



US Army Corps  
of Engineers



## AQUATIC PLANT CONTROL RESEARCH PROGRAM

MISCELLANEOUS PAPER A-88-5

### PROCEEDINGS, 22ND ANNUAL MEETING, AQUATIC PLANT CONTROL RESEARCH PROGRAM

16-19 NOVEMBER 1987  
PORTLAND, OREGON

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June 1988  
Final Report

Approved For Public Release; Distribution Unlimited

Prepared for DEPARTMENT OF THE ARMY  
US Army Corps of Engineers  
Washington, DC 20314-1000

Published by Environmental Laboratory  
US Army Engineer Waterways Experiment Station  
PO Box 631, Vicksburg, Mississippi 39180-0631

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SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE				Form Approved OMB No 0704-0188 Exp. Date: Jun 30, 1986	
1a. REPORT SECURITY CLASSIFICATION <b>Unclassified</b>		1b. RESTRICTIVE MARKINGS			
2a. SECURITY CLASSIFICATION AUTHORITY		3. DISTRIBUTION / AVAILABILITY OF REPORT			
2b. DECLASSIFICATION / DOWNGRADING SCHEDULE		Approved for public release; distribution unlimited			
4. PERFORMING ORGANIZATION REPORT NUMBER(S) Miscellaneous Paper A-88-5		5. MONITORING ORGANIZATION REPORT NUMBER(S)			
6a. NAME OF PERFORMING ORGANIZATION USAEWES Environmental Laboratory		6b. OFFICE SYMBOL (if applicable) WESEP-A	7a. NAME OF MONITORING ORGANIZATION		
6c. ADDRESS (City, State, and ZIP Code) PO Box 631 Vicksburg, MS 39180-0631		7b. ADDRESS (City, State, and ZIP Code)			
8a. NAME OF FUNDING / SPONSORING ORGANIZATION US Army Corps of Engineers		8b. OFFICE SYMBOL (if applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER		
8c. ADDRESS (City, State, and ZIP Code) Washington, DC 20314-1000		10. SOURCE OF FUNDING NUMBERS			
		PROGRAM ELEMENT NO.	PROJECT NO.	TASK NO.	WORK UNIT ACCESSION NO.
11. TITLE (Include Security Classification) Proceedings, 22nd Annual Meeting, Aquatic Plant Control Research Program, 16-19 November 1987, Portland, Oregon					
12. PERSONAL AUTHOR(S)					
13a. TYPE OF REPORT Final report		13b. TIME COVERED FROM _____ TO _____		14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT 345
16. SUPPLEMENTARY NOTATION Available from National Technical Information Service, 5285 Port Royal Road, Springfield, VA 22161.					
17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)		
FIELD	GROUP	SUB-GROUP			
			Aquatic plant control — Congresses Research planning — Congresses		
19. ABSTRACT (Continue on reverse if necessary and identify by block number)  The 22nd Annual Meeting of the US Army Corps of Engineers Aquatic Plant Control Research Program was held in Portland, Oregon, on 16-19 November 1987, 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.					
20. DISTRIBUTION / AVAILABILITY OF ABSTRACT <input checked="" type="checkbox"/> UNCLASSIFIED/UNLIMITED <input type="checkbox"/> SAME AS RPT. <input type="checkbox"/> DTIC USERS			21. ABSTRACT SECURITY CLASSIFICATION <b>Unclassified</b>		
22a. NAME OF RESPONSIBLE INDIVIDUAL			22b. TELEPHONE (Include Area Code)	22c. OFFICE SYMBOL	

**SECURITY CLASSIFICATION OF THIS PAGE**



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## **PREFACE**

The 22nd Annual Meeting of the US Army Corps of Engineers Aquatic Plant Control Program was held in Portland, Oregon, on 16-19 November 1987. 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.

Ms. Billie F. Skinner, Program Manager's Officer, EL, was responsible for coordinating the necessary activities leading to publication. The report was edited by Contract Editor Phyllis Davis, for the Information Products Division, ITL, WES.

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

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# AGENDA

## 22nd Annual Meeting US Army Corps of Engineers AQUATIC PLANT CONTROL RESEARCH PROGRAM

Portland, Oregon  
16-19 November 1987

### MONDAY, 16 NOVEMBER 1987

- 10:00 a.m. Registration—Main Lobby  
-5:00 p.m.  
6:30 p.m. Reception—Umatilla Room

### TUESDAY, 17 NOVEMBER 1987 General Session, Rogue/McKenzie Rooms

- 8:00 a.m. Registration Continued—Entrance to Rogue Room  
8:30 a.m. Call to Order and Announcements  
—W.N. Rushing, Waterways Experiment Station (WES),  
Vicksburg, Mississippi  
8:35 a.m. Welcome to Portland District  
—COL Gary R. Lord, Commander, USAE Division,  
Portland, Oregon  
8:45 a.m. Comments by the Technical Monitor,  
—E. Carl Brown, Operations and Readiness Division, Natural  
Resources Management Branch, Office, Chief of  
Engineers (OCE), Washington, DC  
9:00 a.m. Integrated Control Technology Development,  
—J. Lewis Decell, WES, Manager, Aquatic Plant Control  
Research Program (APCRP), WES

#### *Special Session - Potential New Research Topics* —J. Lewis Decell, WES, Presiding

- 9:20 a.m. Geographic Information System (GIS) Applications for  
Aquatic Plant Management  
—M.R. Waring, WES  
9:40 a.m. A Computer Enhanced Aerial Video Mapping Technique for  
Aquatic Plant Surveys  
—T.M. McNabb, Aquatics Unlimited, N.W. Kent, Washington

NOTE: Computer model demonstrations-  
Wallowa Room  
9:00 a.m.-4:00 p.m. 17 Nov  
9:00 a.m.-Noon 19 Nov

- 10:00 a.m. BREAK

- 10:20 a.m. Evaluating the Benefits and Costs of Alternative Aquatic Plant Control Measures  
—J. Henderson, WES
- 10:40 a.m. New Concepts in Benthic Screens for Aquatic Plant Control  
—J. Plott, Dow Corning, Midland, Michigan
- 11:00 a.m. Influence of Rhizosphere Microflora on Nutrition and Growth of Rooted Aquatic Macrophytes  
—D. Gunnison, WES
- 11:20 a.m. The Potential Role of Endogenous Defenses in Plant-Herbivore Interactions  
—W.C. Kerfoot, University of Michigan, Ann Arbor
- 11:40 a.m. LUNCH

*USAE Division/District Presentations*

- 1:00 p.m. Aquatic Plant Control Operations Support Center (APCOSC) Update and Introduction of New Operations and Presentation Formats  
—W. Zattau, USAE District, Jacksonville, Florida
- 1:15 p.m. Use of Herbicides in Potable Water Reservoirs  
—L. Mason, USAE District, Tulsa, Oklahoma
- 1:30 p.m. Aquatic Plant Control Operations as They Relate to Endangered Species  
—M. Dupes, USAE District, Jacksonville, Florida
- 1:45 p.m. Aquatic Plant Management Advances in British Columbia  
—P.R. Newroth, Ministry of Environment and Parks, Victoria, British Columbia
- 2:00 p.m. Highlights of the Potomac River and Chesapeake Bay Programs  
—G. Earhart, USAE District, Baltimore, Maryland
- 2:15 p.m. BREAK
- 2:30 p.m. USAE Division/District Working Session—Rogue Room
- 3:30 p.m. Federal Aquatic Plant Management Working Group—Umpqua Room

**WEDNESDAY, 18 NOVEMBER 1987**  
**General Session, Rogue/McKenzie Rooms**  
*Ecology of Problem Submersed*  
*Aquatic Plant Species*  
 —J. Barko, WES, Presiding

- 8:00 a.m. Effects of Autogenic Changes in Sediment Chemistry on Aquatic Macrophyte Growth  
—J. Barko, WES
- 8:15 a.m. Habitat Studies: Effects of Aquatic Macrophytes on Water Quality  
—V. Carter, US Geological Survey, Reston, Virginia
- 8:30 a.m. Habitat Studies: Effects of Aquatic Macrophytes on Invertebrates  
—A. Miller, WES
- 8:45 a.m. Habitat Studies: Effects of Aquatic Macrophytes on Fish  
—K.J. Killgore, WES

- 9:00 a.m. Effects of Midwinter Drawdown on Aquatic Macrophytes in  
Eau Galle Reservoir, Wisconsin  
—G.L. Godshalk, WES
- 9:15 a.m. Patterns of Sediment Deposition on the Littoral Region in  
Eau Galle Reservoir  
—W.F. James, WES
- 9:30 a.m. BREAK
- 9:45 a.m. Distribution and Ecology of Aquatic Macrophytes in Devils Lake,  
Wisconsin  
—R.A. Lillie, Wisconsin Department of Natural Resources, Madison
- 10:00 a.m. Carbon Limitation of Submersed Macrophyte Growth as Affected by  
Other Environmental Factors  
—R. Smart, WES
- 10:15 a.m. Overview: Coordination of Control Tactics with Phenological  
Events of Problem Aquatic Macrophyte Species  
—H.C. Westerdahl, WES
- 10:30 a.m. Phenology and Carbohydrate Allocation of Waterhyacinths  
—K. Luu, WES
- 10:45 a.m. Carbohydrate Allocation in Monoecious and Dioecious Hydrilla  
—G. Pesacreta, WES

*Computer-Aided Simulation Procedure for APC*  
—R.M. Stewart, WES, Presiding

- 11:00 a.m. Introduction and Overview  
—R.M. Stewart, WES
- 11:15 a.m. Field Validation and Improvements to INSECT, a Computer Model of  
Waterhyacinths and Associated Biocontrol Agents  
—F.G. Howell, University of Southern Mississippi, Hattiesburg
- 11:30 a.m. Hydrilla and Triploid White Amur Growth Models  
—K.S. Akbay, Marquette University, Milwaukee, Wisconsin
- 11:45 a.m. Development of a Coupled Model-Herbicide Fate and Effects on  
Target Species  
—J.H. Rodgers, North Texas State University, Denton, Texas
- 12:00 noon LUNCH
- 1:30 p.m. Field Trip to Mt. Hood and Timberline Lodge-  
Meet in main lobby

**THURSDAY, 19 NOVEMBER 1987**  
**General Session, Yakima/Umatilla**

*Chemical Control Technology Development*  
—H.E. Westerdahl, WES, Presiding

- 8:00 a.m. Introduction and Overview  
—H.E. Westerdahl, WES

- 8:15 a.m. Development of Herbicide Application Techniques for  
Flowing Water  
—K.G. Getsinger, WES
- 8:30 a.m. Influence of Tides on Herbicide Dissipation  
—A. Fox, Center for Aquatic Plants, University of  
Florida, Gainesville
- 8:45 a.m. Herbicide Adjuvant Evaluation  
—K.G. Getsinger, WES
- 9:00 a.m. Herbicide Concentration/Exposure Time Studies on  
Eurasian Watermilfoil  
—R. Green, WES
- 9:15 a.m. Feasibility of Using Plant Growth Regulators for  
Managing Aquatic Plants  
—C.A. Limbi, Purdue University, West Lafayette, Indiana
- 9:30 a.m. Effects of Selected Plant Growth Regulators on Aquatic Macrophyte  
Stem Elongation and Reproductive Propagules  
—S.J. Klaine, Memphis State University, Memphis, Tennessee
- 9:45 a.m. Cooperative Herbicide Field Evaluations Under Experimental  
Use Permits  
—H.E. Westerdahl, WES
- 10:00 a.m. A New Herbicide for Aquatic Plant Management:  
Benesulfuron Methyl  
—L. Anderson, USDA, Davis, California
- 10:15 a.m. BREAK

*Biological Control of Aquatic Plants*  
—E.A. Theriot, WES, Presiding

- 10:30 a.m. Genetic Engineering Technology Development: Mechanisms of  
Specificity  
—E.A. Theriot, WES
- 10:45 a.m. Genetic Engineering Technology Development: Host Specificity of  
Microflora  
—C. Smith, University of Wisconsin, Madison
- 11:00 a.m. Genetic Engineering Technology Development: Release of  
Engineered Organisms into the Environment  
—S. Lindow, University of California, Berkeley
- 11:15 a.m. Microbial Control of Eurasian Watermilfoil: Formulation  
Development  
—H. Gunner, University of Massachusetts, Amherst
- 11:30 a.m. Microbial Control of Hydrilla: Laboratory Assessment  
—G. Joye, WES
- 11:45 a.m. Update on Triploid Grass Carp Research  
—G. Pauley, University of Washington, Seattle
- 12:00 noon LUNCH

- 1:00 p.m. Impact of Herbicides on Waterhyacinth Biocontrol Agents  
—A.F. Cofrancesco, WES
- 1:15 p.m. Field Study for Validation of the Model, INSECT  
—M.J. Grodowitz, WES and USDA, Fort Lauderdale, Florida
- 1:30 p.m. Quarantine Research on Bagous and Hydrillia—Insects for  
Control of Hydrilla  
—G. Buckingham, USDA, Gainesville, Florida
- 1:45 p.m. Insect Biocontrol Agents for Hydrilla  
—T.D. Center, USDA, Fort Lauderdale, Florida
- 2:00 p.m. Insect Biocontrol Agents for Waterlettuce  
—D. Habeck, University of Florida, Gainesville
- 2:15 p.m. Initial Considerations of the Role of Allelopathy in Aquatic Plant  
Management: Report on Workshop held Sep 87  
—A.F. Cofrancesco, WES
- 2:30 p.m. Report of Tuesdays Division/District Working  
Session and Wrap-up
- 2:45 p.m. Adjourn 22nd Annual Meeting
- 3:15 p.m. 1989 Civil Works R & D Review, Yakima/Umatilla  
Rooms, Directorate of R & D, OCE (Corps of  
Engineers Representatives Only)

# ATTENDEES

## 22nd Annual Meeting US Army Corps of Engineers AQUATIC PLANT CONTROL RESEARCH PROGRAM

Portland, Oregon  
16-19 November 1987

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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	4,046.873	square metres
acre-feet	1,233.489	cubic metres
Fahrenheit degrees	5/9	Celsius degrees or Kelvins*
feet	0.3048	metres
gallons per acre	0.00093	cubic decimetres per square metre
gallons (US liquid)	3.785412	cubic decimetres
inches	25.4	millimetres
miles (US statute)	1.609347	kilometres
ounces (mass)	28.34952	grams
pints (US liquid)	0.4731765	cubic decimetres
pounds (force) per square inch	6,894.757	pascals
pounds (mass)	0.000112	kilograms
pounds (mass) per gallon	0.12	kilograms per cubic decimetre
square feet	0.09290304	square metres
square miles	2.589998	square kilometres
tons (mass) per acre	0.22	kilograms per square metre
tons (2,000-pounds, mass)	907.1847	kilograms

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\*To obtain Celsius (C) temperature readings from Fahrenheit (F) readings, use the following formula:  $C = (5/9)(F - 32)$ . To obtain Kelvin (K) readings, use:  $K = (5/9)(F - 32) + 273.15$ .

**22nd Annual Meeting  
US Army Corps of Engineers**

**AQUATIC PLANT CONTROL  
RESEARCH PROGRAM**

**INTRODUCTION**

The Corps of Engineers (CE) Aquatic Plant Control Research Program (APCRP) requires 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 distributed widely in order to transfer 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 nationwide.

The contents of this report include the presentations of the 22nd Annual Meeting held in Portland, Oregon, 16-19 November 1987.

**SPECIAL SESSION**  
**POTENTIAL NEW RESEARCH TOPICS**

# Geographic Information System (GIS) Applications for Aquatic Plant Management

by  
Michael R. Waring\*

## INTRODUCTION

Managers and planners for many of the nation's water resources projects are faced with a myriad of problems associated with objectionable aquatic plant species. These species include hydrilla (*Hydrilla verticillata*), Eurasian watermilfoil (*Myriophyllum spicatum*) waterlettuce (*Pistia stratiotes*), and waterhyacinth (*Eichhornia sp.*). Growth habits and control methods have been researched and documented extensively. However, very little work has been done using state-of-the-art technology such as Geographic Information Systems (GIS) for aquatic plant management. Managers and planners could benefit from such technology through an increased ability to accurately and effectively predict patterns of colonization. The ability to examine "what if" scenarios and to spatially portray predicted avenues of colonization would be very beneficial in applying preventive control measures prior to serious infestations. When coupled with state-of-the-art remote sensing techniques for collecting data, this ability becomes a highly effective and efficient method for determining alternatives.

## LITERATURE REVIEW

A preliminary review of the literature reveals that although specific GIS methods related to aquatic plants are generally not available, there are methods that have been applied to other aspects of resource management that could be readily adapted to aquatic plant predictions. White (1986) reported on the integration of a GIS into a Decision Support System (DSS) to provide spatial data for forest impact simulation models. The goal of the system is to provide managers with increased capabilities for making assessments of biological, social, and economic impacts for alternative pest management actions. Rowland (1986) used a GIS within the Tennessee Valley Authority to map and analyze the establishment and spread of gypsy moth infestations. The area covered a multistate region. Hertz-Brown and Williams (1981) used GIS to more effectively determine management strategies for controlling the spruce budworm problem in Maine. They concluded that the GIS contributed to benefits such as reduced spray protection costs, increased income through better salvage/utilization of the resource, better budgeting of fixed and regeneration costs, and better identification of areas where other environmental concerns were involved. Waltz and Moore (1986) combined known growing condition preferences with psychological inferences of grower behavior to develop predictive models for narcotic crop growth. Model parameters changed interactively as planting and growth patterns responded to a variety of outside pressures

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

(law enforcement activities, weather conditions, etc.). The ability of the GIS to respond to changes provided officials with up-to-date, accurate information. Aquatic vegetation and water quality were monitored on a GIS at Lake Marion by Welch et al. (1986). They used the system to quantify aquatic macrophyte growth trends and to examine correlations between the distribution of those macrophytes and water quality.

Although little work has been done using GIS to map and analyze aquatic plants, extensive literature does exist on the use of remotely sensed data to identify and evaluate aquatic plants. Such remotely sensed data provide outstanding opportunities for developing cost-effective techniques for identification and control using the unique capabilities of GIS. Martyn et al. (1986) reported on the use of color infrared photography to evaluate the effectiveness of grass carp in controlling aquatic weeds on Lake Conroe, Texas. Extensive studies have been completed by personnel at the US Army Corps of Engineers Waterways Experiment Station (Dardeau 1983, Long 1979) on the use of remote sensing and aerial survey techniques for mapping and monitoring aquatic plant populations. Such techniques, when combined with state-of-the-art imagery from SPOT, could provide managers and planners with an outstanding data base for analysis of "before and after" conditions.

## DISCUSSION

At this point, the question may be asked "What is a GIS?" In very simple terms, it is a computer-based system for storing, retrieving, analyzing, and displaying both spatial (geographically referenced) and textual (attribute) data. A typical system (Figure 1) consists of hardware for data input (digitizer), storage and analysis (computer), and display (monitor, printer), plus various types of software to accomplish such functions.

