegetation in the transition zone. Nevertheless, certain patterns of vegetation do appear to be evident. *Hydrilla* and *Ceratophyllum* at present are the species of the guts and upper tributaries, while *Myriophyllum* and *Vallisneria* are more prevalent in the larger creeks and *Vallisneria* has made significant inroads back into the river proper. The high incidence of hydrilla in this part of the river, considering that this should only be the second season of growth for this species, does not bode well for its growth patterns in the following years. While no rooted populations of hydrilla have been found in the river proper in the transition zone, there are large areas in the Potomac, Aquia, and Nanjemoy Creeks and Port Tobacco River which on the face of the available evidence would be suitable for the growth and colonization of hydrilla. Each of these areas supports marinas and extensive recreational boating. Questions still remain as to whether hydrilla will make an effective migration into the vacant niches of the Virginia side or into the already occupied areas on the Maryland side. The fact that a much greater variety of species and a greater density occur on the Maryland side indicates that in some way this area at the present is more suitable to plant growth. What these favorable factors are cannot be stated on the basis of this study.

Future investigations should include chemical analysis of the waters from each of the different areas, studies of the flow and wind activity in each area, and competition studies with *Myriophyllum*, *Vallisneria*, and *Ceratophyllum*, which appear to be the main competitors of *Hydrilla verticillata* in the transition zone. The data indicating a greater light penetration taken from those areas in which hydrilla was present demonstrate that the effective clearing of water by hydrilla indicated in other studies occurs here also. The distinctive pattern of extensively occupying the narrow niches of the marsh guts indicates that hydrilla either has been present in the area for more than 2 years or that when once present it can in one season occupy all of the one niche very successfully. Studies on the growth and development of hydrilla in a gut will be conducted in the 1985-86 seasons. Three guts on the Maryland side and one in Virginia will be utilized for these studies.

According to Cooley, Dooris, and Martin (1980)* aeration may be used to improve the water quality and reduce the subsequent growth of hydrilla. Certainly, hydrilla was not found in any of the higher energy beaches. Whether aeration is a factor in this lack of growth or whether mere mechanical action precludes growth remains to be studied. Suitable beaches and the present colonies in the King George area will be used for studying wave action phenomena in the growth of hydrilla in 1985.

There appear to be two very distinctive varieties of hydrilla in the transition zone of the river. One population in the Mallows Bay area shows distinctive morphological and growth features from every other population within the zone. Specimens will be collected and biological data such as emergence, growth patterns, time and density of turion, and tuber formation will be followed during the next growing season.

If the southern spread of hydrilla into the transition zone occurred only during the 1984 season, then the migration of hydrilla into the even lower regions of the river can be expected in the 1985 season. The upper portions of the estuaries of the Wicomico and Machodoc Creeks and the resort area of Colonial Beach will become areas of hydrilla growth. If, however, the present populations have been present in the river for some time and were mistaken for *Elodea* in previous surveys, then the southern migration of hydrilla will not likely take place beyond the transition zone as the natural barriers have already acted against this migration.

The appearance of *Vallisneria* in many areas of the river indicates the remarkable comeback of this valuable plant. If the factors that are present in the Virginia waters which preclude growth of submersed aquatic vegetation in any numbers can be determined, then perhaps the vegetational patterns of the river will be changed as remedies are taken and if hydrilla does not move in.

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* T. N. Cooley, P. M. Dooris, and D. F. Martin, 1980. "Aeration as a Tool to Improve Water Quality and Reduce the Growth of *Hydrilla*," *Water Research* (UK), Vol 14, pp 485-489.

RECOMMENDATIONS

Recommendations for the 1985 season include an ongoing survey of the region for further expansion and growth of hydrilla colonies, studies on the competitive effects of hydrilla against other species of the region, studies on the chemical and physical criteria of the two different areas within the zone, studies on the aeration or flow factors influencing the growth of hydrilla within the zone, studies and collections of the differing populations of hydrilla within the zone, and finally a preliminary survey beyond the transition zone for further expansion possibilities. Any studies on the production of turions or tubers or their germination under different salinities should be done with the tubers from both populations until it is determined whether or not there are two distinct populations.

THE POTOMAC RIVER HYDRILLA PROJECT

Incidence and Longevity of Fragments of Submersed Aquatic Vegetation in the Potomac River After Mechanical Harvesting of *Hydrilla verticillata*

by
Ruth Ann Allaire,* Kennard W. Potts,* Thomas P. Sheehan,* and Norman R. Sinclair*

INTRODUCTION

Hydrilla verticillata Royle (hydrilla) is a potentially undesirable plant in certain areas of the Potomac River; therefore, methods for its control are of immense interest to many diverse groups involved in river phenomena. Harvesting of submersed aquatic vegetation (SAV) by mechanical means seems innocuous since it does not involve the side effects of herbicides. Mechanical harvesting may, nevertheless, be self-defeating since it opens the door to further spread of the plant. Myriads of chopped up fragments carried downstream can produce many new generations of plants by vegetative reproduction (Allaire, Flint, and Miller 1985).**

This study undertook periodic sampling downstream of harvest sites in the Potomac River for approximately 30 miles, before, during, and after harvesting of submersed aquatic vegetation, primarily hydrilla. Other species in the study were *Myriophyllum*, *Heteranthera*, *Najas*, and *Vallisneria*. This study was conducted during the fall of 1984 in the areas of Dyke Marsh and Olde Towne Yacht Club, Alexandria, Va., and Swan and Treasure Coves in Maryland.

The purpose of this study was threefold:

- Determine if cutting SAV by mechanical means has an impact upon the incidence of floating vegetation and, if so, to what level.
- Determine how far downriver fragments of SAV after harvesting would travel in significant numbers.
- Determine if any species of SAV survived downstream longer than others.