Data may be obtained from numerous sources, including satellite imagery, field inventories, existing maps, soil surveys, and various secondary sources such as state and Federal resource information systems. In many cases, these data will have to be digitized for spatial analysis and the attribute data entered into a data base management system. Once the data are available within the system, analyses of various combinations of data layers can be performed. These data can be either analyzed separately to aid in management decision making or used in conjunction with various simulation models to further aid in the decision making process (Figure 2). The ability to examine varied data layers with respect to each other allows the manager to obtain a better understanding of complex interrelationships and produce more informed decisions.

Systems are available for the full range of computers, from micros to main-frames and include many off-the-shelf, "turn-key" systems that are useable for a wide variety of applications. Currently, a number of Corps of Engineers District Offices have operational GIS's, with more planned for the future. Additionally, many state, local, and other Federal agencies operate such systems. These systems represent a wealth of expertise and data bases that may be available to others.

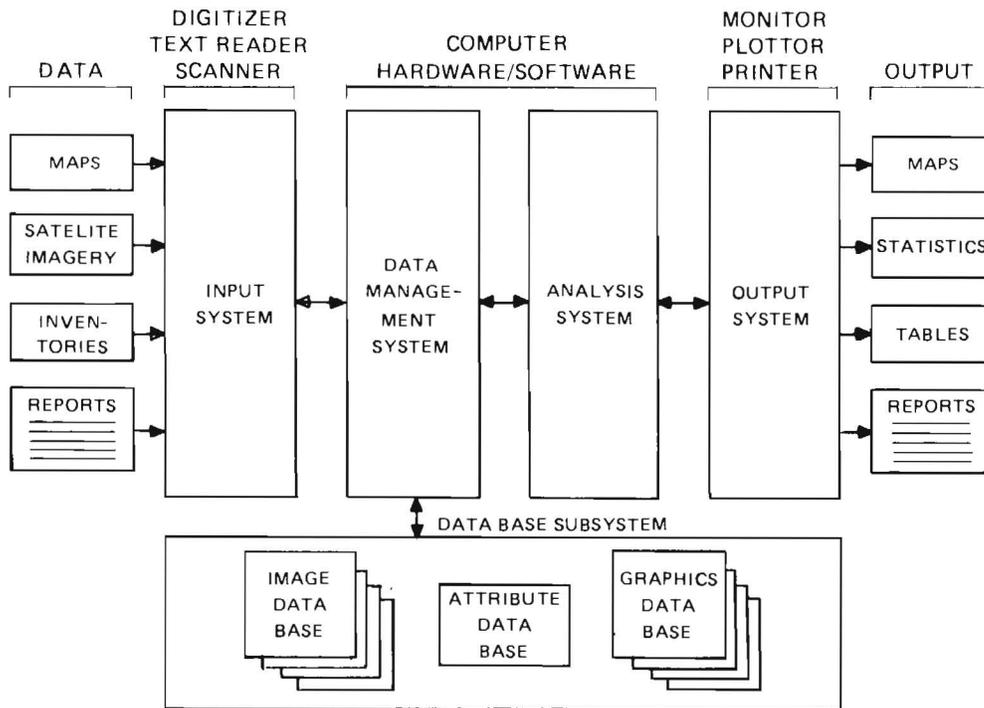


Figure 1. Geographic Information System components

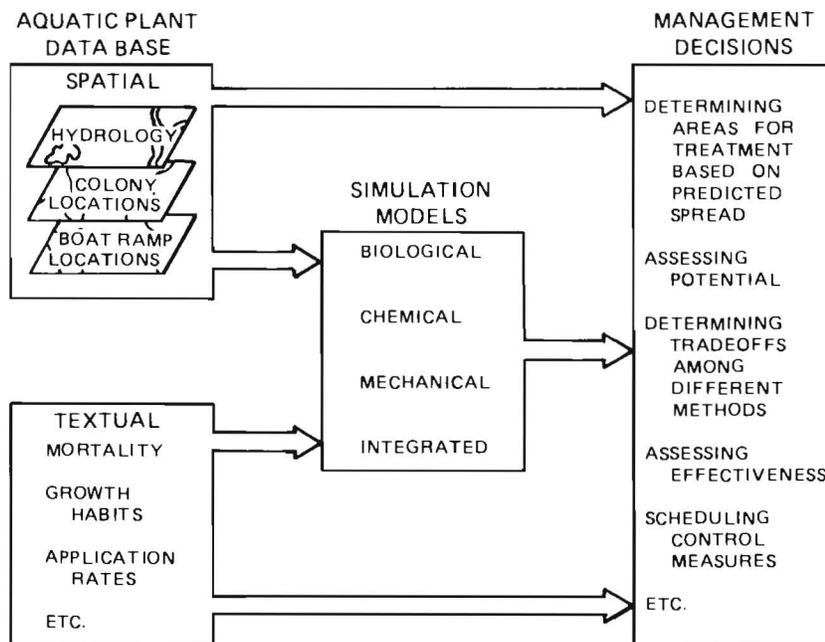


Figure 2. GIS overlay and analysis

## SUMMARY

The ability to bridge the gap between the cost-effective advantages of remotely sensed data and the rapid analysis techniques of GIS is a powerful tool that could provide managers and planners with increased abilities in controlling aquatic plant problems. Serious consideration should be given to adapting these tools and techniques in order to provide benefits such as more accurate analyses at reduced costs.

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# **A Computer Enhanced Aerial Video Mapping Technique for Aquatic Plant Surveys**

by  
T. M. McNabb\*

Aerial photography has been utilized over the years to provide aquatic plant managers and researchers with an accurate picture of environments of concern. Obtaining data from the aerial perspective is useful because many patterns are visible that are not evident from the ground perspective or from on the water.

A recent addition to the tools available for aquatic plant survey, mapping, and detection work is a modification we have made of our Pollution Imaging System (PIMS). This computer based system includes an aerial video camera system for collection of data, and an IBM based computer to process and color enhance video images. The enhancement is based on the ability of the computer to process VHS formatted video images off a color monitor. The processing consists of reading and storing a reflectance value at each point on the image that is displayed on the monitor. The program software will then compare the stored values from the image to a lookup table on floppy disks that assigns colors to different reflectance values. The processed image is generated in a matter of seconds based on these lookup values. This process will define shapes and boundaries of areas with identical reflectance values. For example, species of aquatic plants can be assigned different colors such as pondweeds equal red, milfoil equals blue, hydrilla equals green, and the processed image will color areas with each of these plants red, blue, and green. The process can take many shades of green and recolor them for easy identification.

The aerial camera system is lightweight and can be carried to remote locations on commercial means of travel and installed in rented aircraft. The camera system is comprised of the PIMS Camera, a number of filtration systems that allow the operator to select portions of the spectrum for collection, the PIMS VHS format Video Cassette Recorder (VCR), monitors for both the pilot and the camera operator, and a 24-volt converter to work off the aircraft power system. The PIMS Camera is positioned through a belly hole in the aircraft to collect data in a vertical perspective that can be correlated to a scale. Flight mission profiles are designed to put the aircraft over the targeted areas and collect the data that the staff requires to perform the analysis. Flight lines are determined for the pilot with overlaps of areas photographed outlined as required. The audio track on the VCR can be utilized by the camera operator to record any notes or comments that will be helpful with the interpretation. The VCR collection format allows the operator to review the imagery collected while still airborne and determine if areas of concern were adequately covered. This feature also allows the staff to perform any ground truthing while at the site and limits the number of trips required to obtain a complete data set.

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The imagery is now ready to return to the lab for processing. The processing of the imagery is performed on the PIMS Computer. This computer system has the capability to scan video imagery as it is displayed on the monitor and store a reflectance value for each pixel on the screen. The computer can then be instructed to reference a lookup table that assigns specific colors to specific ranges of reflectance. The video image is then false colored based on the lookup table and the reflectance value stored. Target conditions such as aquatic plant species can be assigned a specific color.

Applications for this system are many. In situations where a large area is to be surveyed for aquatic plant species, the system can be used to fly the targets and record data on video tape. The imagery can then be compared to ground transects, color enhanced, and re-compared to the ground transects. The areas on the transects that show one color are compared to the transect plants at that location, and then the remaining imagery can be evaluated.

For detection work, the lookup tables can be constructed to color problem species such as hydrilla or Eurasian watermilfoil one color, and other aquatic vegetation different colors. The plane then collects data from the area of interest, and each image is processed. Any place the problem species color occurs would be targeted for ground investigation.

Another application for this system is to delineate boundaries to wetlands areas by color coding terrestrial, marginal, and wetland species. This information would be useful to agencies that monitor impacts of development or dredging on wetland areas.

Image collection and processing is cost-effective and rapid, as the aerial platform allows for coverage of a large area fast, and the computer enhancement takes about 20 sec per image. The image collection system is very portable and can be hand carried to remote areas to limit costs of collection. Currently, work is being performed to enhance the capabilities of the equipment and provide onboard color enhancement for specific uses. Software is also being modified to provide quantified information such as the area of specific impacts. There are also a number of terrestrial plant mapping programs that would benefit from this technology such as power line right-of-way mapping and irrigation ditch bank work.

# Evaluating the Benefits and Costs of Alternative Aquatic Plant Control Measures

by  
Jim E. Henderson\*

## INTRODUCTION

Aquatic plant control programs are implemented to improve or allow water use for navigation, recreation, water supply, and other uses (Dardeau and Hogg 1983). The implementation of an aquatic plant control program is intended to change the quality of the affected water resources and the character of man's use of the resources. The value that is placed on the changed conditions may only partly be expressed by the market place. The value of the changed conditions is needed for comparison with the costs involved in implementing the controls.

Information on the economic costs and benefits of aquatic plant control can be used in planning to provide answers to the following questions:

- What is the economic value of aquatic plant control for uses of a waterway, and what are the impacts of different technologies upon the user?
- What public groups or users are affected by aquatic plant control efforts?
- What are the economic trade-offs, e.g. costs, of one control technology relative to another, and what are the economic trade-offs of various levels of control?

## THE VALUE OF AQUATIC PLANT CONTROL

Resources have economic value to the extent they (a) provide consumer satisfaction or enjoyment, i.e. provide a desirable service, and (b) are scarce. Economic benefits arise from people's willingness to pay for services received from a resource, good, or service. For water resources, these services include navigation, water supply, and other project purposes. Land and water resources provide a range of intangible services that are also valued by people. These services include recreation and such benefits as aesthetics. The question—What is the economic value of aquatic plant control?—has an answer: the economic benefits of the navigation, recreation, and other services provided by the resource. The economic value of a particular resource or resource condition can be represented as the total economic value shown graphically in Figure 1. Economic value includes the components that when combined are the total value of a resource, good, or service (Loomis and Peterson 1984).

The navigation, flood control, and other typical project services are fairly well understood by those familiar with Federal water resources projects. The option, existence, and bequest values shown in Figure 1 require explanation.

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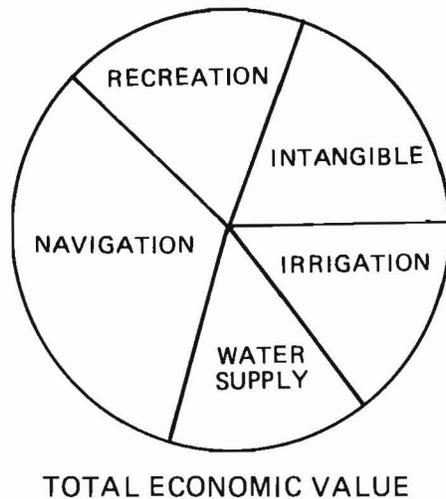


Figure 1. Total economic value

People are willing to pay to know that free-flowing water resources are available, in good condition, and will be available to future generations. Existence benefits arise when people are willing to pay for just knowing something exists. Examples are wilderness areas and endangered species. Existence benefits can be thought of as preservation or conservation value. For aquatic plant control, the existence value is the willingness to pay for a free-flowing waterway or an unobstructed lake. The willingness to pay for the existence services are evident even though there may be no expectation or intention on using the resource. In terms of aquatic plants, persons value free-flowing, more natural conditions even though there may be no intent to use a waterway (Randall 1987, McAllister 1980).

To maintain the opportunity to use a resource in the future, people are willing to pay for an option on this future use, which is known as the option value. This willingness to pay is to preserve the resource without degradation, so that it may be used for some purpose in the future. The willingness to pay strictly for the option value is distinct from the value of the future use, per se (Randall 1987).

Bequest values derive from willingness to pay to provide the resource to future generations. These values are to ensure that benefits of the resource are given to succeeding generations, as opposed to existence and option values which are for present and future consumption by those living today (Randall 1987).

Because the economic evaluation of water resource projects has traditionally focused on the services provided by authorized project purposes, the existence, option, and bequest benefits, and to a large extent aesthetics and recreation, have been ignored. The effect of not considering the total benefits resulting from aquatic plant control should be considered in economic terms of "What is not accounted for in terms of benefits and costs?" To this end, Figure 2 is instructive in showing the relationship of the Total Economic Value to willingness to pay for existence, option, and bequest values. This figure indicates that people's willingness to pay for onsite use may only be one-third of their willingness to pay. By only considering the onsite effects, i.e. recreation use, and not considering the benefits derived from existence, option, and bequest values, a considerable part of the benefits may not be accounted for. For

aquatic plant control, the point is that the value of control efforts to the nation are only partially being considered at this time.

Determining the economic value for an operation and maintenance function requires estimation of the costs and benefits for the service that the function provides. The costs and benefits for the vendable services provided, e.g. water supply, are readily calculated through market evaluation procedures. The economic values accruing from the nonvendable services shown in Figure 1 are valued through nonmarket valuation techniques which determine willingness to pay for the services. The economic costs and benefits of aquatic plant control are determined by the combined costs and benefits of tangible or vendable services and the willingness to pay for recreation and other intangible or nonmarket values.

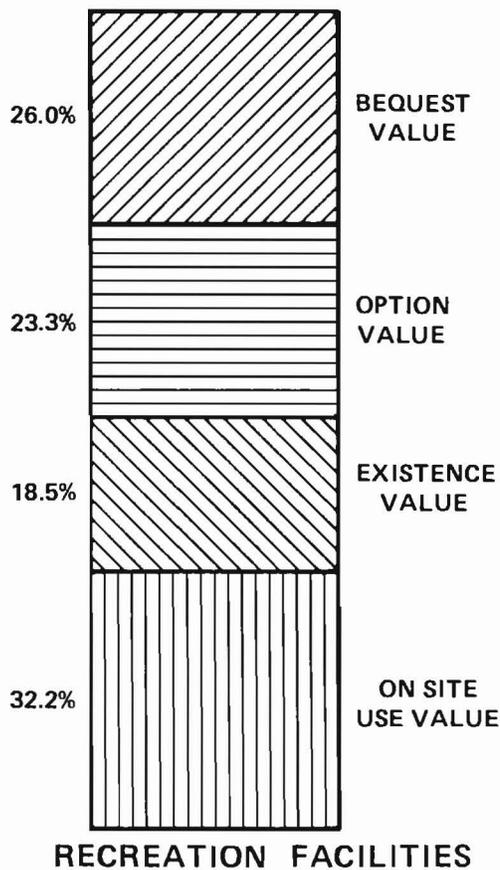


Figure 2. Relation of intangible values to onsite values

Impacts caused by an aquatic plant control technology upon land uses, e.g. access, and water resources result in changes in the magnitude of the monetary benefits associated with man's use of the resource. The costs associated with these service benefits are the costs of lost opportunities (i.e. benefits foregone) or the negative impact of an increase in something not desired, e.g. increases in problem aquatic plant conditions (McAllister 1980). The direct costs of aquatic plant control are the costs of the chemicals, biological control development, and application, and these costs are relatively easy to obtain. The costs for such things as loss of the use of a lake or effects on use due to water quality degradation, reflecting recreation and the intangible values, are more difficult to determine.

## VALUATION OF AQUATIC PLANT CONTROL SERVICES

### **Navigation, water supply, and irrigation**

The value of aquatic plant control for navigation, water supply, irrigation, and other such purposes is the contribution to national economic development resulting from navigation and water supply (Engineer Regulation 1105-2-40). Benefits from navigation, water supply, and irrigation accrue from increased value of goods and services which result from water use. The value of these services can readily be calculated because a market exists and/or a market analysis can be developed. The navigation, water supply, and other services have tangible costs components. The costs for water supply, navigation, and other purposes are the costs of equipment, time, and other resources used for aquatic plant control that cannot be put to other purposes. The benefits for navigation and similar purposes are the increase in national economic development or reduction in costs.

### **Recreation and intangible values**

Answering the value question for recreation and the intangible services is more problematic because there is a lack of market information. Markets for recreation and the intangible services are not readily available and must be derived using nonmarket valuation techniques. Nonmarket valuation techniques attempt to estimate the net economic value for resources for which market prices are inadequate or unavailable (Stoll, Loomis, and Bergstrom, in preparation). Recreation benefits are measured by willingness to pay for the recreation services (Engineer Regulation 1105-2-40). The benefits from existence, option, and bequest services are also determined by willingness to pay.

To date, the only study to identify valuation of recreation benefits related to aquatic plant control was the study of fishing conducted at Orange and Lochloosa Lakes, Florida (Milon, Yingling, and Reynolds 1986). This study used the contingent valuation method (CVM) to determine the willingness to pay of fishermen for various levels of aquatic plant control. CVM is one of three recognized methods for calculating recreation benefits of Corps activities (Vincent, Moser, and Hansen 1986). CVM, the Travel Cost Method, and Unit Day Values are used in different situations depending on the availability of certain data on recreation use and the recreation activities involved.

Less work has been done to evaluate such benefits as option, existence, and bequest services due to their intangible nature and lack of past consideration in agency decision making. However, because the public has expressed an interest in preservation or conservation through legislation for specific resources, efforts are being directed toward estimating willingness to pay for these services. Bergstrom, Dillman, and Stoll (1985) determined the values for preservation of prime farmland in South Carolina. Bowker and Stoll (in preparation) have examined such values for whooping crane populations, and Walsh, Loomis, and Gillman (1984) have examined preservation values for wilderness areas. These studies have identified the attributes of the resource that are important, quantified the attributes, and then elicited

willingness to pay values for changes to the resources. The key to this process is to identify, define, and quantify the intangible attributes so the economist can begin to determine values for them.

## **WHO IS AFFECTED BY AQUATIC PLANT CONTROL?**

Determining the economic value for an operation and maintenance function requires estimation of the costs and benefits for the services and identifying who pays and who receives the benefits of the services. A review of work done under the Aquatic Plant Control Research Program indicates that little work has been done to determine the full range of impacts that control efforts have on recreation and other uses of the affected waterway. Because the focus has been on the plants in the water, little emphasis has been placed on the impact the effort has on land resources. For example, what happens when access to a boat ramp is impeded because of problem levels of aquatic plants?

The “who pays” question has received increased attention under the present administration, resulting in changes to cost-sharing arrangements. Cost-sharing partners are increasingly interested in identifying exactly which groups benefit from a project. Given the benefits and costs of aquatic plant control as explained above, we should identify the appropriate level at which to analyze these costs and benefits. Navigation and water supply contribute to National Economic Development, increasing the value of the nation’s goods and services. The willingness to pay for recreation and for the intangible values varies among regions, states, and projects. The appropriate level for evaluating the recreation and intangible benefits for aquatic plant control, however, is at the national level also, because providing outdoor recreation is a national priority and benefits accrue to the nation as a whole.

The point for aquatic plant control is that to determine economic values for control efforts, some thought should first be given to exactly what impacts, or changes in resource or use conditions, occur because of aquatic plant control, and what groups are affected by those changes.

## **PLANNING FOR IMPLEMENTATION**

Implementation of an aquatic plant control program requires planning, organization, and monitoring of the program to prevent, maintain, or control aquatic plants at nonproblem levels. In identifying the range of impacts and resource uses, it is important to identify what are considered problem and nonproblem levels of aquatic plants. The quantification of economic costs and benefits can be used to prioritize treatment location and specify levels of treatment, i.e. prevention, maintenance, or control. The economic information can be used to augment the evaluation of the aquatic plant problems and the alternatives for control.

In planning an aquatic plant control program, the identification of the appropriate technology is addressed. The answer to “What are the economic trade-offs of one control

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

technology relative to another?" requires more information than just the direct costs of dollars per acre\* of control. The public perceptions of control technologies and the willingness to pay for a preferred technology can affect public and/or political support for an aquatic plant program. The value of one technology over another and willingness to pay is determined by the public user of a particular project and the benefits received. The appropriate level of analysis is regional or national.

Collection of economic information can be readily integrated into the management procedure set out by Killgore (1984). In Task IV: Inventory Water Uses, the questionnaires or surveys used in Step 1: Identify and Characterize Water Use can be used to incorporate questions about: (a) amount of recreation or other use; (b) willingness to pay for and the desirability of various levels of control; and (c) perceptions and willingness to pay for different control techniques, e.g., biological versus chemical. These questionnaires can address the intangible values as well. Obtaining this information would require a more extensive survey design than is necessary to determine water use alone.

The information on willingness to pay and preferences can assist in prioritizing the treatment locations since "treatment locations must be prioritized according to benefits derived from keeping plant populations at nonproblem levels" (Task VI, Killgore 1984). The willingness to pay and preference information can also provide better data on high and low use areas, affecting the cost effectiveness of alternative treatment techniques. Responses to questionnaires used to elicit the willingness to pay data can reveal public preferences for different control techniques.

## SUMMARY

This paper has identified some of the information important for economic evaluation of aquatic plant control which is summarized as follows:

- The value of aquatic plant control can be determined through existing market and nonmarket valuation techniques. The integration of these techniques in implementation plans requires determination of willingness to pay for aquatic plant control by the public.
- Decisions on aquatic plant control programs can be improved by use of economic information to better evaluate benefits and costs of different levels of control and different control technologies.
- The challenge is to quantify the intangible attributes of water systems, aquatic plant populations and communities, and control or management application so that the economist can begin to develop techniques to assign values to them.

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# NEW CONCEPTS IN BENTHIC SCREENS FOR AQUATIC PLANT CONTROL

by

John E. Plott\* and Dr. G. Douglas Pullman\*\*

## ABSTRACT

The professional lake manager and concerned riparian property owner has a growing number of nuisance aquatic vegetation management options to choose from. Benthic barriers provide some distinct advantages over other methods of aquatic weed control when used appropriately. Ideal benthic barrier application sites are around docks and piers, swimming beaches, marinas, and potable water intakes. They are also valuable for use in irrigation ponds and fishing lanes. Benthic barrier economic value is based upon the longevity of the device, required maintenance, and the size of the effective treatment area or area of principal management concern, relative to other lake vegetation strategies.

Research conducted by Dow Corning Corporation and the Dow Gardens indicates that silicone benthic barriers are likely to exhibit a low buoyant potential in lakes with high organic bottom sediment concentrations and high algal colonization rates. Buoyant potential is described as the tendency of a barrier to "float" and is defined as a function of the device's specific gravity, gas diffusion, ion permeability, and surface filamentous algae colonization potential.<sup>+</sup> Silicone barriers were tested against unslitted Dartek™, Texel™ TAC 150, and Aquascreen™ at the Dow Gardens Aquatic Ecosystems Management Research Laboratory, Midland, Michigan (1987). A field test site was installed in Michigan in the fall of 1987; field monitoring of silicone benthic barrier performance is expected to continue through 1988.

## INTRODUCTION

Since 1981, Dow Corning Corporation (located in Midland, Michigan) has taken a strong interest in the development of silicone fabrics for use as benthic barriers for nuisance aquatic weed control. As recently as 1986, Dow Corning and Dr. Douglas Pullman, Dow Gardens, received a patent for silicone benthic (or bottom) barriers for the control of nuisance aquatic weeds. The patent claims cover the ability of silicone rubber coated fabric to control aquatic plant growth by restricting light, and preventing root penetration while allowing gases that form below the barrier to pass through the silicone coating and escape. Dow Corning Corporation owns all rights to this patent.

Silicones are made from quartz rock and are therefore inert and highly resistant to

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\*Dow Corning Corporation, Midland, Michigan.

\*\*The Dow Gardens, Midland, Michigan.

+Pullman, G.D. 1986. "An Evaluation of Several Benthic Barriers for Aquatic Weed Control," Ph.D. Dissertation, Michigan State University, East Lansing, Mich.

chemical and microorganism attack and the effects of ultraviolet light. The polymer structure of a silicone rubber allows high gas diffusion rates.

These unique features for silicones are successfully incorporated in a wide range of Dow Corning products, from controlled drug release membranes, to silicone treated surgical gowns and surgical draperies that retard the growth of bacteria and reduce the chance for post-operative infections; to silicone coated architectural roofing fabrics for stadiums, arenas, and open air pavillions. Market studies were performed, and field and laboratory investigations were used to evaluate the relative value of several commercial benthic barriers and a silicone benthic barrier.

## DISCUSSION

### Marketing study findings

A Dow Corning marketing study was recently completed on silicone benthic barriers (February, 1987). Interviews were conducted with lake property owners, aquatic weed control contractors, and state and federal government officials. The results of this study identified a market opportunity for a benthic barrier for nearshore recreational lake areas and ponds that is described as:

- a. Lightweight.
- b. Easy-to-install and maintain.
- c. Durable (5-8 year life).
- d. Environmentally "safe" (e.g. nonchemical).
- e. Provides immediate, localized weed control.
- f. Priced from \$0.80-1.00/sq ft installed (economics).

Key product features require that the barrier be opaque (e.g. to block light and restrict weed growth), resist vegetal and root penetration by a variety of submersed aquatic weeds (e.g. Eurasian watermilfoil, pondweed, cutgrass, torpedo grasses, etc.), and have a low buoyant potential so that the barrier stays on the bottom and does not float to the surface. Target applications include shallow water areas especially around boat docks and piers, marinas, water intakes, irrigation reservoirs, and swimming areas.

The four methods commonly used to control nuisance aquatic weeds are:

- a. Weed harvesting (mechanical).
- b. Aquatic herbicides (chemical).
- c. Benthic barriers (physical).
- d. Biological controls (biological).

According to those contractors interviewed, weed harvesting is effective but limited by shallow water depths (6 ft or less). Weed harvesting equipment has limited maneuverability around boat docks and piers and may become damaged by underwater pilings.

Aquatic herbicides are also effective but may limit the of recreational lakes and irrigation reservoirs for up to 30 days following application. Several chemical treatments may be required, and some herbicides are restricted to areas at least one-half mile

away from potable water intakes to protect drinking water supplies.

Benthic barriers can be subdivided into three basic types:

- a. Continuous films (unslitted Dartek).
- b. Porous fabrics (Texel TAC 150).
- c. Open Cell Screens (Aquascreen).

The Dartek™ barrier is a black pigmented nylon sheet similar to PVC plastic and slitted at intervals to permit gas escape; Texel™ TAC 150 is a blend of polyester and polypropylene plastic fiber (grey color) with the feel of a wool blanket; and Aquascreen™ is a PVC coated fiberglass mesh and is very similar to common window screen.

Two major problems with leading benthic barriers are gas entrapment and plant attachment (or penetration) through the barrier. Gas entrapment below the barrier can cause some barriers to float to the surface. Algae can also attach to the upper surface of porous fabrics (e.g. Texel TAC 150) and open cell screen type barriers (e.g. Aquascreen) causing these devices to trap gas and eventually float. Plant attachment can minimize the efficacy of the treatment.

Biological controls, such as triploid grass carp, are also effective, but grass carp may be too nonselective and may contribute to excessive nutrient cycling and cause algae blooms.

Very few of these available options represent the total answer for aquatic weed control. Several methods are effective but not selective; others are selective but not convenient-to-use by the average lake property owner. Several aquatic herbicides and biological controls may require permits and additional field monitoring programs to ensure proper application and performance control.

### **Silicone benthic barriers**

Research conducted at the Dow Gardens (from 1981 through 1986) indicated that silicone barriers, a continuous silicone rubber-coated fabric, may overcome many of these field installation and maintenance problems normally associated with other barrier devices. Silicone barriers were found to be gas permeable, resistant to plant attachment, and, as a result, had a low buoyant potential when compared to other types of commercially available bottom barrier products.\*

The buoyant potential is defined as the combined effect of its specific gravity, its gas and ion permeability, and its ability to resist filamentous algae colonization and plant attachment.\* Lakes where benthic barriers may be effective include USA regions outlined in an Aquatic Plant Control Research Program publication.\*\* Areas include the North Central and Northeastern United States and Eastern Canada; the Mid-Atlantic States; the Southern Gulf States and Florida; and the Pacific Northwest and California.

### **Dow Corning buoyancy test program**

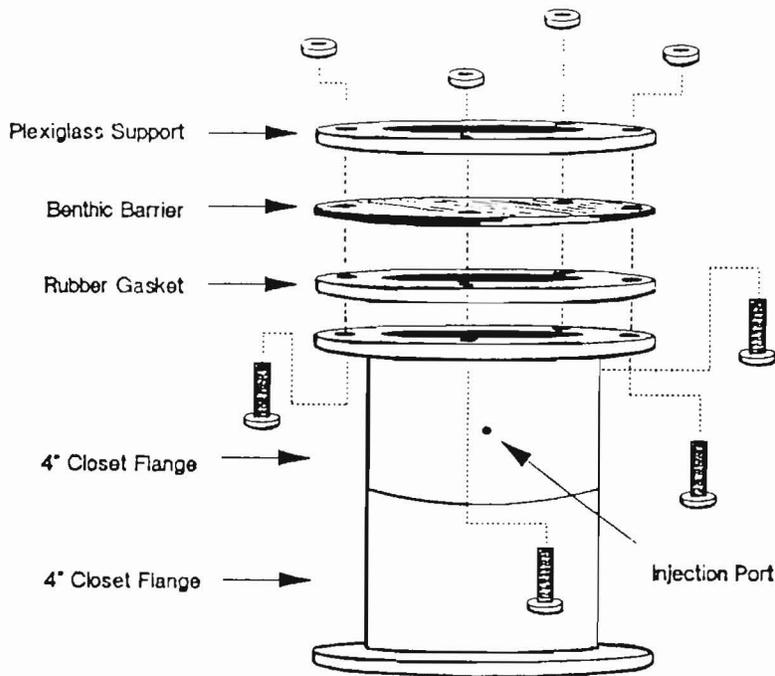
In June 1987, followup laboratory tests were conducted at the Dow Gardens to evaluate the relative buoyant potential of three commercial benthic barriers and a silicone

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\*Pullman, op. cit.

\*\*Decell, J. L. 1986. "A View of Interests in Aquatic Plant Control Problems and Technology."

benthic barrier. Water incubation tanks and lights were installed at the Dow Gardens Aquatic Research Lab located in Midland, Michigan. Individual buoyancy chambers were made of 4-in.-diam. PVC pipe, a benthic barrier test material, and a clear pressure ring and a rubber gasket. The assembled device is described in Figure 1.



**Figure 1. Benthic barrier buoyancy test chamber, Dow Gardens**

Leading products tested included unslitted Dartek™, Texel™ TAC 150, Aquascreen™, and a silicone benthic barrier. An absolute barrier was also used to measure the equivalent amount of gas generated below each device for comparison purposes; aluminum foil or saran was used as the absolute barrier for these trials. Six repetitions of each barrier type were made to reduce experimental error in this testing.

The test program run at the Dow Gardens was subdivided into two principal phases:

- Phase I measured the buoyant potential of each device without attendant filamentous algae growth.
- Phase II measured the buoyant potential of each device with the affect of attendant algae growth.

Each chamber was dosed with approximately 1,200-g dry weight per meter squared of Eurasian watermilfoil to initiate gas development below each device. Water temperatures were held at a constant 20°C (68°F) throughout the test. Phase I samples were incubated in the dark to limit surface algae growth; in Phase II, 14 hr of artificial sunlight was used to support algae growth while holding water temperatures constant. The amount of Eurasian watermilfoil used in these tests is considered a “high” dosage rate for a northern lake region.\*

\*Hutchinson, G.E. 1975. *Limnological Botany*, Vol. 3, J. Wiley and Sons, New York.

### Phase I test results

Phase I test results plotted as “submerged weight” on the Y-axis and time across the X-axis showed three statistically significant groupings (see Figure 2).

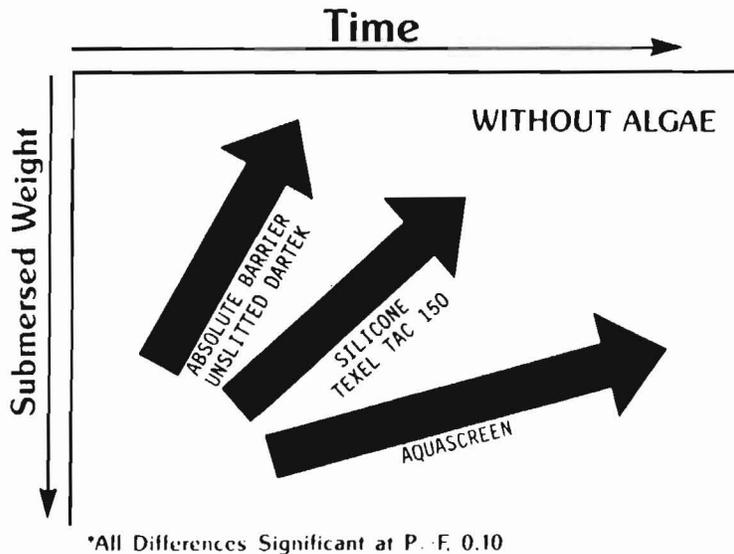


Figure 2. Benthic barrier Phase I test results (without algae)

The absolute benthic barrier and the unslitted Dartek™ barrier became buoyant, while the silicone and the Texel™ TAC 150 barriers were able to pass gas, and the Aquascreen™ barrier remained relatively unchanged throughout the duration of Phase I. Recall again that the Phase I studies were conducted in the absence of light and attendant algae growth. All data were statistically validated to a 90 percent confidence level using “Repeated Measure Analysis of Variance” (SAS Institute, Raleigh, North Carolina).

From Phase I test results, two major findings were confirmed for silicones.

- First, silicones offer increased gas permeability over other continuous film type barriers such as unslitted Dartek™.
- Second, silicones have comparable gas permeability to porous fabrics such as Texel™ TAC 150 in the absence of light and attendant algae growth even though the silicone rubber itself is a continuous film.

### Phase II test results

In Phase II, samples were again loaded with Eurasian watermilfoil at a rate approximately equal to 1,200-g dry weight per meter squared and exposed to 14-hr light/dark cycles so that filamentous algae growth could occur. Water temperatures were again held at a constant 20°C (68°F). Submerged weight was again plotted on the Y-axis; time was across the X-axis. Test results are shown in Figure 3.

Based on the results of Phase II, there were four (4) statistically distinct groupings. The absolute benthic barrier and the unslitted Dartek™ were the most buoyant (as in Phase I). The Texel™ TAC 150 and the Aquascreen™ barriers both showed increased buoyancy

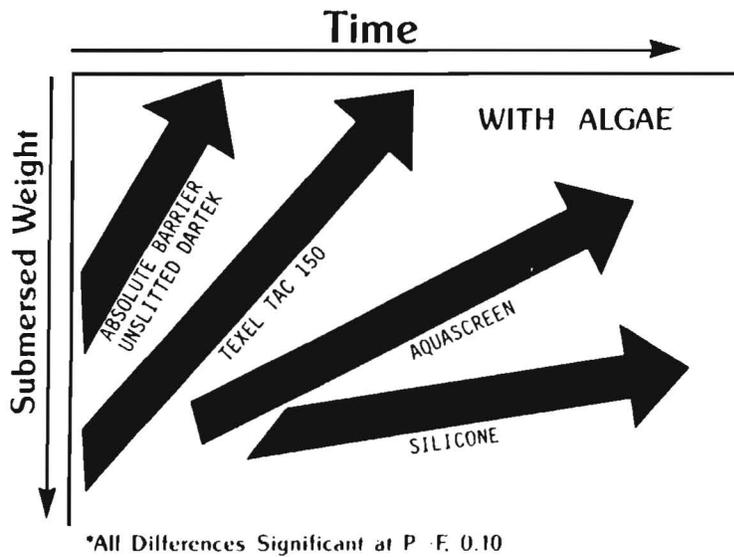


Figure 3. Benthic barrier Phase II test results (with algae)

potentials relative to Phase I test results as algae and fungi clogged the barriers. The silicone barrier remained relatively unchanged when compared to Phase I. These results were again validated statistically to a 90 percent confidence level using "Repeated Measure Analysis of Variance."

Previous field installations using Dartek™, Texel™ TAC 150, and Aquascreen indicate that these barriers do not withstand extended outdoor weathering and ultraviolet light exposure. Therefore, extended outdoor trials against silicones will be conducted over the Winter/Spring/ Summer (1988) period at selected test sites.

Phase I and Phase II buoyancy tests results, therefore, indicate that (under laboratory conditions) silicones had the lowest buoyant potential and would likely outperform all other barrier types in more extreme lake exposure conditions, especially in nearshore areas with high organic bottom sediment conditions and high plant attachment rates.

### Silicone barrier field trial

For limited field trials this Fall, Dow Corning Corporation produced approximately 5,000 sq ft of silicone benthic barrier from its coating facilities near Atlanta, Georgia. A 24-by 40-ft test section was installed in October 1987 in a 7-acre pond in Midland, Michigan. The barrier was placed in approximately 1 to 8 ft of water; aquatic weed types included pondweed and a niad weed complex. The bottom was comprised of approximately 20 cm of organic sediment deposited over hard pan clay. The silicone fabric was floated across the control area and was allowed to sink to the bottom (taking approximately 10 min). Gladiola stakes were used to anchor the barrier along the shoreline. A special staking device was fabricated that made this step of the installation very fast and efficient. The dark brown silicone benthic barrier blended well with the surrounding lake bottom. Results of this test and adjacent test sections using Texel™ TAC 150 will be monitored throughout the Winter/Spring/Summer periods (1988).