MATERIALS AND METHODS

To sample vegetational fragments, plankton tows and purse seine collections were conducted 10 times during 21 days before harvest, 4 times during the 5 days of harvest, and 5 times during 15 days after harvest, providing 19 sample days at each of 10 sites along the river (Figure 1). Since the epicenter of the hydrilla colony was in Dyke Marsh and Belle Haven Marina, the first sample was taken upriver from

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** R. A. Allaire, M. L. Flint, and P. Miller. 1985. "Viability of Fragments of *Hydrilla verticillata* Royle After Mechanical Harvesting," Manuscript.

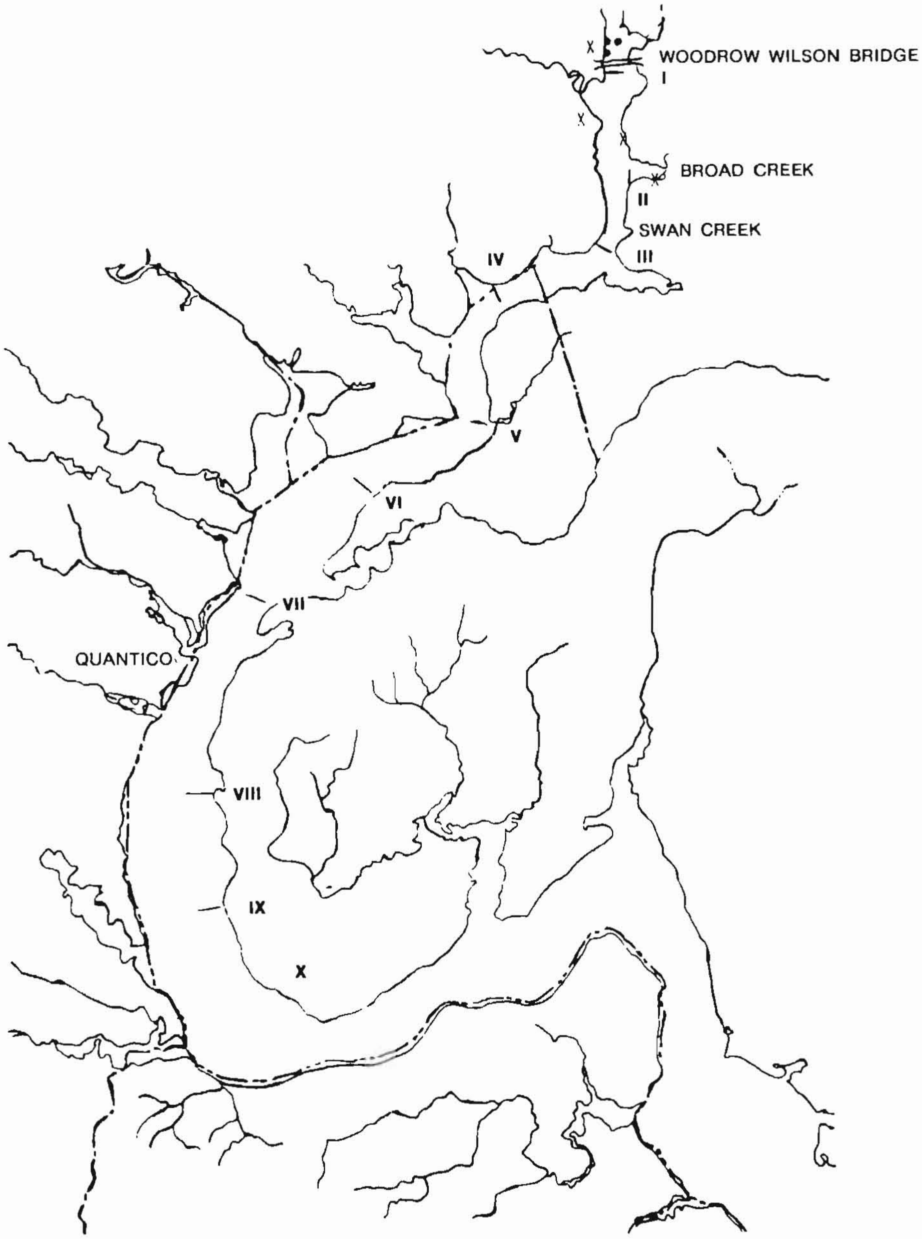


Figure 1. Location of sample sites

this point at the Woodrow Wilson Bridge. The other sites were staggered for 35 miles downstream in order to determine how far fragments would travel in significant numbers.

CONCLUSIONS AND RECOMMENDATIONS

Mechanical harvesting of submersed aquatic plants had a significant impact on the incidence of floating vegetation in the Potomac River for 30 miles downstream and for a period of several days. The increase in vegetative fragments was 10 times greater than the normal incidence established during preharvesting.

The incidence of hydrilla was even more significant with a 300-fold increase in the number of floating fragments by the fifth day of harvest. While the numbers of hydrilla and *Myriophyllum* were somewhat comparable before harvest, after harvest the numbers of hydrilla were greater both in number and in ability to float longer downstream in the river. The species showing the least change in either numbers or shift in numbers downstream was *Vallisneria*. Both *Najas* and *Heteranthera* showed slight increases in incidence of fragments with cutting, but not nearly the changes that occurred with either hydrilla or *Myriophyllum*.

Over a period of 13 days the incidence of fragments in the river returned to the preharvest level. However, since the only species with significant numbers increasing in downstream sites was hydrilla, the impact on future colonization of downstream areas cannot be ascertained without further study. Of extreme importance is the fact that fragments of hydrilla appeared on beaches in the lower study area for 3 weeks after cessation of the tow sampling (personal observations) in greater quantities than had occurred prior to the harvest.