## CONCLUSIONS

Specific areas for future lab and field studies for the silicone benthic barrier will likely include:

- Silicone barrier effectiveness over selected aquatic weed types such as hydrilla, Eurasian watermilfoil, pondweed.
- Silicone barrier effectiveness to eliminate tuber regrowth and seed revegetation.
- Silicone barrier plant penetration studies and required maintenance levels to contain subsequent shoreline overgrowth.
- Silicone barrier effects on bottom sediment bio-geochemistry.
- Silicone barrier ecological impact studies (e.g. fish, aquatic macrophytes, water-fowl habitat, water quality).
- Silicone barrier field installation methods development for both deep and shallow water areas.
- Silicone barrier cost/benefit analysis versus other forms of aquatic weed controls such as herbicides, weed harvesting, grass carp, and bottom dredging, especially in nearshore areas.
- Model selection criteria by benthic barrier type and lake conditions for use in lake management practice.

The application of silicone barriers to “large scale” aquatic weed control projects should also be evaluated, e.g., potable water intakes on Lake Okeechobee, Florida, earthen dam water control projects, soil stabilization studies, and wastewater treatment impoundments. The integrated use of silicone barriers with mechanical, chemical, and biological controls should also be evaluated.

# **Influence of Rhizosphere Microflora on Nutrition and Growth of Rooted Aquatic Macrophytes**

by  
D. Gunnison\* and J. W. Barko\*

## **ABSTRACT**

The root systems of higher plants and the assemblages of microorganisms associated with them constitute the rhizosphere. Plant-microbe interactions in the rhizosphere have not been extensively examined in freshwater plants. However, information based on studies conducted in other environments indicates that these interactions may be important in freshwater systems. Understanding the microbial processes occurring in the rhizosphere is necessary to better elucidate nutritional relationships affecting aquatic plant productivity and distribution. This paper, based on a previous extensive literature review (Gunnison and Barko 1987), highlights two aspects of the rhizosphere microbiology. These aspects include the microbial transformation of nitrogen and phosphorus in the rhizosphere and the overall role of the rhizosphere microflora in affecting plant growth regulation. In contrast to what is known about the rhizosphere microbiology of terrestrial and wetland plants, very little is known about freshwater plants. However, based on the literature examined, there are two areas that appear to warrant investigation. These areas include the assessment of the role of rhizosphere microflora in aquatic plant nutrition with an emphasis on nitrogen, and the production of plant growth regulating substances by rhizosphere microflora.

## **INTRODUCTION**

Through their functions in food production and providing habitat diversity, aquatic plants play an important role in aquatic ecosystems (Pennak 1971, Wiley et al. 1984). Aquatic plants cause concern when they occur at nuisance levels in these same habitats. Our current understanding of how the effects of environment relate to the growth and distribution of these plants is limited, but improving. A large amount of information on the physiology and ecology of rooted aquatic plants has been developed in recent years; however, investigations in this area have primarily been limited to abiotic factors, including light, temperature, nutrients, and sediment composition. The influence of biotic factors, especially plant-microbial interactions in the rhizosphere (the root systems of higher plants and the microorganisms associated with them) is poorly understood.

Information obtained to date on agronomically important terrestrial plants indicates that rhizosphere microorganisms are important in nutrition and therefore affect plant survival and growth. The limited evidence available for aquatic plants indicates that the rhizosphere microflora likewise play a critical role in the nutrition of these plants. It is

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

also suspected, based on what is known about the rhizosphere of terrestrial plants, that rhizosphere microorganisms may play a direct role in the growth of their host plants. This growth potentially occurs in two ways through production of plant growth hormones or other growth-stimulating compounds, and through production or decomposition of growth-inhibiting substances. The present report, based on a previous extensive review of the literature (Gunnison and Barko 1987), highlights findings related to microbial transformation of nutrients in the rhizosphere and microbial formation of plant growth regulators.

## NUTRIENT TRANSFORMATIONS

### Nitrogen

The symbiotic relationship between the nitrogen-fixing bacteria of the genus *Rhizobium* and leguminous terrestrial plants has been known for a considerable time. When terrestrial leguminous crops are inoculated with nitrogen-fixing rhizobia prior to planting, plant survival and crop yields increase. More recent work has demonstrated the existence of associative (nonstructural) relationships between nitrogen-fixing bacteria other than rhizobia and nonleguminous terrestrial plants. Within the past few years, knowledge in this area has expanded to include several wetland and a few marine plant species.

In the specific case of salt marsh cordgrass (*Spartina alterniflora*), nitrogen-fixing bacteria have been found both on the root surface (rhizoplane) and within the plant roots themselves (McClung et al. 1983; Whiting, Gandy, and Koch 1986). The importance of this relationship to the growth of salt marsh cordgrass has not been established; however, it is known that a tight coupling does exist between plant productivity and the activity of the nitrogenase enzymes in the rhizosphere (Whiting, Gandy, and Koch 1986). There is reason to believe that a similar relationship occurs in the seagrass *Halodule wrightii*, where invasion of the roots by the nitrogen-fixing bacterium, *Klebsiella*, has been found (Schmidt and Hayasaka 1985).

Another area where rhizosphere microorganisms contribute to the nitrogen economy of rooted aquatic plants is in the formation of ammonium. Ammonium is the preferred form of inorganic nitrogen for uptake by aquatic plants from the sediment. As part of a study demonstrating the importance of seagrass rhizoplane bacteria in recycling organic nitrogen, Smith, Hayasaka, and Thayer (1984) examined the relative rates of ammonification of glutamate, alanine, valine, and glycine by rhizosphere bacteria from *Zostera marina* and *Halodule wrightii*. The authors demonstrated that excess ammonium released by the rhizosphere microorganisms accumulated entirely within the plants and water column.

### Phosphorus

It has been known for some time that certain bacteria in the rhizosphere of terrestrial plants participate in the mobilization of phosphorus from the surrounding soil. This step is accomplished through two mechanisms: release of inorganic phosphate from organic compounds containing phosphate, and solubilization of inorganic phosphate from phosphate-containing minerals in the soil. Craven and Hayasaka (1982) demonstrated

that during periods of active growth of the seagrass *Zostera marina*, there was a steady increase of soluble phosphate that was accompanied by a corresponding increase in the numbers of phosphate-solubilizing bacteria. This process was an order of magnitude lower during periods of plant dormancy. Since rooted aquatic plants appear to require soluble inorganic phosphate for uptake and growth, this solubilization process may be of significant value to the plant. The significance may be greater for those aquatic plants that transport excess oxygen to their roots and oxidize the immediately surrounding sediment. This process results in the formation of ferric oxyhydroxides, and these compounds strongly bind inorganic phosphates rendering them unavailable for plant use.

## PRODUCTION OF PLANT GROWTH REGULATORS

### Plant growth hormones

The ability of certain rhizosphere microorganisms to form plant growth hormones has been known for some time, although the bulk of this understanding is limited to a few crop plants. Tien, Gaskins, and Hubbell (1979) examined plant growth hormones produced by the nitrogen-fixing microorganism *Azospirillum brasiliense* on pearl millet and found production of indole acetic acid (auxin, IAA), gibberellic acids, and cytokinin. They subsequently examined the effects of treating millet roots with varying levels of IAA, gibberellic acids, and kinetin individually and in combination, and contrasted results with those obtained by inoculation of millet roots with *A. brasiliense*. Addition of hormones singly or in combination increased plant shoot growth. However, none of these increases were as effective as that resulting from inoculation of plant roots with *A. brasiliense*; this treatment resulted in nearly a doubling of the plant shoot biomass. The microbial and combined hormone treatments apparently were able to increase plant shoot growth because they each vastly increased the number of laterals and root hairs. These changes increased the root sorptive surface area and improved plant nutrient uptake.

### Other plant growth regulators

Other (organic) compounds that stimulate or inhibit plant growth include aliphatic di- and tribasic acids, oxy acids, and aromatic acids (Takijima 1964a), phenolic acids (Wang, Chang, and Chaung 1967; Vaughn, Sparling, and Ord 1983), and nicotinamide, mугenic acid, and arenic acid (Walker and Welch 1986). Inorganic compounds include carbon dioxide and hydrogen sulfide (Sheikh 1970). Mixtures of compounds include a variety of plant, peat, and soil extracts (Kimber 1967, 1973; Takijima 1964b; Dooris and Martin 1980; Juttner and Schroeder 1982). These substances may be produced by rhizosphere microorganisms or by organisms living in the soil outside of the rhizosphere. In either event, the compounds must pass through the rhizosphere as they diffuse toward the roots. During this passage, these compounds are available for modification by the rhizosphere microflora.

## DISCUSSION

The presence of plant-microbe interactions has not been examined in detail for freshwater plants. We know a considerable amount about wetland plants, but only a fair amount about marine plants. Nonetheless, these two environments are not very far removed from freshwater habitats; wetland plants are often emergent aquatic plants, and several marine species are capable of growth in freshwater. Based on what is known about other environments, these interactions probably also occur with rooted freshwater plants. However, until aquatic plant rhizospheres are thoroughly investigated, we will remain uncertain of the role of interactions in the rhizosphere on the growth and distribution of nuisance aquatic plant species.

Better information on the interactions between aquatic plants, sediments, and the rhizosphere microflora will be important in improving our understanding of the ecology of rooted aquatic plants. Durako and Moffler (1987) and others have suggested that the rhizosphere microflora-root relationship may be required for the nutrition (particularly nitrogen economy) of certain plants; however, the extent to which microbial processes replenish nutrient supplies in sediments depleted due to aquatic plant uptake needs to be assessed (Barko et al., in press). A thorough understanding of factors affecting sediment nutrient replenishment will assist in the development of innovative, ecologically-oriented plant management techniques. For example, if the presence of nitrogen-fixing bacteria is mandatory for growth of a nuisance plant in a nitrogen-poor sediment, then altering the rhizosphere environment to produce conditions unsuitable for growth of these bacteria may make the sediment unsuitable for the growth of the nuisance plant. Likewise, colonization of the root surface by plant growth hormone-producing microorganisms may stimulate increased production of root laterals and hairs. Increased laterals and hairs enlarge the sorptive surface area of the plant's roots, thus improving the plant's ability to do well in certain nutrient-poor sediments. If the rhizosphere environment can be managed to discourage proliferation of hormone-producing microbes or encourage multiplication of antagonistic species, the beneficial effects of the growth hormone-producing microorganisms may be prevented. Consequently, the nuisance plant may do poorly or not survive at all in the sediment of interest.

## CONCLUSIONS

Based on our understanding of the rhizosphere microbiology of aquatic plants in freshwater habitats, the following areas appear to offer the highest level of information return from research investments. The role of the rhizosphere microflora in aquatic plant nutrition should be investigated with special emphasis on the importance of nitrogen fixation to submersed aquatic plants. Based on the literature we examined, it is through the process of nitrogen fixation that rhizosphere microflora have the greatest influence on the nutritional ecology of rooted aquatic plants. As a consequence, an improved understanding of nitrogen fixation in aquatic plants also has the highest potential to yield improved control techniques. Another important area for research is the production of plant growth regulating hormones. It

is becoming increasingly apparent that there is a positive relationship between plant growth hormone production by certain rhizosphere microorganisms and increased root surface area in terrestrial plants. This same relationship may well occur in rooted aquatic plants. If so, perhaps this relationship can be exploited to discourage the growth of nuisance aquatic plants.

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# Role of Endogenous (Chemical) Defenses in Plant-Herbivore Interactions

by  
W. Charles Kerfoot\*

## INTRODUCTION

The use of the term “chemical defenses” implies active evolution of toxic, noxious, or unpalatable compounds for purposes of deterrence. In the case of animals, the evolution and elaboration of glands specifically designed to aid delivery of noxious substances seems evidence enough of purpose. Additional behavioral or morphological adaptations, e.g. aposematism, often provide convincing evidence that the use of compounds is intimately related to protection from potential consumers (Kerfoot 1982, Scrimshaw and Kerfoot 1987). Palatability tests are used to confirm initial impressions and to quantify relative vulnerability. Unfortunately, these obvious clues are often absent in macrophytes. The mere presence of noxious compounds is not sufficient to demonstrate defensive design, per se. Three levels of proof seem necessary: (a) Are noxious substances present in tissues? (b) Are they of sufficient quantity to influence palatability and hence herbivory? and (c) Have they evolved specifically as a defense against herbivory?

The importance of chemical deterrents is acknowledged for predator-prey and plant-herbivore interactions in terrestrial and marine environments (e.g., Rosenthal and Janzen 1979; Futuyma and Slatkin 1983; Bell and Carde 1984; Bakus 1981; Norris and Fenical 1982; Hay, Fenical, and Gustafson 1987). The apparent scarcity of comparable findings in the freshwater literature stands in stark contrast. To what extent is this contrast real?

While many terrestrial plant families utilize noxious compounds against herbivory, generalizing to aquatic macrophytes is not simple. Freshwater aquatic species represent a highly biased sample, constituting less than 1 percent of all described plants, and are relatively rich in monocot genera (Sculthorpe 1967, Hutchinson 1975). Certain species are closely related to predominantly terrestrial families (e.g., water cress, *Rorippa*, in the family Cruciferae; aquatic buttercups and marsh marigolds, *Ranunculus* and *Caltha*, in the Ranunculaceae; water hemlock and water parsnip, *Cicuta* and *Sium*, in the Umbelliferae), yet many other emergent, floating, or especially submersed species belong to highly diversified or specialized aquatic taxa.

Until quite recently, freshwater macrophytes were considered impoverished in secondary compounds, at least typical terrestrial substances (Sculthorpe 1967, Li and Willaman 1968, Hutchinson 1975, Sheldon 1987). The exact origin of this widespread belief is puzzling. It seems more a consequence of underrepresentation in detailed plant surveys and general neglect. For example, a recent review by Hutchinson (1975) states:

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An examination of any of the large surveys of phytochemistry . . . indicates that the water plants have been unduly neglected by organic chemists; when they have been studied, secondary compounds, particularly those such as alkaloids, glycosinolides, cardenolides, and cyanogenic substances, which may constitute chemical defenses against herbivores, are rarely found.

Curiously, circumstantial evidence has long argued to the contrary, suggesting the presence of pungent compounds and several interesting side-effects including deterrence. The most important observations are: (a) many herbivorous animals attack aquatic macrophytes, yet few cause extensive damage (Gaevskaia 1966, Anderson and Sedell 1979, MacKay and Wiggins 1979, Wetzel 1983); (b) certain macrophytes emit strong or unpleasant odors when crushed, e.g., the penetrating wintergreen smell of sweet rue, *Acorus*, or the rank, pungent smell of skunkweed, *Chara* (Wium-Anderson et al. 1982); (c) water conditioned by certain macrophytes will repel zooplankton and insects, e.g., *Daphnia* repulsed by extracts from *Elodea*, *Myriophyllum*, and *Nitella* (Pennak 1973), and avoidance of *Chara* and *Nitella* stands by egg-laying mosquitoes, and (d) relatively few species will consume fresh leaves, whereas many herbivores and detritivores will consume partially decomposed material (Smock and Stoneburner 1980).

Secondary compounds in aquatic macrophytes are diverse from a qualitative standpoint and certain types concentrated from a quantitative standpoint. Relatives of terrestrial families with known noxious compounds seem to carry related chemical substances, although relative potency remains a question in some species. In the more highly evolved aquatic taxa, three general classes of compounds seem to merit special treatment: alkaloids, sulphated or sulphur-containing substances, and phenolics.

## AQUATIC RELATIVES OF TERRESTRIAL FAMILIES

Some of the best-known and most potent substances come from aquatic members of the Umbelliferae. Several species are listed as dangerous or poisonous plants, including the water hemlock (*Cicuta maculata*), water parsnip (*Sium suave*), and water dropwort (*Oenanthe corocata*) (Kingsbury 1964, Tampion 1977, Stephens 1980). Often mistaken for parsnip, these species exude unsaturated higher alcohols as a toxic yellow oil, or juice that turns yellow when exposed to air (cicutoxin, *t*-heptadeca-8:10:12-triene-4:6-diyne-1:4-diol, which is a violent convulsant that acts on the central nervous system; oenanthetoxin, both a surface irritant and a highly toxic poison, suspected to be a long chain acetylenic alcohol, along with oenanthetal and oenanthetone). These species are responsible for major losses of livestock that forage among shoreline emergents.

Additional cases come from the family Ranunculaceae. Here several aquatic buttercups, genus *Ranunculus*, and the marsh marigold, *Caltha palustris*, produce an irritant, poisonous yellow volatile oil, protoanemonin. This oil, a lactone of 2-hydroxy-vinylacrylic acid, is liberated through enzymatic action from a glycoside precursor, anemonin, when buttercup tissues are crushed. After a few days protoanemonin reverts to anemonin, which is harmless, making only fresh plants poisonous (Poulsen 1916, Shearer 1938, Verdcourt and Trump 1969).

Several marginal emergents contain alkaloids, resembling their terrestrial relatives. Amphibious species of *Polygonum* (Polygonaceae), *Decodon* (Lythraceae), and *Ranunculus* are the best known cases (Hegnauer 1969, Hutchinson 1975). The pungent-taste of *P. hydropiper* apparently comes from the alkaloid polygodial.

Our own investigations, however, have centered on a member of the Cruciferae, water cress. Like other crucifers, water cress (*Rorippa nasturtium-aquaticum*) contains the glycosinolide gluconasturtin, a glucoside of a phenyl-ethyl mustard oil (Schultz and Gmelin 1952). When tissue is damaged, either through chewing or freezing, this glucosinolate will undergo hydrolysis, mediated by myrosinases, to form two important flavor components: phenylethylisothiocyanate and hydrocinnamitrile (3-phenylpropionitrile). These two compounds constitute between 30 and 58 percent of the volatiles released from ruptured or decomposing tissue (Spence and Tucknott 1983, Spence et al. 1983). Tests conducted at the University of Michigan Biological Station showed these two compounds to be quite toxic to *Gammarus pseudolimnaeus*. Both crushed and frozen tissue would kill amphipods, and authentic compounds gave the following lethal doses: 48 h LC<sub>50</sub> of 3.6 ug/l for phenylethylisothiocyanate and a 48 h LC<sub>50</sub> of 130 ug/l for hydrocinnamitrile. Amphipods normally do not consume healthy leaves, but live in the roots of this macrophyte. However, like many other potential consumers, they will ingest decomposing leaves, after a suitable period of leaching. During this interval, presumably the toxic compounds have been leached away from leaf tissue.

## ALKALOIDS

Alkaloids have been reported from a wide variety of macrophytes, but usually only in modest concentrations. Examples from primitive plants include the stonewort family Characeae (*Chara*) and the horsetail Sphenopsida (*Equisetum*). Dicotyledons include the families Nymphaeaceae (*Nymphaea*, *Nuphar*), Nelumbaceae (*Nelumbo*), Cabombaceae (*Cabomba*), Ceratophyllaceae (*Ceratophyllum*), Lythraceae (*Decodon*), and Haloragaceae (*Myriophyllum*). Monocotyledons include the families Hydrocharitaceae (*Vallisneria*, *Elodea*), Potamogetonaceae (*Potamogeton*), Pontederiaceae (*Heteranthera*), Arales (*Calla*), Lemnaceae (*Lemna*), Sparganiaceae (*Sparganium*), Typhaceae (*Typha*), and Cyperaceae (*Carex*). Highest concentrations and greatest number of compounds occur in the Nymphaeaceae (Su, Staba, and Abul-Hajj 1973b; Wrobel 1967). Su, Staba, and Abul-Hajj (1973a) reported positive Dragendorff reactions in 12 of 22 surveyed species. More refined methods indicate even greater incidence of alkaloids, but in relatively low concentrations, 0.13-0.56 mg g<sup>-1</sup> dry weight (Ostrofsky and Zettler 1986). While the latter authors surveyed only 14 species in six genera (*Cabomba*, *Ceratophyllum*, *Elodea*, *Heteranthera*, and *Potamogeton*), TLC spots indicated the presence of alkaloids in all species, ranging from a low of two (*Cabomba*) to a maximum of nine (*Potamogeton*).

## SULPHATED OR SULPHUR-CONTAINING COMPOUNDS

Sulphur-containing compounds have been described from several families, notably the Characeae, Nymphaeaceae, Potamogetonaceae, and Hydrocharitaceae, with many of these representing uniquely aquatic substances. Sulphur-containing compounds are

also found in marine macrophytes, e.g., crystalline sulphur and cyclic polysulfides in red algae (Ikawa 1973, Wratten and Faulkner 1976), and sulphated flavones and caffeic acid esters in seagrasses (Harborne 1975, Harborne and Williams 1976; McMillan, Zapata, and Excobar 1980; McMillan 1983).

Perhaps the most striking example of sulphur-based freshwater compounds occurs in the Characeae. Certain species of the stonewort *Chara* emit a rank garlic-like pungent odor, giving rise to the common name "skunkweed". These plants repel mosquito larvae and egg-laying adults (Caballero 1919, 1922a, 1922b; Pardo 1923; and several other references reviewed by Hutchinson 1975), stimulate avoidance in the cladoceran *Daphnia* (Pennak 1973), and inhibit phytoplankton growth (Steeman-Nielson 1973, Crawford 1979, Wium-Anderson et al. 1982). In attempting to identify the noxious, volatile compounds, Amonkar (1969) believed the primary compounds allied to garlic oil, i.e. mainly allicin or allyl thiosulfinate, and demonstrated that the active larvicidal properties of the latter came from the related diallyl or trisulfide (Amonkar and Banerji 1971). Recent isolation and characterization of these sulphur-based compounds has instead revealed two simple compounds, a dithiolane and a trithiane (4-methylthio-1,2-dithiolane and 5-methylthio-1,2,3-trithiane) as shown by Anthoni et al. (1980) and Wium-Andersen et al. (1982). Confirmed in several species, both the foul odors and the repellent properties of stoneworts seem attributable to the presence of these two compounds.

## MACROPHYTE PHENOLICS

Compared with terrestrial species, external physical deterrents to herbivory are greatly reduced in aquatic macrophytes (Sculthorpe 1967; Hutchinson 1975; Kerfoot, submitted manuscript). Moreover, aquatic macrophytes are generally high in protein, micronutrients, and water content (Boyd 1969, 1970; Hutchinson 1975; Muztar, Slinger, and Burton 1978a; Wetzel 1983). Average water content is elevated above terrestrial values: emergents (79 percent) floating-leafed (82 percent), and submersed (88 percent) according to Straskraba (1968). As a group, aquatic macrophytes have sufficient protein and micronutrient concentrations to rank them high in food value relative to most terrestrial plants, and even relative to many standard cultivated forage crops (Gortner 1934; Boyd 1968; Hutchinson 1975; Muztar, Slinger, and Burton 1978b, Wetzel 1983). Plants with floating leaves have higher percentages of proteins (26.5 percent AFDW) and ether-extractable material (4.0 percent) than either submersed (22 and 2.2 percent) or emergent (13 and 2.1 percent) categories (Straskraba 1968, Wetzel 1983).

Combining the two attributes (reduced external deterrents, high basic food value), one might judge macrophytes a good forage source. However, major attempts to develop macrophytes as either food supplements or staples have failed for two reasons: (a) high water content with rapid spoilage, and (b) low palatability and low livestock weight gain (Gopal 1987). Many of the macrophytes have unpleasant tastes and odors (Gopal 1987).

Phenolic compounds have been identified in freshwater macrophytes prior to our surveys (Boyd 1968; McClure 1970; Su, Staba, and Abul-Hajj 1973a; Hutchinson 1975; Planas et al. 1981), but relative concentrations and qualitative aspects were unclear. Our surveys of soluble phenolics, based on Folin-Denis assays (Ribereau-Gayon 1972, as modified by Mole and Waterman 1987a) and TLC analysis (Harborne 1984), illustrate

major differences between various taxa and growth forms (Table 1, Figures 1 and 2).

Table 1  
Simple Phenolics and Tannins Identified from Macrophytes\*

	<i>p-H</i>	<i>SY</i>	<i>VA</i>	<i>TA</i>	<i>GA</i>	<i>EL</i>	<i>FE</i>	<i>CA</i>	<i>pC</i>	<i>SI</i>	<i>CH**</i>	<i>Tannins</i>
DICOTYLEDONS												
<i>Nymphaea</i>	+	+		+	+	+	+	+	+	+		+ hydrolyzable
<i>Nuphar</i>	+	+		+	+	+	+	+	+	+		+ hydrolyzable
<i>Brassenia</i>	+	+			+	+	+	+	+	+		+ hydrolyzable
<i>Ceratophyllum</i>	+	+	+				+	+	+		+	
<i>Myriophyllum</i>	+	+		+	+	+	+	+	+	+		+ hydrolyzable, condensed (some species)
<i>Hippuris</i>						+	+	+				+
<i>Ludwigia</i>						+			+			+
<i>Jussiaea</i>						+			+			+
MONOCOTYLEDONS												
<i>Elodea</i>								+			+	
<i>Vallisneria</i>								+	+			+ condensed
<i>Alisma</i>	+						+	+	+			
<i>Sagittaria</i>							+	+	+	+		
<i>Aponogeton</i>	+						+	+	+	+		
<i>Potamogeton</i>	+	+		+			+	+	+			+ condensed (some species)
<i>Zostera</i>	+	+	+	+	+		+	+	+			+
<i>Eichhornia</i>							+	+				+ condensed
<i>Sparganium</i>							+	+	+	+		+ condensed
<i>Typha</i>							+		+	+		
<i>Carex</i>							+	+	+	+		+ condensed
<i>Eleocharis</i>												+ condensed
<i>Scirpus</i>							+		+			

\*McClure 1970; Kerfoot (unpublished data).

\*\**p-H* *p*-hydroxybenzoic acid; *SY*, syringic acid; *VA*, vanillic acid; *TA*, tannic acid; *GA*, gallic acid; *EL*, ellagic acid; *FE*, ferulic acid; *CA*, caffeic acid; *pC*, *p*-coumaric acid; *SI*, sinapic acid; *CH*, chlorogenic acid.

As might be expected from their taxonomic affiliations and exposure, shoreline emergents (e.g., *Scirpus*, *Carex*, *Sagittaria*, *Eleocharis*, *Phragmites*, *Polygonum*, *Sparganium*, *Decodon*) are similar to terrestrial herbs. Relative to this standard, concentrations in rooted, floating-leaf macrophytes (e.g., *Nymphaeae*, *Brassenia*, *Nuphar*, *Potamogeton natans*) are unexpectedly high, rivaling values found in leaves of many terrestrial trees (e.g., oaks, sycamore, birch, willow, some maples). Lowest values are found for fully submersed genera and species (e.g., *Najas*, *Elodea*, *Ceratophyllum*, *Hydrilla*, *Egeria*, *Vallisneria*, some *Myriophyllum* and *Potamogeton* sp.). Even the exceptions among submersed and marginal emergent categories reinforce the general pattern. High values for shoreline emergents (the closely related *Ludwigia* and *Jussiaea* in the family Onagraceae, *Decodon* in the family Lythrales, *Cabomba* in the family Cabombaceae) and for submersed species (*Myriophyllum verticillatum*) include plants that either are capable of producing floating leaves or are related to taxa that do (Hutchinson 1975).

The Folin-Denis tests are general tests for soluble phenolics, so we had to identify the qualitative nature of compounds. Soluble phenolic compounds in macrophytes fall into a number of categories: (a) low molecular weight, free or attached simple phenolic acids and phenols, many important as precursors (e.g., *p*-hydroxybenzoic, syringic acids in

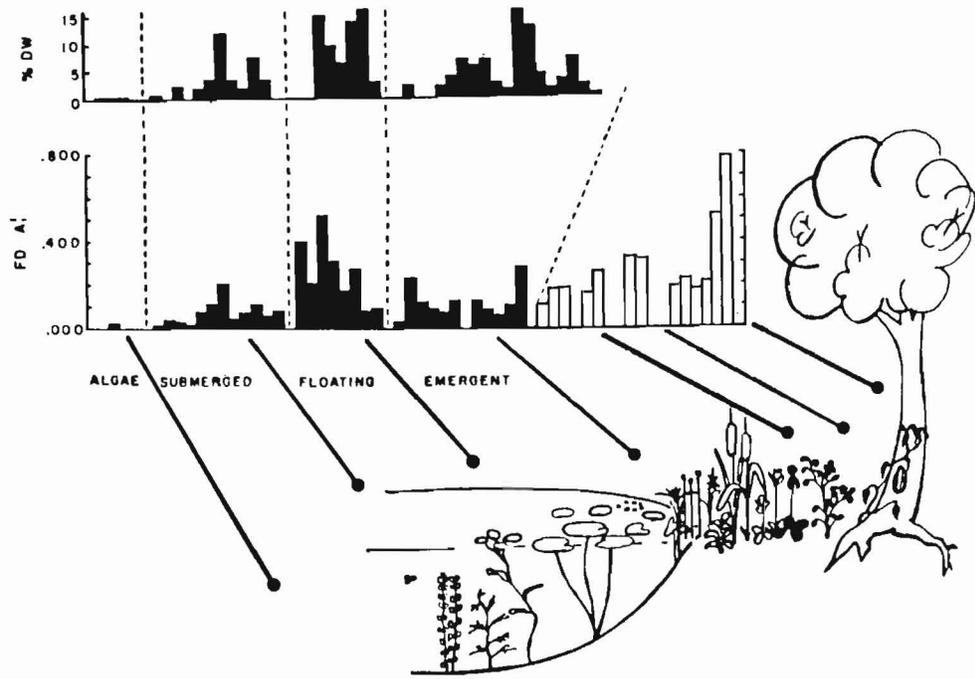


Figure 1. Soluble phenolic concentrations (Folin-Denis assay) along a transect into Third Sister Lake, Michigan. Trees sampled include maples, oaks, beech, and willow; vines include grape and raspberry; herbs include Queen Anne's lace, bull thistle, goldenrod, etc. Upper portion gives corresponding values from Boyd (1968), expressed as percent dry mass; whereas bottom assay values are expressed in absorbance units, following the recommendation of Mole and Waterman (1987a)

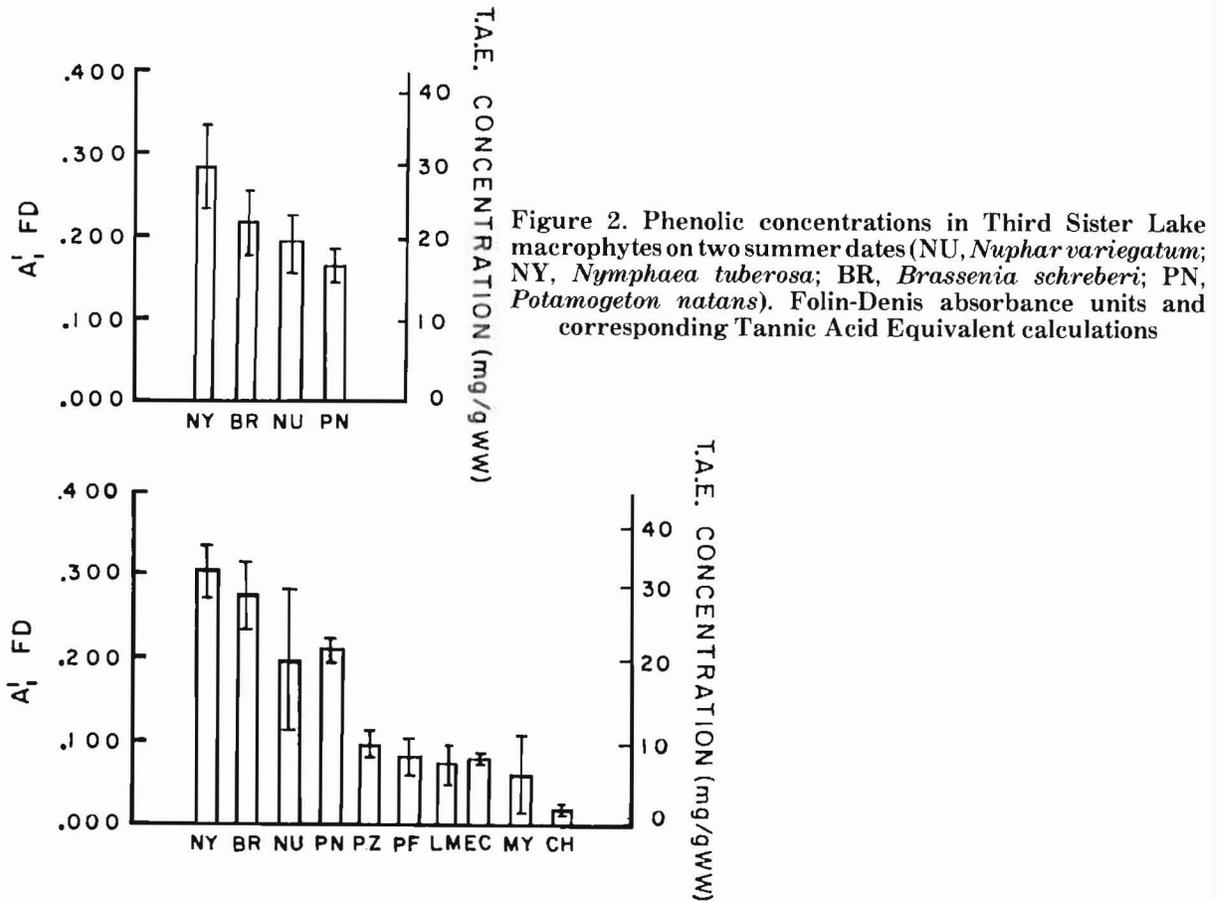


Figure 2. Phenolic concentrations in Third Sister Lake macrophytes on two summer dates (NU, *Nuphar variegatum*; NY, *Nymphaea tuberosa*; BR, *Brassenia schreberi*; PN, *Potamogeton natans*). Folin-Denis absorbance units and corresponding Tannic Acid Equivalent calculations

lignins) or unpalatable compounds (e.g. tannic acid); (b) phenylpropanoids (e.g., the hydroxycinnamic acids ferulic and *p*-coumaric acids, hydroxycoumarins, and other lignin building blocks); (c) flavonoid pigments (including anthocyanins, proanthocyanins, flavonols, flavones, and others); and (d) polymers such as hydrolysable and condensed tannins (Harborne 1984). In terrestrial studies, tannins have received special attention. These are polymers of appreciable molecular weight, with condensed tannins derived from flavan-3-ols, whereas hydrolysable tannins come from gallic or ellagic acid esterified to a sugar moiety (Ribereau-Gayon 1972; Harborne 1984; Beart, Lilley, and Haslam 1985; Haslam 1986). Tannins are moderately soluble in water, produce a characteristic astringent taste, and are distinguishable by their ability to precipitate protein, although the link between concentration and protein precipitation ability is not simple (Martin and Martin 1982, Mole and Waterman 1987b). These compounds are frequently considered to lower both the palatability and food value of leaf tissue (Feeny 1976, Coley 1986).

In our surveys, elevated phenolic values coincided with the presence of hydrolysable tannins. Alkaline and acid hydrolysis of leaf tissues, followed by two-dimensional TLC (methods of Harborne 1984) with authentic compounds, confirmed high quantities of gallic and ellagic acid (Kerfoot, submitted manuscript). Highest yields were for species of lily pads (families Nymphaeaceae, Nelumbaceae, and Cabombaceae), although other high-scoring dicot genera (*Ludwigia*, certain *Myriophyllum*) and one monocot species (*Potamogeton natans*) gave positive indications. High scores for lily pad species agree with the only previous survey for general phenolics (Boyd 1968) and the specific results of Su, Staba, and Abul-Hajj (1973b). Su and his co-workers identified three important compounds in *Nymphaea* and *Nuphar*: tannic acid, gallic acid, and ethyl gallate. The incidence of ellagic acid was used to evaluate the position of the family Nymphaeaceae in the Ranales, quite independent of any functional role (Bate-Smith 1968).

The discovery of high tannin concentrations in lily pad genera and the marked contrast with submersed species represent unexpected and exciting developments. Lily pad genera (*Nymphaea*, *Nuphar*, *Nelumbo*, *Brassenia*, etc.) are the Methuselahs of the macrophyte world, with recorded lifespans exceeding 20-50 years (Sculthorpe 1967). These long-lived species also contain appreciable concentrations of alkaloids and sulphated compounds (Table 2; Wrobel 1967; Su, Staba, and Abul-Hajj 1973b; Hutchinson 1975) making them broadly protected against a variety of potential consumers and pathogenic organisms. In many respects, despite their outward appearances, these species may represent the "trees" of the littoral zone. And like terrestrial trees, they are subject to insect attack despite their noxious compounds.

## PLANT-HERBIVORE RELATIONSHIPS

Relatively few insects are known to attack the leaves of water lilies, but those that do can cause substantial damage (Scott 1924, Smirnov 1960, Wallace and O'Hop 1985). Consumers include the beetles *Pyrrhalta* (*Galerucella*) *nymphaeae* and *Donacia crassipes* (Chrysomelidae), aphids (*Ropalosiphum* sp.), fly larvae (*Hydromyza* sp., some chironomid larvae), and various lepidopteran larvae (Smirnov 1960, Gaevskaya 1966). The chrysomelid beetle *Pyrrhalta nymphaeae* is perhaps the most abundant and interesting consumer.