Hydrilla fragments remained in viable condition for at least 32 miles. Since the numbers of *Myriophyllum* and hydrilla were somewhat equal before harvesting and since only large numbers of hydrilla appeared to make the journey downriver in large quantities, a question must be asked as to the longevity and biodegradability of *Myriophyllum* as it contributes a decreasing portion of samples at downriver sites.

In correlating the data from harvesting samples with wind data, there appeared to be no direct correlation with high winds and the incidence of fragments in the river. The wind data used were the maximum wind velocities measured at National Airport above the sampling area and at Fort Belvoir in the middle of the area. The chop on the river was greater during some days of sampling than during the harvesting period.

Tow site III showed the greatest amount of fragments and would have picked up all fragments floating down. Tow site II across the mouth of Broad Creek showed increased numbers of fragments only during the last day of harvest and the first day of postharvest, while tow site III downstream showed increased numbers from the first day of harvest. Tow site I, just below the first harvesting site, did not show a large increase until the fourth day, after harvesting in that particular area. No attempts were made to correlate the flow up river with the tide of floating fragments.

The time of the year in which harvesting was conducted, i.e. when the vegetation was brittle and already breaking up into mats; the absence of long-term data on the incidence of fragments in the river; and the lack of constant monitoring of upstream versus downstream patterns of fragment migration preclude any definite conclusions on the long-term effect of the harvesting. However, such studies as this should continue until such questions as downstream migration effects of harvesting are determined. Certainly, many mats float down the river in the fall and what effect they have on localities downstream is yet to be examined. Whether chopped up fragments have a better chance of dispersing the species than the large mats needs to be examined. Finally, questions as to the buoyancy and biodegradability of hydrilla in comparison to other species of submersed aquatic vegetation should be answered.

THE POTOMAC RIVER HYDRILLA PROJECT

Preliminary Laboratory Research Results on Monoecious Hydrilla

by
Kerry K. Steward* and Thai K. Van*

The female dioecious biotype of hydrilla was introduced into Florida probably from India in 1958 or 1959. Since that time it has spread throughout peninsular Florida, westward through the sunbelt states into the Imperial Valley California irrigation system and northward up the western seaboard into the Sacramento area. Simultaneously the plant has moved up the eastern seaboard into Georgia, Alabama, and South Carolina.

A monoecious biotype has been reported from Virginia, Maryland, District of Columbia, and Delaware and apparently is the result of a separate introduction. The monoecious plant may also be in Pennsylvania since it has been reported to be established in the flats of the Susquehanna River in the upper reaches of Chesapeake Bay.

BIOLOGY

Hydrilla is a multimillion dollar problem when considering the costs of control and management and the losses caused by the infestations due to decreased utilization of the water resources. Because it is rooted to the bottom, this plant is able to obtain its nutrition from both the bottom muds and the surrounding water. The plant is able to dominate an entire body of water rapidly through its very efficient methods of vegetative reproduction, i.e. through runners and stolens over the surface of the bottom muds, and through fragments of the stem which break loose from established colonies, sink to the bottom, and become rooted to form new colonies. The plant produces tuberlike propagules deep within the bottom muds which enable it to survive hostile environments and adverse conditions such as low temperatures or dewatering during drawdowns, and to regrow after herbicide applications for control.

The plant has unique physiological characteristics which enable it to outcompete native species. It is able to utilize carbon from both dissolved carbon dioxide (CO_2) and bicarbonate (HCO_3^-) in solution for photosynthesis. It has a low light requirement enabling it to grow from great depths where light penetration is reduced and it is able to start utilizing carbon earlier in the day than native species, thus reducing the supply of carbon and other nutrients available for competing species.

* US Department of Agriculture, Fort Lauderdale, Florida.

The characteristic that enables hydrilla to be a survivor and so successful in colonizing new areas, and subsequently make it so difficult to control, is its ability to regrow from tubers in the hydrosoil. Tuber formation in the Florida biotype has been observed to start 19 days after planting of apical fragments. Tubers are produced in this biotype in response to short days and the results of studies under controlled environmental conditions indicate this is true for the monoecious plant as well.

PRELIMINARY RESULTS

Collections of the monoecious plants were obtained by the US Department of Agriculture, Aquatic Plant Management Laboratory, in Fort Lauderdale, and stock cultures established in fall of 1981 (Kennilworth Gardens) or fall of 1982 (Trapp Pond, Del.; Lake Anne, N.C.; Lily Ponds, Md.)

A series of studies to compare the monoecious and dioecious biotypes was initiated in order to gain a better understanding of the biology of the monoecious plants. A summary of the results of these studies follows:

Investigate growth and reproductive response to photoperiod. Monoecious and dioecious biotypes produce flowers and tubers mainly in response to short days. Biomass of monoecious biotypes declines and eventually disintegrates under short days. Biomass of the dioecious biotype declines but persists under short days.

Investigate growth response to temperature. Tuber germination of both biotypes was inhibited the first week at 15°C. Tubers of monoecious plants germinated and continued to grow at 15°C after 2 weeks. Germination of tubers from dioecious plants continued to be inhibited at 15°C. Germination and development of tubers was not retarded at 22°C and 30°C. Increase in stem length and dry weight at 15°C was greatest in the dioecious biotype. There were no differences in growth between biotypes at medium (22°C) and high (30°C) temperatures. Contrary to earlier preliminary results, monoecious biotypes do not tolerate lower temperatures except for tuber germination.

Determine light compensation point (light requirement for photosynthesis). This value was found to be 8 $\mu E/m^2/sec$ for the monoecious and 10 $\mu E/m^2/sec$ for the dioecious biotype—approximately 0.3 percent of full sunlight at this location.

Determine partitioning of photosynthate between shoots, roots, and propagules. Under long days, biomass allocation was 91 percent or 81 percent to shoots of dioecious or monoecious biotypes, respectively. Under short days, allocation of biomass to propagule production was 2 percent for dioecious and 52 percent for monoecious biotypes. The number of propagules produced was nearly four times greater by the monoecious biotype, indicating much greater productivity and efficiency in terms of reproductive and survival potential.

Investigate tuber production in outside aquaria. Monoecious biotypes were most productive producing two to three times more tubers per square foot over similar growth periods than the dioecious biotype.

Determine salinity tolerance in order to evaluate potential for colonizing Chesapeake Bay. The threshold level appeared to be approximately 13 ppt since biomass was reduced after 6 weeks to 71 percent of controls and to 29 percent at 14 ppt. This would allow growth to occur in the bay near the mouth of the Potomac River as bay waters are diluted by spring discharges, but not during the fall low discharge period when salinities increase.

Investigate survival and growth of fragments as a means of propagation. The number of new shoots from fragments increased threefold in the monoecious and fivefold in the dioecious biotype after 15 weeks. A total of 12 percent of monoecious and 16 percent of dioecious biotypes produced rooted plants which attached to the bottom.

Evaluate the response of monoecious hydrilla to EPA-registered aquatic herbicides. The results indicate that response is similar to that of dioecious hydrilla, i.e., the same effective herbicides can be used on both biotypes.

THE POTOMAC RIVER HYDRILLA PROJECT

Preliminary Research on Monoecious Hydrilla

by
Lars W.J. Anderson*

Recent findings of monoecious hydrilla (*Hydrilla verticillata* (L.f.) Royle) in the Potomac River and other northeastern locations (Steward et al. 1984; Langeland and Smith 1984) are cause for concern in the western United States. Due to the high proportion of irrigated cropland in the west (Figure 1) and the attendant dependency on an extensive and complex water transport network, there is tremendous potential for economic impacts from hydrilla. Millions of dollars have already been spent to control past and current infestations of dioecious hydrilla in California (Anderson and Dechoretz 1982).

The fact that monoecious hydrilla is proliferating in the Potomac River (Rybicki et al. 1985a, b) adds further concern since flowing water represents a major potential for hydrilla infestation (e.g. Sacramento Delta, California Aqueduct, Delta Mendota Canal, Colorado River, and thousands of miles of lesser irrigation canals). This report summarizes initial results on the vegetative reproduction of monoecious hydrilla.

MATERIALS AND METHODS

Outdoor cultures

Three apical cuttings were obtained from monoecious or dioecious hydrilla that had been grown from tubers in the greenhouse. Four sources of monoecious plants and three sources of dioecious plants were used. Each group of three cuttings was planted in UC Mix in plastic cans (ca. 0.62 × 0.44 × 0.15 m), and kept outdoors in cement tanks (ca. 2.2 × 0.77 × 0.6 m). After 2 weeks, the most vigorous of the three shoots was kept and the other two removed. Water levels were maintained throughout the culture period (3 April 1984 to 15 February/2 March 1985). At harvest, all propagules were collected from each tank by washing through a screen sieve (ca. 3 mm). Total numbers and total weight of tubers and turions were determined.

Photoperiod and tuber production

Plants of each type (monoecious and dioecious) were grown under 10-, 12-, 14-, and 16-hr photoperiods in the greenhouse for 8 weeks. The total number of plants for each type was 128. Plants were harvested at 2-week intervals and the biomass of shoot, tubers, and roots determined. Leaves were also analyzed for pigment content.

* US Department of Agriculture, Agriculture Research Service, Aquatic Weeds Research, University of California, Davis, California.