**Table 2**  
**Numbers of Identified Alkaloids, Sulphated Compounds, and Phenolics in Various Macrophyte Families**

Family	Alkaloids	Sulphated Compounds	Phenolics		
			Simple	Tannins	Flavonoids
<b>PRIMITIVE PLANTS</b>					
Characeae	+	2 dithiolane, trithiane	-	-	-
Sphenopsida	2 palustrin, palustridin	-	-	-	-
<b>DICOTYLEDONS</b>					
Nymphaeaceae	11	5 neothioinuparidine, thioinupharidine, etc.	5	+++	8
Nelumbaceae	14	-	-	+++	14
Cabombaceae	+	-	-	+++	-
Ceratophyllaceae	+	-	4	++	3
Haloragaceae	+	-	3	++	3
Callitrichaceae	-	-	2	-	3
Hippuridaceae	-	-	4	+	1
Menyanthaceae	-	-	2	-	3
Ranunculaceae	2	-	-	-	-
Saururaceae	-	-	-	++	-
Polygonaceae	+	-	-	++	4
Cruciferae	-	4 isothiocyanates, thiocyanates, sulphides	-	-	+
Lythraceae	7	-	-	++	3
Onagraceae	3	-	2	+++	5
<b>MONOCOTYLEDONS</b>					
Butomaceae	-	-	3	-	+
Hydrocharitaceae	+	-	3	-	5
Alismaceae	+	-	-	-	+
Aponogetonaceae	-	-	4	-	4
Zosteraceae	+	+	2	++	-
Potamogetonaceae	+	+	9	+++	+
Najadaceae	-	-	-	-	1
Pontederiaceae	?	-	4	-	3
Lemnaceae	-	-	-	-	43
Sparganiaceae	?	-	4	++	2
Typhaceae	?	-	4	-	5
Cyperaceae	1	-	4	++	5
Gramineae	5	-	3	-	2

Members of the Chrysomelidae have adapted to feeding on the roots of such noxious plants as Indian hemp (*Cannabis*), belladonna (*Atropa*), and dogbane (*Apocynum*), or on the leaves of milkweed (*Asclepias*). A closely related congener (*Pyrrhaltra luteola*) feeds on elm leaves.

The waterlily leaf beetle (*Pyrrhalta nymphaeae*) is unusual among the Galerucinae in that both larvae and adults feed upon the upper surface of floating lily pads. Development is rapid, with eggs hatching in 4-6 days, larvae passing through three instars in 2-5 days each, and pupae hatching after about 5 days (Scott 1924, Smirnov 1960, Wallace and O'Hop 1985). The exposed position of all life stages suggests the use of noxious compounds by the larvae and adult stages, a presumption confirmed by our initial investigations (Kerfoot, submitted manuscript). Eggs are laid in small masses, about 7-19 (mean 14). Larvae are jet-black against the green of leaves, showing yellow bands and underbelly if molested. First instar larvae are gregarious, feeding in small groups, and have incompletely developed defensive glands. Second and third instar

larvae possess complete rows of lateral tubercles on the mesothorax, metathorax, and abdominal segments. These tubercles discharge droplets of a noxious substance, while the body fluid itself appears a bright yellow and unpalatable (rejected by fish and invertebrates in feeding trials). The segmentally arranged glands are refined in the pupal stage, as the developing pupa cloaks itself within the larval integument. During initial pupal formation, the pupa is brilliant yellow and during emergence the adult is also bright yellow. The yellow hemolymph of a related beetle (*Pyrrhalta luteola*) has been identified as a mixture of anthraquinones and anthrones (Howard et al. 1982).

Our initial investigations involved three types of tests (tissue preference, feeding trials, demographic studies) designed to investigate a relationship between tannin content and preference. The tests were simple modifications of the leaf disk assay or simple field manipulations. In the behavioral tissue preference trials, one adult or third instar larva was given a choice of three different plugs (*Nuphar*, *Nymphaea*, or *Brassenia*) laid on moistened filter paper. In the feeding trials, one adult was given a choice of three plugs (as above), or three larvae were initially placed on each of the pads. Controls were run with only a single type of plug. In the demographic trials, egg masses deposited on *Nuphar* and nearby *Nymphaea* were marked, cleared of surrounding leaves, and larvae followed after emergence.

The initial results demonstrated strong preference for *Nuphar* leaves, the ones lowest in total phenolics (Figure 3). Choice and feeding preferences were strongest among larvae, weakest among adults. Adults and third instar larvae would consume all three tissue types, but clearly preferred *Nuphar* (Figure 3). The most sensitive stage appeared to be the first instar larvae. In the field, these larvae would disperse from *Nymphaea* leaves soon after initial nibbling, rather than feed in the customary manner. If dispersal was blocked, larvae died of weight loss and dehydration without moulting into the second instar (Figure 4).

While correlation between phenolic content and feeding preference does not prove that tannins are solely responsible, the results encourage further investigation. Recall that another chrysomelid beetle, *Agasicles hygrophila*, the alligatorweed flea beetle, is one of the chief biological control agents for the alligatorweed, *Alternanthera philoxeroides*. Both the specificity of host plant preference and the enhanced survivorship of introduced beetles are likely a consequence of chemical defenses.

## IMPLICATION

Four generalizations seem to be emerging from our recent investigations:

- a. Noxious, toxic unpalatable compounds are varied and common among marginal emergents and shoreline species. Many of these species are related to predominantly terrestrial families known for their potent compounds.
- b. Alkaloids and especially phenolics are concentrated in rooted macrophytes that produce floating leaves. Many of these species are long-lived plants (e.g., *Nuphar*, *Nymphaea*, *Brassenia*), analogous to "trees," and thus represent "apparent" species in the littoral (Feeny 1976, Rhoades and Cates 1976). Alternatively, floating leaves may simply be more susceptible to insect attack, with the elevated

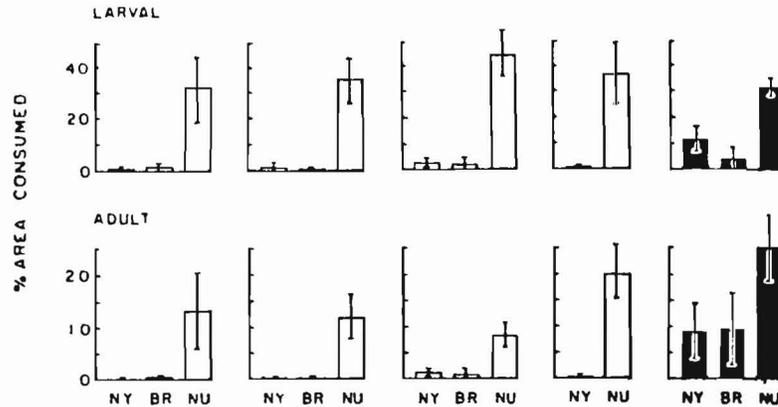


Figure 3. Feeding preferences of third instar larvae and adult lily pad flea beetles. Standard leaf disk presentations; black, single species trials; unshaded, simultaneous presentation of three disks (NY, *Nymphaea*; BR, *Brassenia*; N, *Nuphar*). Larvae and adults fed for 18-24 hours. Preference trials run on four different dates

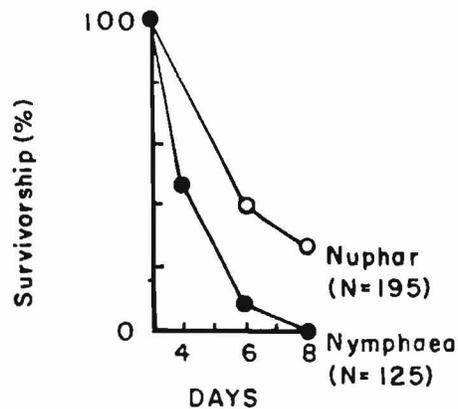


Figure 4. Survivorship of first instar larvae hatched on white lily pads (*Nymphaea*) compared with yellow lily pads (*Nuphar*). Cannibalism contributed to the decline in controls, whereas experimentals died of dehydration without moulting into second instars

levels of phenolics, especially hydrolyzable tannins, reflecting this attribute.

- c. Submersed macrophytes possess lower levels of defensive compounds, with lower concentrations and fewer kinds of phenolics and alkaloids. This pattern is consistent with lowered risk from insectivorous herbivory.
- d. Clonal or "weedy" species, i.e., those that exploit the high growth/high propagation life history mode, show reduced levels of phenolics in tissues and greater susceptibility to fish, snail, and insect damage in standardized tests (Sheldon 1987, snails). These patterns conform to the "resource availability" hypothesis (Coley, Bryant, and Chapin 1985, Coley 1987), i.e., that macrophytes which opt for a high growth strategy commit less energy to defensive compounds.

Immediate implications are in two areas: (a) defensive compounds as an explanation for supposedly "allelopathic" effects, and (b) use of enzyme blocks in the shikimic acid pathway to prevent the production of defensive phenolics. The latter is promising because monocotyledons such as *Hydrilla* rely on relatively few phenolic compounds in low concentrations for defense.

Phenolic compounds are especially important because, as quantitatively rich substances, they probably contribute to many of the supposedly "allelopathic" effects ascribed to aquatic macrophytes. The term allelopathy was originally used to describe the inhibitory effects of secondary plant compounds, that accumulate in substrate or that are released in water, upon nearby competitors (Rice 1974, Mueller 1970). Many phenolic compounds are surprisingly refractile, e.g., *p*-coumaric, *p*-hydroxybenzoic, vanillic, and ferulic acids (Guenzi and McCalla 1966; Whitehead, Dibb, and Hartley 1982). These compounds become differentially enriched in decomposing plant tissues and surrounding sediments as less refractile compounds decompose and leech away (Boon et al. 1983; Valiela, Teal, and Allen 1985; Wilson, Valiela, and Swain 1985). In contrast, more soluble phenolics, such as gallic or tannic acids, leak from living tissues, or are released soon after tissue destruction and decomposition. For example, soluble phenolics from *Nymphaea*, *Nuphar*, and *Myriophyllum* are known to inhibit a variety of bacteria, fungi, and algae (Su, Staba, and Abul-Hajj 1973a; Planas et al. 1981). Antimicrobial inhibition is strongest in three identified phenolics: tannic acid, gallic acid, and ethyl gallate (Su, Staba, and Abul-Hajj 1973).

Phenolics are widespread in aquatic macrophytes and potentially influence palatability and digestibility. Certain compounds are located within vacuoles in special cells dispersed within palisade or vascular layers, e.g., protocatechuic, caffeic, vanillic, *p*-coumaric and chlorogenic acids in *Eichhornia* (Martyn and Cody 1983; Martyn, Samuelson, and Freeman 1983); ferulic acid in *Spartina* (Valiela et al. 1979), whereas others are bound by esterification to lignins, cellulose, and hemicelluloses, e.g., hydroxycinnamic acids in *Spartina alterniflora* (Hartley and Jones 1977; Collins, Wilson, and Swain 1981). Ferulic acid in *Spartina* has been demonstrated to have antimicrobial, antiherbivore, and antidetrivore properties (Valiela et al. 1979; Reitsma, Valiela, and Buchsbaum, in press).

Low concentrations of phenolics in fast-growing submersed or rootless, floating-leaf species (e.g., *Hydrilla verticillata*, *Myriophyllum spicatum*, *Eichhornia crassipes*, *Lemna* sp.), and fewer varieties open the way toward blocking plant defenses. Production

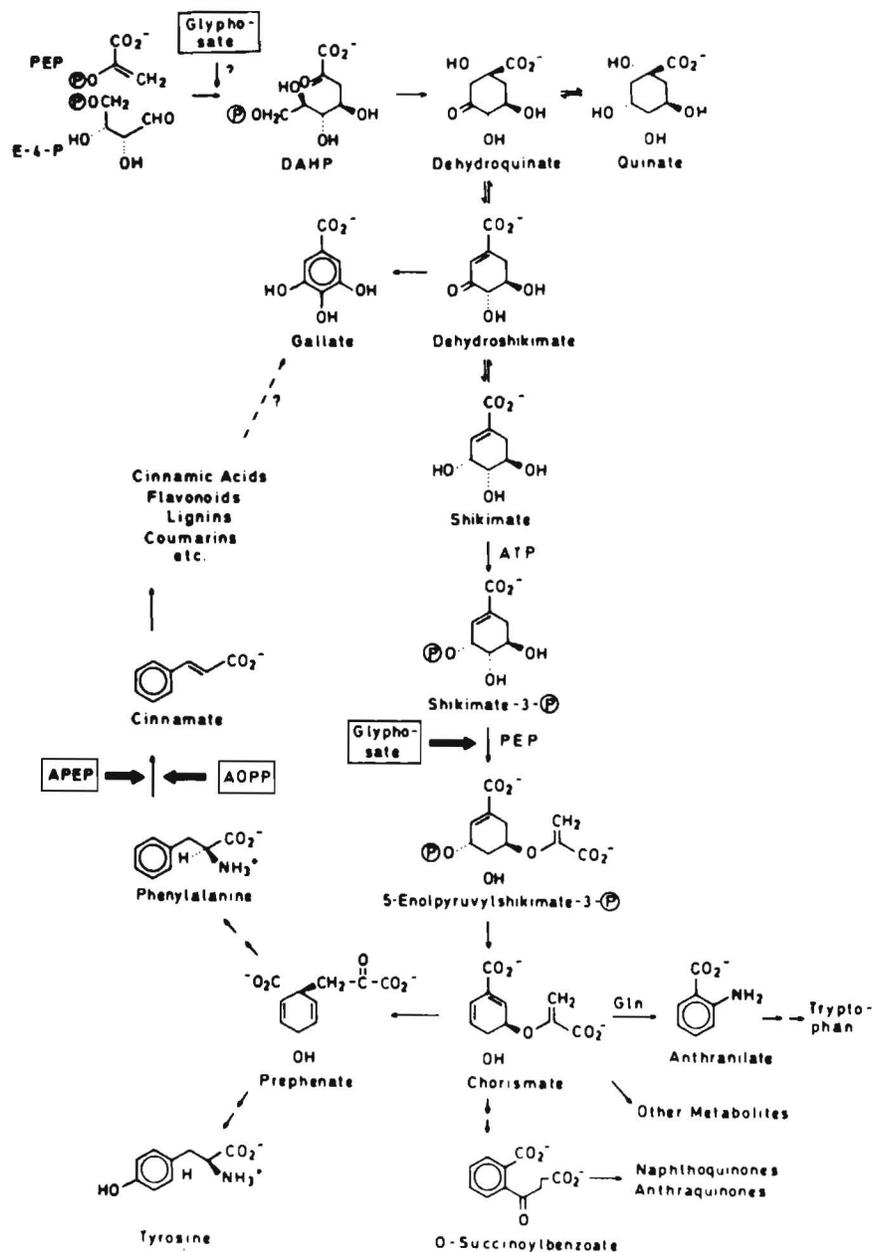


Figure 5. The shikimate pathway, showing targets of inhibitor action (the herbicide Glyphosate; L-AOPP, L-aminooxy-phenylpropionic acid; APEP, (R)-(1-amino-2-phenylethyl)phosphonic acid). The latter two compounds are inhibitors of PAL (phenylalanine ammonia-lyase) (from Amrheim 1986)

of many phenolic defensive compounds is related to the crucial shikimic acid metabolic pathway (Figure 5). The very successful herbicide glyphosate inhibits the enzyme which catalyzes the conversion of shikimate 3-phosphate into 5-enolpyruvylshikimate 3-phosphate. It was initially tested on *Lemna gibba* (Amrhein 1986). Search is underway for compounds that specifically block much more limited steps in the shikimic acid pathway, without harming vital functions (Conn 1986). For example, the compounds L-AOPP and APEP represent potential compounds that influence more terminal pathways leading to several defensive phenolics, although responses of tannins seem complicated (Haslam 1986). Blocks on ferulic acid, anthocyanins, and tannins might produce defenseless plants, ones susceptible to local grazers.

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**USAE DIVISION/  
DISTRICT PRESENTATIONS  
AQUATIC PLANT PROBLEMS—  
OPERATION ACTIVITIES**

## Lower Mississippi Valley Division, New Orleans District

by  
Glen N. Montz\*

The aquatic plant management program at the New Orleans District consists of the Aquatic Plant Control Program and the Removal of Aquatic Growth Project. The Aquatic Plant Control Program is the cooperative cost-sharing (50/50) agreement between the Federal government and the State of Louisiana. Field operations under this program are conducted in-house by Corps personnel and by the Louisiana Department of Wildlife and Fisheries. Emphasis on in-house Corps work under this program is on waterhyacinths and hydrilla, while in-house Corps work under the Removal of Aquatic Growth Project is mainly treating waterhyacinths in Federally maintained waterways.

The very nature of the plants involved and their growing habits make this a project that does not lend itself to a firm engineering plan established in advance.

Between FY 70 and FY 80, emphasis was placed on waterhyacinths and alligatorweed. Treatments with 2,4-D and mechanical sawboats were used to control these two plant species. Beginning in FY 81 to present, the program has changed drastically.

The introduction and spread of new problem plants such as hydrilla, salvinia, and *Najas minor* has resulted in additional control methods to treat these species. In FY 81 the District initiated a small scale operation using Diquat-Cutrine Plus and Aquathol K to control hydrilla. In FY 85 this operation was expanded to include broadcasting granular Aquathol and spraying Aquathol K. Both granular and liquid Aquathol gave good results which lasted for 1 year, but experimental plots by Elanco using Sonar gave excellent results which lasted for 2-3 years. In FY 87 granular Sonar was broadcast on hydrilla with excellent results.

Treatments on salvinia were begun in FY 84 using Diquat. Aquathol K was used during FY 86 on salvinia without success. *Najas minor* has been controlled using water drawdowns by the Louisiana Department of Wildlife and Fisheries in several lakes in north Louisiana. No chemical treatments to date have been used on *Najas minor* by the State. Rodeo has been used by in-house Corps personnel on waterhyacinths and water paspalum in areas where 2,4-D is banned, such as the Jean Lafitte National Historical Park and Plaquemines Parish.

Waterhyacinth remains as the most serious aquatic pest in Louisiana, followed by hydrilla, alligatorweed, salvinia, egeria, water paspalum, Eurasian watermilfoil, frogbit, watershield, American lotus, coontail, and *Najas minor*.

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To shade hydrilla, floating aquatics in Lake Boeuf near Raceland and Lake Theriot near Houma have been permitted to grow from FY 84 to present in several large tracts of each lake. A mild winter in 1985-86 allowed many acres of floating aquatics in these tracts to effectively shade and reduce hydrilla and other submerged plants. When these floating plants are eventually sprayed, the submerged vegetation will again become a problem.