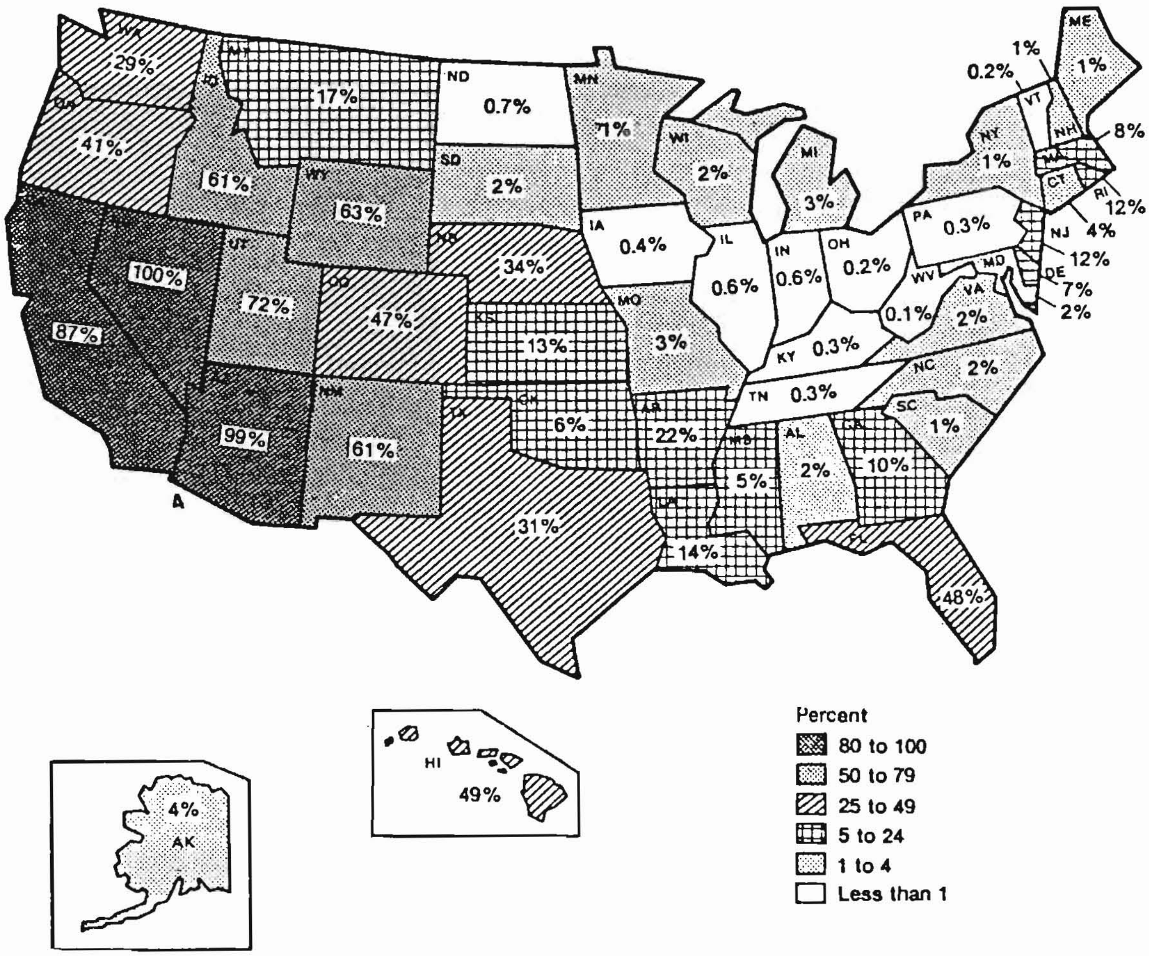


Figure 1. Proportion of irrigated cropland in the United States. (Source: California-Arizona Farm Press 10/6/84)

RESULTS AND CONCLUSIONS

Monoecious and dioecious propagule production

Outdoor culture. The number of subterranean turions (tubers) produced by the different sources varied considerably, but the averages show that the moneocious plants produced over seven times more propagules than did the dioecious plants (Table 1). The prolific production in the monoecious plants was evidenced by the over 2,000 tubers found in the Violets Lock culture. However, the size (weight) of tubers from the monoecious plants was only about one third that of the dioecious plants. In spite of the wide variability in numbers of tubers formed by either of the biotypes, the within-biotype weight per tuber was quite uniform (note standard errors in Table 1). This suggests that both types of hydrilla control their respective propagule size closely and thus allocate a "set" amount of storage capacity to each propagule, at least under the growing conditions in this experiment.

The other major difference in the propagule production between the two biotypes was the near absence of axillary turion production in the dioecious plants. The four axillary turions found in the dioecious cultures were about three times the weight of

Table 1
Tuber and Turion Production from Dioecious and Monoecious Hydrilla Outdoor Cultures*

	Tubers			*Turions*		
Source	*No.*	*Weight G*	*MG/ Tuber*	*No.*	*Weight G*	*MG/ Turion*
			Monoecious			
Violets Lock	2076	281.1	135.4	77	3.89	50.5
Lily Ponds	573	100.8	175.9	272	9.31	34.2
Dyke Marsh	1064	253.6	238.3	936	35.40	37.8
Aquatic Gardens	186	228.5	192.7	487	23.29	47.8
MEANS	974.5±817	215.7±79	185.6±43	443±3.69	17.9±14	42.6±7.8
			Dioecious			
Imperial Valley	267	149.7	560.7	3	0.41	136.7
Texas (Lake Conroe)	65	3.43	527.6	1	0.1	100.0
Florida (Fort Lauderdale)	52	28.9	555.8	0	0.0	
MEANS	128	60.7	548±18	1.3	0.3	118.3±25.9

* All cultures were started on 3 April 1984 from single apical cuttings in plastic trays containing soil (UC Mix) and maintained in outdoor cement vaults. All cultures were harvested between 15 February and 2 March 1985.

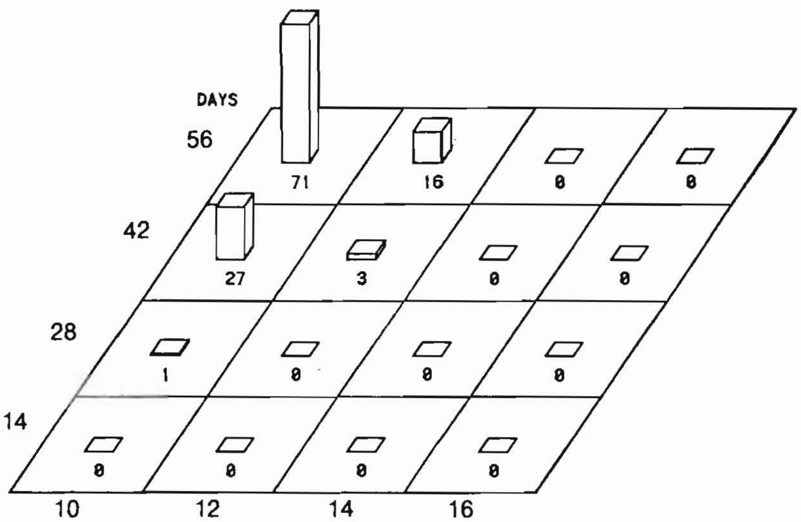
the monoecious axillary turions — approximately the same relationship seen with the subterranean turions (tubers).

Though these data are preliminary, the overall differences are similar to what has been reported in studies at the Agriculture Research Service Fort Lauderdale laboratory, and show that the monoecious plants are probably far more capable than the dioecious plants in expanding their population densities and in occupying new substrate from one year to the next. The smaller size of the monoecious turions (both subterranean and axillary) may indicate that they do not have the multiyear longevity noted in the dioecious turions. Studies are under way to determine this longevity.

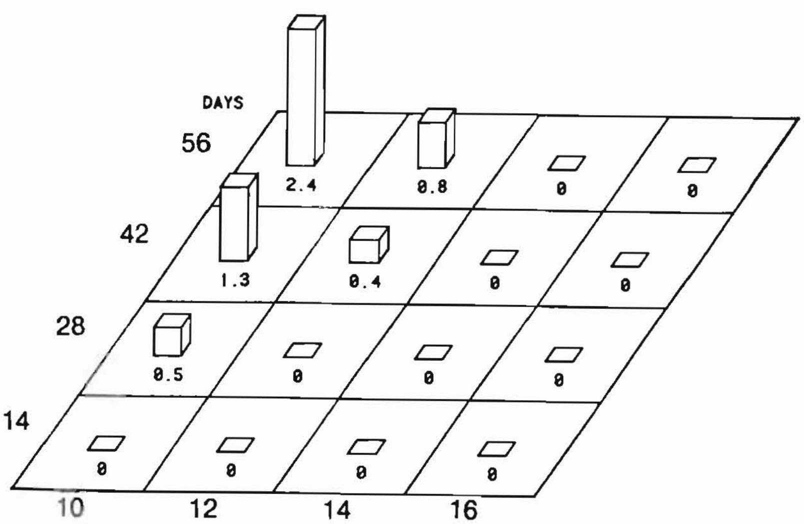
Photoperiod influence. Results showed that monoecious hydrilla produced tubers under 10- and 12-hr photoperiods after 28 to 42 days (Figure 2). (Figure 3 shows results for dioecious hydrilla.) These initial results suggest that the monoecious plants are well suited to northern latitudes where changes in day length are most rapid. The plant's ability to form tubers as quickly as American pondweed (*Potamogeton nodosus*) and Sago pondweed (*P. pectinatus*), both of which are already problematic weeds in the west, indicates that it could successfully invade northwestern waters (Anderson and Spencer 1985).

ACKNOWLEDGMENTS

Drs. Richard Yeo and David Spencer assisted in obtaining portions of the results presented here. The technical assistance of Ms. Doreen Gee, Mr. Randy Sedlacek, Mr. John Shaff, Ms. Barbara Bailey, Mr. Jon Thurston, and Ms. Cathy Cowan is also appreciated.



a. Number of tubers per clone



b. Fresh weight of tubers

Figure 2. Tuber production in monoecious hydrilla grown under four photoperiods. Plants were grown initially for 2 weeks under 14-hr photoperiod