Most of the New Orleans District's total in-house effort (72 percent of herbicide costs and 98 percent of acreage treated in FY 87) is directed toward treatment of mainly waterhyacinths with 2,4-D and Rodeo. The use of Diquat on salvinia accounted for 4 percent of herbicide costs in FY 87. The use of Aquathol on submerged aquatics, other than hydrilla, accounted for 4 percent of herbicide costs in FY 87. The use of Aquathol, Aquathol K, Sonar, and Diquat on hydrilla accounted for 20 percent of herbicide costs in FY 87.

Federal crews have experimented with 2,4-D and additional surfactant on water paspalum, waterlettuce, and salvinia. Solutions consisted of 15 gal of 2,4-D plus 1 gal of surfactant sprayed at 1 gal of 2,4-D per acre. Recorded kills on water paspalum averaged 40 percent; those on waterlettuce and salvinia gave poor results. These tests were conducted to see if 2,4-D could be substituted for Diquat and Rodeo on these species. Rodeo with 2 gal of surfactant per 30 gal drum gave better and faster kills on waterhyacinths and water paspalum than with less surfactant.

Water samples are collected each FY by Corps personnel and analyzed by a Contractor for 2,4-D residue. Samples are collected at various intervals of time after spraying to correlate time to residue of herbicide.

Numerous barricades (fences and gates, pilings, etc.) are being constructed in waterways in Louisiana. There are legal questions remaining concerning access and entrance into these waterbodies. Historically, Federal and State crews sprayed waterhyacinths in most of these waterways prior to the barricades. Treatment of private or posted waterbodies is normally prohibited. However, if the District determines that treatment of these waterbodies is beneficial to the project, approvals are obtained from the waterbody owner. Federal spray crews do not treat unwanted aquatics in waterways with barricades unless the drifts of vegetation coming from these waterways are adversely affecting nearby navigable waterways. No spraying is done in barricaded waterways if landowners request that we do not treat the aquatics there.

All Corps of Engineers personnel active in aquatic growth control operations are certified as commercial pesticide applicators by the Louisiana Department of Agriculture.

The New Orleans District plans to purchase a 30- to 35-ft mechanical harvester in FY 90 to remove mainly hydrilla from several lakes and waterways in south Louisiana. Disposal of harvested plants will be on adjacent spoil banks, shorelines, or on floating mats in large lakes. Rights-of-way will be obtained from landowners for all shore disposals. In many cases, it is projected that disposal of plants can create small islands on floating mats in large lakes that can eventually be vegetated with shrubs and can become rookeries for various bird life.

An After Action Report for the A-76 Commercial Activities study was completed during FY 87 and concluded that the District is in compliance with the Most Efficient Organization (MEO) study recommendations and the Performance Work Specifications (PWS).

The Aquatic Plant Control, Information Exchange Bulletin, by the Operations Support Center in Jacksonville District, is an excellent means of disseminating information about the Corps mission regarding aquatic plant management.

## Southwestern Division/Galveston District

by  
Joyce Johnson\*

The Galveston District is responsible for the federally funded Aquatic Plant Control Program (APCP) within the state boundaries of Texas. The District's jurisdiction is generally along the Texas Gulf coast to about 200 miles inland. However, the statewide program was assigned to the Galveston District because at the inception of the program in Texas the problem plants were waterhyacinth and alligatorweed, which grew generally in a fringe along the coast. The Fort Worth and Tulsa Districts are responsible for the operation and maintenance of their respective projects within Texas, which includes some aquatic plant control. However, this operational effort is not part of the cost-shared APCP. The control of noxious aquatic plants has been managed by the Galveston District since 1968. A General Design Memorandum and Environmental Statement for the control of alligatorweed and waterhyacinth were published in the early 1970's. As stated, most of the control work in the past has been along the coastal region. However, a submersed exotic species, hydrilla, is becoming a prevalent aquatic problem throughout the south and eastern portions of Texas.

In 1986, a Supplement No. 1 to the General Design Memorandum and an Environmental Assessment were completed to incorporate the treatment of hydrilla and Eurasian watermilfoil in the Texas APCP. Herbicide treatment of hydrilla has been accomplished at three lakes.

During the 19-year history of the APCP in Texas, the Galveston District has had four contracts with the State of Texas, which provide for 70 percent reimbursement of the control costs. The current contract has been modified to a 50 percent cost-sharing level in accordance with the Water Resources Development Act of 1986.

The Texas Parks and Wildlife Department plays an active, vital role in the Texas program, performing most of the field work and all of the herbicide spraying. The herbicide 2,4-D is used to control waterhyacinth; endothall and fluridone are used to control hydrilla. Alligatorweed is controlled predominately by *Agasicles hygrophila* in Texas at the present time. Populations that dwindled during the late 1970's have been supplemented by releases during the past 6 years by the Waterways Experiment Station (WES) with insects provided by Florida or collected in Louisiana. In addition, WES has introduced other biological agents including *Neochetina bruchi*, *Neochetina eichhorniae* and *Sameodes albiguttalis* for the control of waterhyacinth and *Vogtia malloi* and *Amynothrips andersoni* for alligatorweed control. The State of Texas has continued the work started by WES by incorporating the biological agents in the cost-sharing program. Several nursery areas for these species have been established during the WES effort. The acceptance by the Texas Parks and Wildlife Department of a renewed emphasis on biological control of aquatic species has been due, in large part, to the quality of the work done by the initial WES team, including Ed Theriot, Al Cofrancesco,

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

and Mike Stewart, and the continued close coordination with the State Coordinator from subsequent WES personnel.

During the heaviest months of the herbicide spray program (April through September), the Texas Parks and Wildlife Department has five crews stationed in three areas treating waterhyacinth. Prior to 1985, there were seven crews in Texas during the summer period. More aerial spraying occurs to compensate for the reduced boat coverage caused by reduction of Texas Parks and Wildlife Department staff. The treatment of hydrilla is done on an as-needed basis by the field crews. During 1987, about 17,900 acres of waterhyacinth and 90 acres of hydrilla were chemically treated as part of the APCP.

It is expected that since the number of problem-level infestations of hydrilla are increasing within the state, the program will continue to expand for the foreseeable future. The proposed control of hydrilla is limited to treatment of boat ramps and access in 11 presently infested lakes in Texas. However, since the herbicide costs involved in treatment of hydrilla are so much greater than costs associated with treatment of waterhyacinth, the program will cost nearly three times more than in the past. It is clear that less costly, environmentally compatible methods of hydrilla control are needed in the Texas APCP. There is an active interest within the State to solve the problems caused by hydrilla, and a Regional Task Force has been formed by several State agencies in recognition of the severity of the hydrilla problem.

During 1988, the Galveston District will complete the negotiation of a 50 percent cost-shared Cooperative Agreement with the State of Texas. The District is also aiding in the State's effort to develop greater public awareness of the problems associated with the spread of hydrilla and the purpose and need for aquatic plant management to ensure the continued multipurpose use of the lakes and rivers within Texas.

# Southwestern Division, Tulsa District

by  
Loren M. Mason\*

## INTRODUCTION

A chemical control program was conducted on Pat Mayse Lake on 15 June 1987. The control program was initiated in response to the excessive growth of Eurasian watermilfoil (*Myriophyllum spicatum*). A water quality monitoring program was also performed in conjunction with the control program. Purpose of the monitoring program was to collect pertinent water quality data to evaluate the impacts of applying a herbicide to a municipal potable water supply lake. A similar chemical control program was conducted in 1983 with no adverse biological or water quality impacts on the lake or its primary uses. Pat Mayse Lake is a 5,990-acre Army Corps of Engineers lake project located in Lamar County, Texas, approximately 15 miles north of the city of Paris, Texas. Project authorized purposes include water supply for the city of Paris, flood control, fish and wildlife, and recreation. Growth patterns of watermilfoil within Pat Mayse Lake are shown in Figure 1.

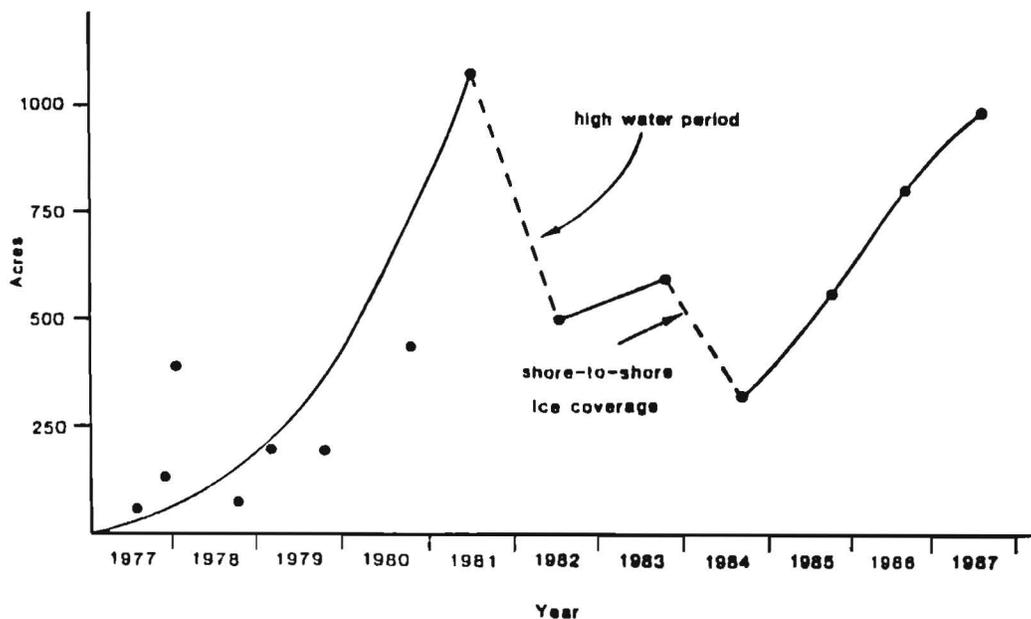


Figure 1. Invasion of *Myriophyllum spicatum* in Pat Mayse Lake

\*US Army Engineer District, Tulsa; Tulsa District

## COORDINATION

In order to assure a treatment program in June 1987, it was necessary to begin coordination with State and local agencies as well as public interest beginning in January 1987. Because Pat Mayse Lake serves as the primary water supply source for the city of Paris, and local fishing clubs were concerned as to the effects of the control program on the fishery, extensive coordination activities were initiated by the District to ensure technical compliance.

Concurrence was obtained from the Environmental Protection Agency, Texas State Department of Health, Texas Water Resources Board, Texas Parks and Wildlife Commission, and the city of Paris, Texas. Numerous planning and information meetings were conducted to ensure that all aspects of the chemical and water monitoring programs were adequately coordinated prior to initiating treatment.

## CHEMICAL TREATMENT

To minimize treatment impacts and reduce public concerns, the control program was limited to specified recreational shorelines, designated swimming beach areas, and boat ramps. As a result, the treatment acreage was reduced to 86 acres out of the total 960 acres overgrown with watermilfoil. To further minimize application impacts within the 86 acres, a granular formulation of dipotassium salt of endothall (Aquathol) was selected. Based upon the watermilfoil biomass, shoot density, and 6- to 10-ft water depths, the granular endothall was applied at a rate of 200 lb/acre to achieve the desired concentration of 2.0 ppm.

The treatment areas were marked with clear 1-gal plastic jugs every 100 ft secured to 1/4-in. nylon cord and anchored. The outer limits of the treatment areas along the shoreline were marked with 3-ft wooden stakes and red survey tape for easy visibility. The treatment areas were marked and signed in a highly visible manner to ensure that the public knew the location of the actual treatment sites and to aid the application crews from the Texas Parks and Wildlife Department in applying the endothall. The treatment areas are shown in Figure 2.

## CONCLUSION

Based upon results of the 1987 water quality monitoring study conducted by North Texas State University, there are sufficient data to verify that the endothall rapidly dissipated within 72 hr from the treatment areas, and no endothall was detected in the vicinity of the city of Paris water intake.

There was a 100 percent kill of the Eurasian watermilfoil within the treated areas with no detectable adverse impacts occurring in the treated or untreated areas of the lake and no effects on the fishery.

## FUTURE PROGRAMS

The existing restrictions placed upon the use of endothall in Pat Mayse Lake limit the

**LEGEND:**

○ - Treatment Areas for 1987.

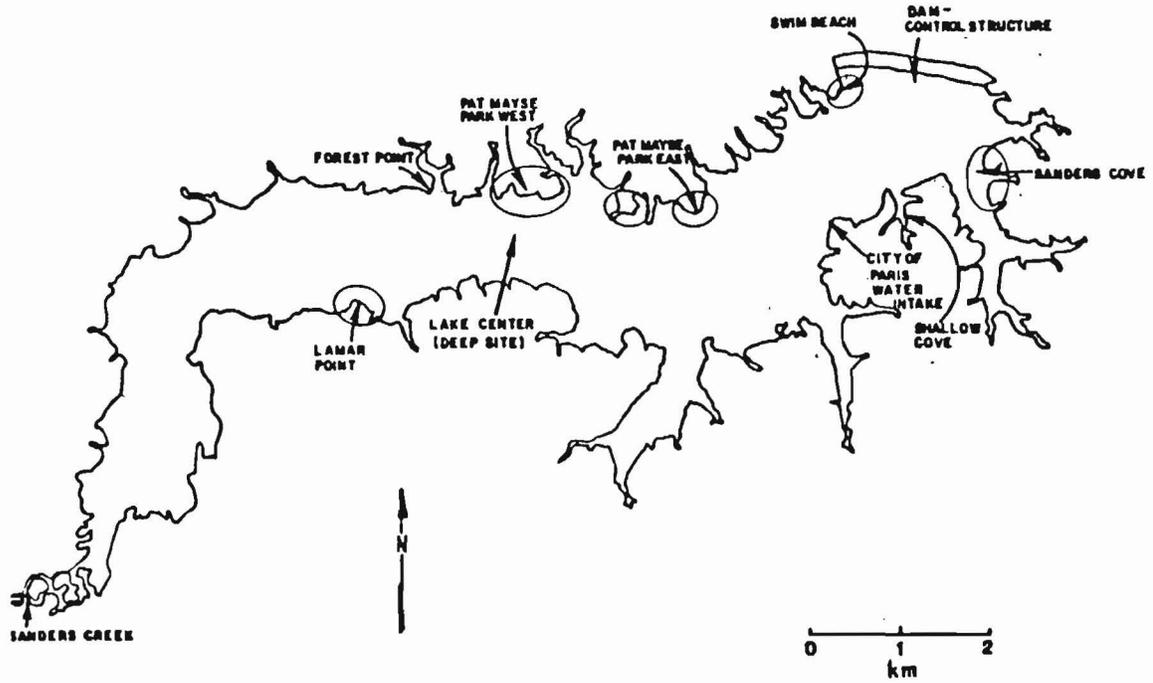


Figure 2. Pat Mayse Lake

District's chemical control program to a two-week time frame in June of each year, during which Campbell Soup is shut down for their annual maintenance program. In order to eliminate the restrictive two-week time period, the District plans to conduct an experimental chemical control program in 1988 using a less restricted use herbicide for potable water supply lakes.

# Aquatic Plant Control Operations Support Center

by  
William C. Zattau\*

## INTRODUCTION

In October 1980, the Aquatic Plant Control Operations Center (APCOSC) was formally established in the Jacksonville District in recognition of the District's knowledge and expertise gained through the administration of the largest and most diverse aquatic plant management program in the Corps. The APCOSC assists other Corps elements and Federal or State agencies in the planning and operational phases of aquatic plant control. The specific duties, relationships with other Corps APC programs, and guidelines for the utilization of the APCOSC are outlined in Engineer Regulation (ER) 1130-2-412.

## FY 1988 ACTIVITIES

The Center has had a busy and innovative year. In addition to the usual information and assistance contacts (Table 1) from throughout the country, the Center initiated publication of the APCOSC Information Exchange Bulletin, the Annual Aquatic Plant Control Program District Survey, and issued an updated APCOSC Aquatic Plant Control Program Contact List.

Table 1  
Breakdown of APCOS Contacts for FY 1987

<i>Type Assistance</i>	<i>Corps OCE</i>	<i>Corps WES</i>	<i>Corps Div</i>	<i>Corps Dist</i>	<i>Other Fed</i>	<i>Other Country</i>	<i>State Local</i>	<i>Industry</i>	<i>Private</i>	<i>Total</i>
Planning	5	1	0	10	1	0	1	0	5	23
Operations	4	1	3	13	2	0	4	2	6	35
Research	0	5	0	0	3	0	5	1	1	15
Training	0	0	0	4	2	0	0	0	0	6
Totals	9	7	3	27	8	0	10	3	12	79

The new Information Exchange Bulletin serves as a forum to keep readers up-to-date on developments and advances in the areas of biological, chemical, and mechanical aquatic plant management. At this time, in excess of 300 subscribers receive the nationally-distributed, quarterly publication. The success of this venture depends on those of you in the audience whom hopefully will offer both constructive criticism and articles for publication.

The first annual Aquatic Plant Control Program District Survey is being distributed at this meeting. This publication details aquatic plant management activities in 14 Corps District APC programs. The information presented was provided by Corps operations

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

personnel in attendance today and compiled by the Center. This information is very valuable and will serve as the foundation for a data base on Corps APC activities.

The Center is offering to sponsor an Aquatic Plant survey and Identification course this spring if there is enough demand. Please contact the Center if you are interested.

During the last Operations Breakout Working Session, there was a consensus that the format of the operations formal presentations needed to be modified. The Center has worked closely with the Districts and OCE this past year and came up with today's format. After these presentations, another Working Session will be held to hear comments on whether to retain this format, modify it, or return to the previous format.

For the first time since 1985, the APCOSC gave its Pesticide Applicator Training Course. Since certificates must be renewed every 3 years, it would appear that more Districts might require the course.

The Center staff this year collected approximately 70,000 alligatorweed flea beetles for distribution to requesting agencies.

## CONTACTS

The Center assisted OCE in revising ER 1130-2-413 (Pest Control Program for Civil Works Projects). This regulation assigns responsibilities and prescribes procedures concerning the use of pesticides (including aquatic herbicides) at all civil works projects. Center staff also participated in reviewing drafts of ER 1130-2-412 (Aquatic Plant Control Program).

The APCOSC hosted a visit by Carl Brown (APCRP Technical Monitor). Site visits to Lake Rodman and Lake Okeechobee gave him a first-hand view of aquatic plant management practices and problems.

Staff assisted the U.S. Army Engineer Waterways Experiment Station in the release of three new biocontrol agents in Florida. The waterlettuce weevil (*Neohydronomus*) was released in Lake Okeechobee, the hydrilla tuber-feeding weevil (*Bagous*) in Lake Toho, and the hydrilla fly (*Hydrellia*) in Lake Marion.

As you can see from Figure 1, the majority of Center contacts were related to operations activities in various Corps Districts. Figure 2 illustrates that Corps Districts made the most requests.

## CONCLUSION

As first stated, the Center has had a busy year. It hopes that all of you will take advantage of the services offered, and suggests improvements which would permit better support for Corps APC activities.