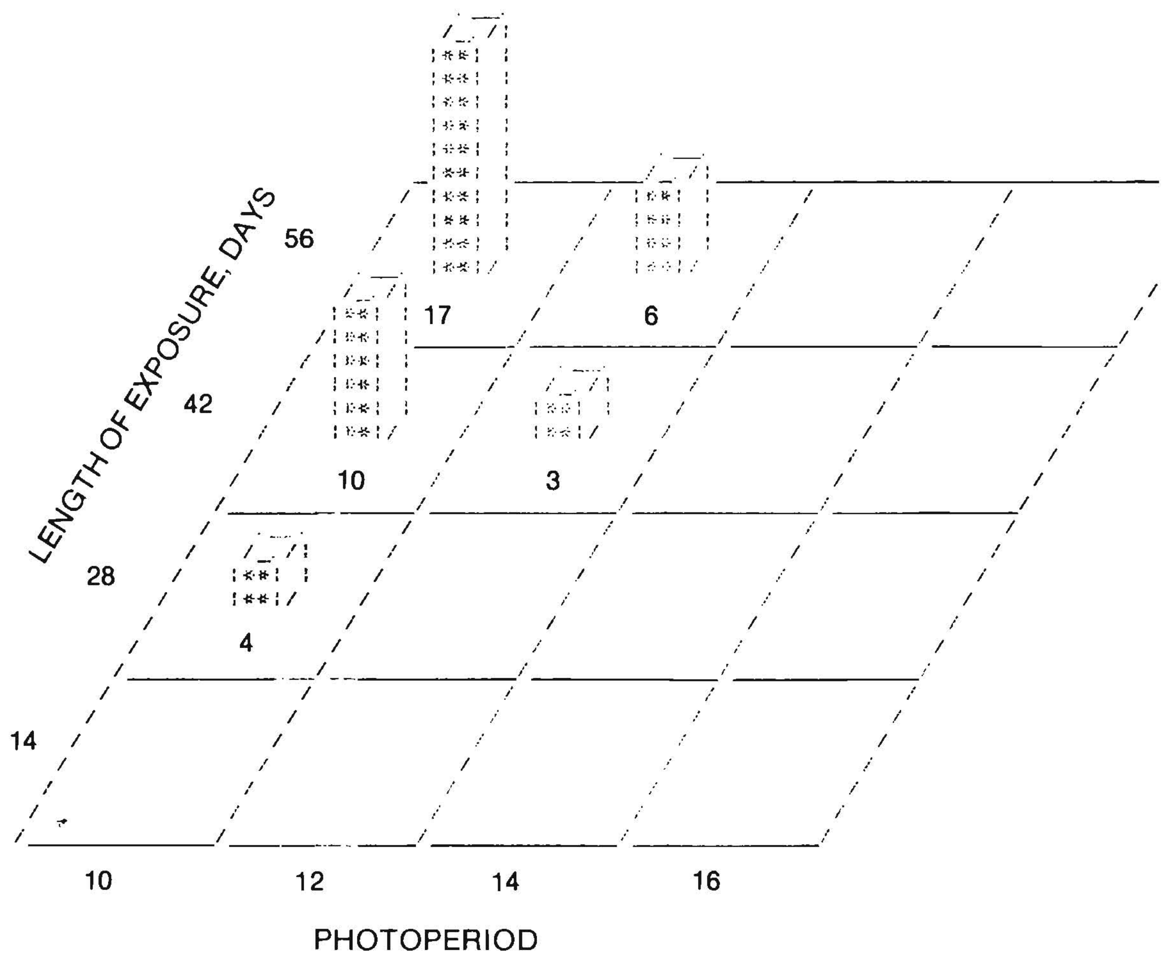


Figure 3. Photoperiod versus vegetative propagule production (number of tubers) for dioecious hydrilla, July-September 1984

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THE POTOMAC RIVER HYDRILLA PROJECT

Potomac River Project FY 84 Demonstration Trials

by
William N. Rushing* and Bruce M. Sabol*

In order to respond to instructions for immediate action on the Potomac River in the summer and fall of 1984, a series of demonstration trials were planned based on aquatic plant control technology currently available. Plans were formulated to perform individual trials with an Environmental Protection Agency approved aquatic herbicide, mechanical harvesting, a diver-assisted dredge, and bottom-covering materials. Only the mechanical harvesting and bottom-covering material demonstrations were performed during the 1984 time period. Lack of approvals to proceed from the various State and Federal agencies prevented the other demonstration trials.

MECHANICAL HARVESTING

Mechanical harvesting operations were conducted from 5 to 14 October 1984 at the five sites shown in Figure 1 and listed below:

- Old Town Yacht Basin—In Alexandria, Virginia, just north of the Woodrow Wilson Memorial Bridge (WWMB).
- Belle Haven Marina—On the Virginia side of the river just south of WWMB.
- Treasure Cove—On the Maryland side of the river 2 miles south of WWMB and just south of the entrance to Broad Creek.
- Broad Creek (two sites)—On the Maryland side of the river 3 miles south of WWMB.

A Mud Cat H6-300 harvester, a shore conveyor, and a dump truck were obtained under contract with Aqua Services of Franklin, N. J., who also provided the equipment operators. Characteristics of the harvesting system are given in Table 1.

Data-collection efforts associated with the harvesting trials included general productivity rates, harvester performance, site characteristics, hydrilla tuber densities, and fragmentation resulting from the harvesting operation. The Waterways Experiment Station and Baltimore District personnel cooperated in collecting the first three items, and North Virginia Community College contractor personnel collected information on tuber densities and fragmentation.

Table 2 contains a summary of the harvesting operations, site conditions, and types of data obtained at each site. At all sites, operational records were kept including total hours worked, area harvested, and mass of material harvested. Harvesting productivity rates were computed from this information. Performance parameters for the Mud Cat harvester were measured at the Broad Creek sites and

* US Army Engineer Waterways Experiment Station, Vicksburg, Mississippi

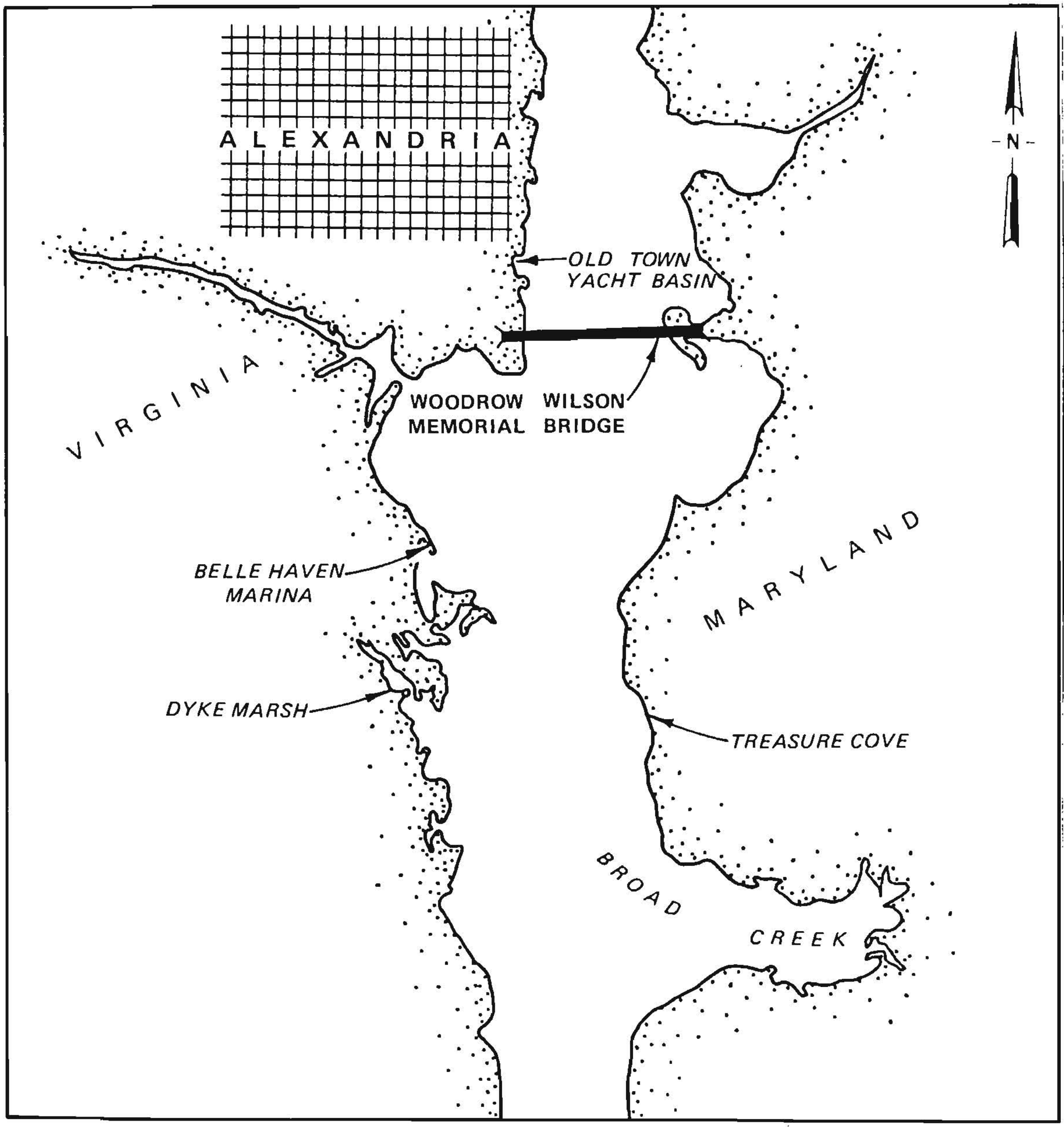

Figure 1. Locations of mechanical harvesting operations on the Potomac River

Table 1
Equipment Used in Harvesting System

Equipment	*Description*
Mud Cat H6-300 harvester	6-ft-wide cutter 300-cu-ft storage capacity
Shore conveyor	12-in width Not designed specifically for use with the harvester or with aquatic plants
Dump truck	1 ton (4 cu yd)

Table 2
Summary of Mechanical Harvesting Operations - Potomac River, Fall 1984

		Site Conditions		*Harvesting Accomplished*			*Time, hr*				*Overall Productivity per hour*		
Location	*Date October 1985*	*Density tons/acre*	*Species**	*Area acres*	*Loads***	*Mass tons*	*Total*	*Harvesting*	*Transport*	*Other*	*Tons*	*Acres*	*Types of Data Obtained†*
Old Town Yacht Basin	11-12	2.4	H	1.4	4.0	3.4	4.9	2.1	0.5	2.3	0.7	0.29	G, T, P, S
Belle Haven Marina	10	3.2	H	0.7	3.8	2.2	4.2				0.5	0.16	G, T
Treasure Cove	5-7††	8.4	S, N, M, H	3.9	34.0	33.0	18.0				1.8	0.22	G, S
	14	8.4	S, N, M, H	0.5	0.4	3.8	5.0	1.2	3.0	0.8	0.8	0.10	G, S
Broad Creek (north)	13	3.2	M, H	1.4	4.3	4.5	2.9	1.5	1.1	0.3	1.6	0.48	G, P, S
Broad Creek (south)	13-14	14.2	H, F	0.5	6.5	7.1	5.0	2.1	2.3	0.6	1.4	0.10	G, T, P, S
TOTALS				8.4	56.6	54.0	40.0						

* Species listed by decreasing order of predominance. Entries defined as follows: H-hydrilla; M-milfoil; S-stargrass; N-naiad; F-filamentous algae.

** One harvester load - one truckload.

† Data types: G-general productivity rates; T-tuber density; P-detailed harvester performance; S-site characteristics.

†† Operations financed by Friends of the Waterfront.

the Old Town Yacht Basin for use in the HARVEST mechanical simulation model. Measurements at Old Town Yacht Basin reflect performance under tight maneuvering conditions, which heretofore have not been measured.

Physical characteristics of the harvested plant material, including harvestable density (tons per acre) and stacked density (pounds per cubic foot), were measured. Additionally, measurements were taken on the dewatering characteristics of freshly harvested plant material.

The results of the mechanical harvesting trials showed a general productivity rate of approximately 1+ tons and 0.2 acre per hour. It should be emphasized that this was a demonstration trial and that the harvesting sites were, for the most part, in areas where maneuvering a floating machine was most difficult.

The trials showed that mechanical harvesting is a feasible alternative for submersed aquatic vegetation (SAV) control in the Potomac River. Most of the areas where SAV might become more of a problem in the future are generally accessible to harvesting equipment.

Results of the tuber density counts will be compared to densities determined during the 1985 growing season to assess the effects of harvesting, if any, on tuber production and regrowth. Results of the fragmentation studies are reported elsewhere in this document.

BOTTOM-COVERING MATERIALS

Several types of screen and polyethylene material are on the market for small-scale aquatic plant control. Although rather costly when used on a large scale, it was considered worth the effort to test some of these materials in the Potomac situation. Two materials were used in the demonstration tests: Aquascreen, a small-mesh screen, and Dartek, a black polyethylene material.

The site is on the north shore of Swan Creek, about 200 ft from the entrance of the creek into the Potomac River. Swan Creek flows into the Potomac from the Maryland side of the river approximately 5 miles south of the WWMB.

Local citizens cooperated with WES and the Baltimore District personnel in placement of the following materials:

Aquascreen	1 strip	14 by 50 ft
	2 strips	8 by 50 ft
Dartek	1 strip	16 by 50 ft

All strips were placed directly on top of the *hydrilla* with long axes perpendicular to the shoreline and with 8-ft buffer zones between the strips. The materials were anchored with rocks and other debris. Edges of the strips were marked buoys and were surveyed to aid in future location. In addition to placing the materials, contractor personnel collected information on tuber densities in the area for future comparison and assessment of the effects of the bottom-covering materials.

The effects of these materials on germination and regrowth of *hydrilla* will be assessed beginning in the 1985 growing season, and observations will be continued as long as valuable information can be obtained.