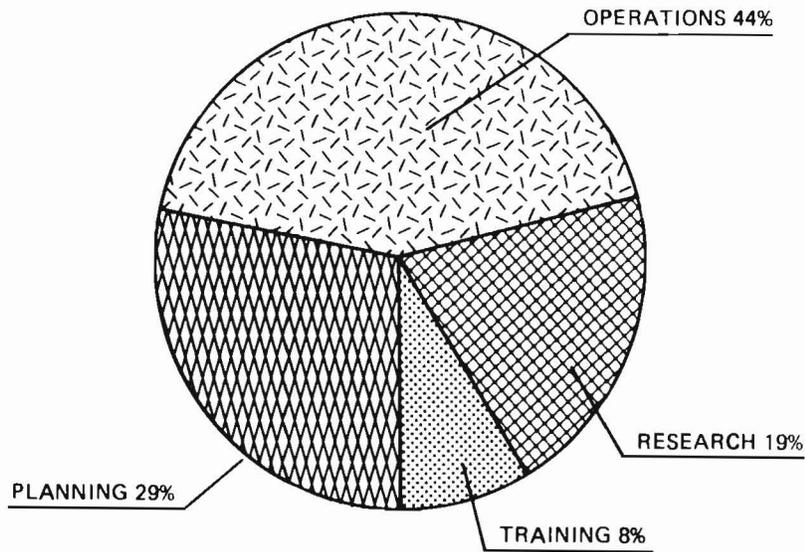


Figure 1. Types of information requested

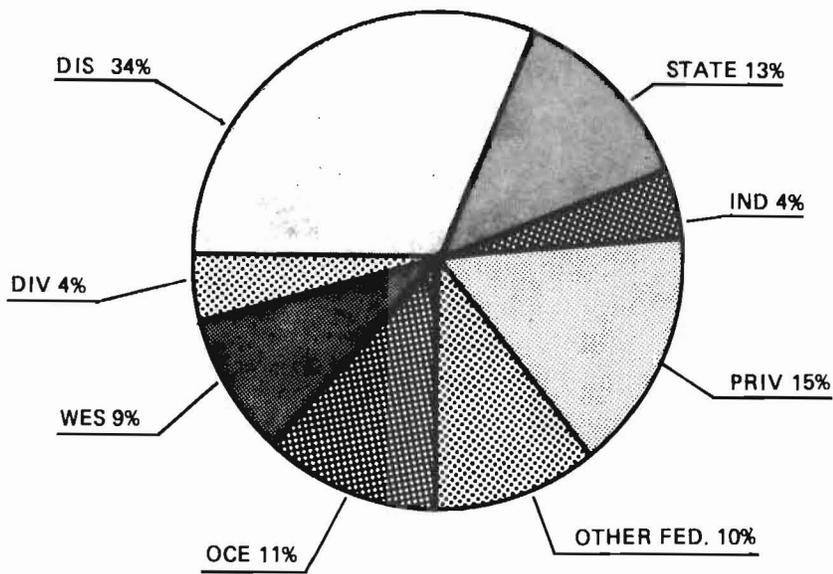


Figure 2. Breakdown of requests for information

# Herbicide Application in Potable Waters

by  
Loren Mason\*

## PUBLIC'S PERSPECTIVE

While the use of chemicals for weed control dates back to ancient times, the modern use of chemicals started in Germany about 1850. The first chemicals used were lime and salt. From that humble beginning, we have progressed to an arsenal of specific herbicide compounds for controlling undesirable weeds.

Along with the development and use of these chemicals has developed a strong public concern relative to the harmful potential that many of the herbicides pose to the environment. As a result, we must be prepared to defend and justify our control programs to the public no matter how long we have been in the business. Like it or not, all chemical control programs are fair game to anyone at any time; therefore, how well we educate the public and address their concerns will ultimately determine the success of our programs and the public's perceptions of our professional competency. This fact is particularly true when the water body being treated is a high quality potable water source.

## COORDINATION CONSIDERATIONS

The following actions are suggested as minimum steps in preparing for a chemical control program in potable waters:

- a. Identify the plant species causing the problem.
  - Identify the problem species.
  - Identify all anticipated problems related to the species (project purposes, health and safety, taste and odors, etc.).
  - Review the range of solutions.
  - Choose a tactic to address the problem.
  - Choose a third party to monitor and evaluate the control program.
  - Choose a third party to apply the chemical.
- b. Identify the Federal, State, and local interest involved with the water body and regulatory interest.
  - Conduct coordination meetings with agencies first.
  - Develop a time table for implementing the control plan.
  - Compile list and notify agencies and other interested parties by letter.
  - Notify Congressional representatives of need to control and prior planning with regulatory agencies.
  - Issue a series of news releases prior to treatment date to allow public response.

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

- Conduct public meeting when necessary to answer questions by using a neutral controlling Government body such as a City Hall meeting or State agency commission meeting.
  - Attend meetings with specific groups who object to program.
  - Always make yourself available to the press.
  - Remember be open and cooperative but firm and committed.
- c.* Implement Control Program.
- Conduct program according to the approved plan.
  - Document, document, document.
  - Follow up with letters and news release.
- d.* Start preparation for next year's program.

# Pesticides and Endangered Species

by  
Michael Dupes\*

The Endangered Species Act (ESA) of 1973 requires that all federal agencies (e.g., the Environmental Protection Agency) ensure that agency actions will not jeopardize the existence of any endangered species or their habitats. The ESA is administered by the Fish and Wildlife Service (FWS) which determines whether a species should be listed as endangered, threatened, or of special concern. The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) of 1972 requires that all pesticides be registered and is designed to minimize their environmental impacts. FIFRA is administered by the Environmental Protection Agency (EPA).

A consultation process, conducted by the EPA, screens the product environmental data to determine if a potential pesticide registration will impact (effect) an endangered species or its habitat. If a "may effect" determination is made, a formal consultation request is made to the FWS which then conducts a formal jeopardy assessment for use of that pesticide in the range of the species. If jeopardy to the species exists, the EPA cannot register that use until the jeopardy opinion is lifted.

In 1980, the FWS first proposed to institute a new plan designed to protect endangered species and their habitats. In response, the EPA initiated consultation in 1982 with the FWS using groupings or "clusters" of pesticides. A cluster consists of those pesticides registered for a similar use. Clusters examined in FY 1986 included Forestry, Mosquito Larvicides, Pasture/Rangeland, and Cropland (corn, cotton, soybean, sorghum, and small grains). These four clusters were selected because the majority of previous jeopardy opinions received were for pesticides associated with these uses. These clusters have been completed by the EPA and have received formal consultations. In the Forestry Cluster, 24 pesticides received jeopardy opinions with 58 endangered species considered jeopardized; the Mosquito Larvicide Cluster, 9 pesticides with 78 endangered species jeopardized; the Rangeland Cluster, 33 pesticides with 182 endangered species jeopardized; and the Crop Cluster, 68 pesticides with 45 endangered species jeopardized. Clusters in preparation include Non-Cropland, Aquatics, Alfalfa, and Rice.

To date, the EPA has received jeopardy opinions on about 250 species that occur in approximately 900 of the 1600 counties throughout the country. This figure includes clusters that have not yet been completed.

For each product containing an active ingredient on which a jeopardy opinion has been issued for an endangered animal species, a county-by-county list must appear on the product label. A supplemental, county-specific Information Bulletin must be obtained by the user prior to use. This Information Bulletin will be readily available

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

from county extension agents, farm supply stores, other outlets as appropriate (e.g. state conservation agencies), or direct mail in some cases. For active ingredients determined to cause jeopardy to endangered plant species, counties must also be listed on the product label, but users in those counties must contact the USFWS prior to use. This requirement is necessary to protect specific location(s) of endangered plants, which might lead to extinction by overzealous collectors if publicized. (Note that a pesticide may be used in a labeled county if it is used outside the range of the endangered species).

EPA anticipated implementing the plan for the first four clusters on February 1, 1988. However, the number and types of comments received from State and Federal agencies concerning the accuracy of EPA's proposed range maps will likely delay that date. In addition, the EPA is allowing States to implement their own plans. California, Florida, Georgia, and New Mexico have committed to formulate State plans.

The aquatics cluster is currently being developed, and a draft biological opinion has been written by the FWS. Of the more than 30 herbicides labeled for aquatics which EPA has requested an opinion from the FWS, all have been listed as jeopardizing an endangered species. In Florida, every county has been targeted primarily due to the wood stork. According to the draft biological opinion, 114 species have been listed as being jeopardized by herbicides in the aquatics cluster.

In conclusion, it appears that the current draft biological opinion indicates a misunderstanding of herbicide selectivity and aquatic plant management techniques. The drafters of the opinion should be aware of the variety of techniques and herbicides used which allow for the selective control of nuisance aquatic vegetation with minimal impacts to fish and wildlife habitats. I recommend that aquatic plant managers in the Corps of Engineers and their cooperators keep up-to-date on the development of the aquatics cluster and request input into the FWS and the EPA review process.

# Review of Current Aquatic Plant Management Activities in British Columbia

by  
P.R. Newroth\*

## EXECUTIVE SUMMARY

The 1987 British Columbia aquatic plant management program included treatments of 13 lakes to control Eurasian watermilfoil and one lake to control native aquatic plants. Six cost-share agreements between local agencies and the British Columbia Ministry of Environment and Parks permitted implementation at a operational cost of about \$465,000 (25 percent local contribution, 75 percent Provincial contribution). Control equipment and technical guidance for control projects were provided by Ministry staff, in addition to surveillance and public information activities. The total area treated was about 264 ha (114 ha by harvesting, 103 ha by rototilling, 31 ha by cultivating, 2 ha by bottom barrier applications, and 14 ha by diver-operated dredging).

## INTRODUCTION

Aquatic plant management in British Columbia, Canada, is focussed on control of Eurasian watermilfoil (*Myriophyllum spicatum* L.), as this species has become a major nuisance since introduction about 1970. This report summarizes British Columbia Ministry of Environment and Parks aquatic plant management activities in 1987. Reviews of the management program from 1972 to 1985 were presented in Information Bulletin Volume XII issued by the B.C. Ministry in 1986 and Newroth (1981, 1986a, 1986b).

Aquatic plant management has been implemented primarily by the Provincial Government in British Columbia waters. Private contractors offering control services have seldom been available, probably because of low demand for their services due to factors such as relatively limited conflicts of aquatic vegetation with recreational use, limited funding for control programs, and the presence of the Ministry of Environment and Parks aquatic plant management program. This program has incorporated a technical advisory role with provision of province-wide surveillance, aquatic plant documentation, and development and testing of new control technologies and major control equipment (five harvesters, two rototillers, three diver-operated dredges, one amphibious cultivator and support equipment). Since about 1980, nearly all programs have been implemented under Ministry guidance and with cost-sharing (75 percent Provincial, 25 percent local) to perform control requested by local authorities.

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\*Ministry of Environment and Parks, Victoria, British Columbia.

## REVIEW OF COST-SHARED CONTROL PROJECTS

Table 1 lists seven aquatic plant control projects implemented in 14 lakes under cost-sharing in 1987. The target of control activities was Eurasian watermilfoil in all projects except for Swan Lake. A broad spectrum of control projects was implemented ranging from cosmetic harvesting (Swan Lake) to semi-intensive control (Shuswap Lake) and to intensive control (Christina and Champion Lakes). Objectives and results of each project are presented in the following paragraphs.

Table 1  
Cost-Shared Aquatic Plant Control Projects Implemented in 1987

<i>Location</i>	<i>Surface/ Littoral Area (ha)</i>	<i>Approximate Area Affected (ha)</i>	<i>Method and Area Treated (ha)</i>	<i>Approximate Cost of Control*</i>
1. Okanagan Valley (7 interconnected mainstem lakes and the Okanagan River)	42,308/3,024	1,000	Harvesting — 103** Rototilling — 72.1 Cultivating — 8.2 Bottom barrier — 0.2 Total — 183.5	\$220,000
2. Shuswap Lake (incl. Shuswap River and Mara Lake)	31,000/1,400	133	Rototilling — 21 Cultivating — 23 Bottom Barrier — 1.2 Diver dredging — 14 Total — 59.2	\$160,000
3. Cultus Lake	627/37	20	Rototilling — 6.6	\$ 19,000
4. Long Lake, Vancouver Island	43/18	16	Rototilling — 3.5	\$ 12,000
5. Christina Lake	1,608/51	0.2	All known colonies treated with bottom barrier or diver—hand removal (0.3 ha)	\$ 40,000
6. Champion Lake	12/3.8	0.1	All known colonies treated by divers— (hand removal)	\$ 4,000
7. Swan Lake	600/200	25	Harvesting — 11	\$ 10,000

\*Control costs do not include costs of Provincial monitoring or administration, or capital equipment costs, and include 25 percent local, 75 percent Provincial Government funding.

\*\*Includes about 6 ha harvested in the Okanagan River at 100 percent Provincial cost.

### Okanagan Valley

Operational Eurasian watermilfoil control programs have been performed annually in Okanagan Valley lakes since 1972; the 1987 treatments were similar in scale to those performed since 1979. Area treated by root tillage methods (cultivation and rototilling) has increased since 1982, with some reduction in area harvested (see Maxnuk 1986, for details on tillage control methods). While substantial areas of seven lakes affected by Eurasian watermilfoil cannot be treated due to control resource limitations, nuisance populations are controlled in important public beach and marina areas, as determined by local priorities. Harvesting can be performed only when growing vegetation is nearing the water surface (June-September), while tillage may be employed in October, November, and February through April, weather permitting. Tillage methods provide

more long lasting benefit, and since most plant parts are removed, this treatment is preferred in high public use areas.

Control strategies vary from lake to lake; a semi-intensive approach combining diver dredging, rototilling, and bottom barrier applications is employed only in Kalamalka Lake where Eurasian watermilfoil is relatively sparse. In the other six mainstem Okanagan Valley lakes (Ellison, Wood, Okanagan, Skaha, Vaseux, and Osoyoos) cosmetic harvesting is combined with root tillage methods and effectively reduces the most critical nuisance problems. Priorization of the areas to be treated each year is the responsibility of the local, cost-sharing agencies. Since only about 18 percent of the affected area can be treated with available equipment and funding, the control program remains unsatisfactory to some local users.

### **Shuswap Lake**

Eurasian watermilfoil was first found in Shuswap Lake in 1981 and has shown rapid expansion with the affected area doubling nearly every year. In 1987, newly established colonies were found in the Shuswap River and Mara Lake, upstream of Shuswap Lake. The focus of control efforts (up to 1987) has been to reduce the rate of spread, while preventing the development of nuisance conditions. By using intensive management techniques such as diver-operated dredges and bottom barrier applications in selected areas, large Eurasian watermilfoil colonies have been prevented from encroaching in some parts of this lake system.

Unfortunately, the spread of viable fragments by currents and movement by boaters from infested areas to more remote, uninfested areas has continued. Now the affected area is substantially larger than that which is practical for annual treatment with existing financial and equipment resources; the control strategy has been modified to limit intensive control methods (diver dredging, bottom barriers) to selected areas. Semi-intensive control methods (root tillage) will be applied to alleviate nuisance conditions only in areas of high public use, and harvesting may be employed in 1988 (for the first time) in Shuswap Lake sites where navigation might be impeded by surfacing Eurasian watermilfoil colonies. Since there is considerable potential for major expansion of Eurasian watermilfoil in the Shuswap system, the use of relatively costly, preventive control methods is still warranted in specific areas. Local interests strongly support the maximum control effort combined with action to reduce further spread wherever possible.

### **Cultus Lake**

Eurasian watermilfoil was first found in Cultus Lake in 1977; probably this plant was introduced by boaters or waterskiers travelling from the Okanagan Valley. Control projects in this valuable recreational lake (over one million user beach-days each year) initially included diver-operated dredging, but the rapid rate of Eurasian watermilfoil expansion led to discontinuation of this approach in 1981 (Truelson 1986). About one-half of the small littoral area is affected by this plant, including public beach areas and some shore spawning areas utilized by sockeye salmon. The highest priority nuisance areas now extend to about 8 ha of littoral zone, and each year retreatment of about 4 ha is required. The remaining area is treated every second year.

Bottom barrier materials have been used in swimming areas since 1981, and about 0.5 ha is now effectively controlled by this method; annual barrier cleaning by divers is required to remove plant fragments. Rototilling has become the main control method, despite gravelly substrates, following removal of obstacles (sunken logs) from some management areas.

The Cultus Lake program has become routine, with a gradual increase in the size of affected areas, treatment costs, and management areas. Because of the limited littoral zone, a larger scale program is not required and the major concerns about adverse impacts of aquatic weeds on public recreation areas have been alleviated.

### **Long Lake**

The identification in 1985 of Eurasian watermilfoil from a number of private ponds, the Cowichan River, and in Long Lake at Nanaimo led to increased control and preventive public information efforts on Vancouver Island. Cost-shared control was first implemented in Long Lake in cooperation with the City of Nanaimo in February-March, 1986, and was repeated in January-February, 1987. While the area affected by Eurasian watermilfoil increased from about 13 ha to about 16 ha during this interval, only about 3.5 ha was rototilled during each of these projects.

Intensive control methods were not considered for Long Lake, because Eurasian watermilfoil was well established in most littoral areas by the time of its discovery. Nuisance impacts are largely prevented since the main public use areas are effectively treated and because most areas infested have not yet developed dense surfacing populations. An expanded control program may be required in the future if more dense growth occurs and as urban development increases around the shoreline. Since boater use of this lake is restricted by absence of boat launching ramps for trailered boats, there is a reduced risk that plant fragments will be spread to other uninfested lakes by boat movement.

### **Christina Lake**

Eurasian watermilfoil surveillance was performed annually in Christina Lake since 1977; the first populations of this plant were located on June 18, 1986, growing scattered in a marina, occupying a 700-m<sup>2</sup> area. Introduction of this plant probably resulted in about 1985 from fragments transported on a trailered boat from the Okanagan Valley lakes. Intensive scuba surveys in 1986 located several small infestations, and all Eurasian watermilfoil plants found were removed by hand or covered by Texel bottom barrier material. This work was performed by Ministry staff at 100 percent cost to the Province.

In 1987, under a cost-shared control program with the Kootenay Boundary Regional District, a three-man scuba diver team was hired to provide intensive control of Eurasian watermilfoil in Christina Lake, with periodic assistance from Ministry staff. This plant was found in 21 sites, all of which were controlled by hand removal and bottom barrier applications. About 0.3 ha was treated, and repetitive inspections of all shoreline areas ensured that control was maintained.

Because of the recreational value of this lake, strong local support and the need to prevent spread from Christina Lake to many nearby uninfested lakes, a high priority has

been placed on this control program. A number of factors determined the choice of an intensive control strategy in Christina Lake. Water quality is excellent and transparency is high, so that diver surveys are not impeded by poor visibility. Also, the littoral area is relatively limited and the lake perimeter (44 km) is a practical length, permitting intensive surveys in a short time period. Unfortunately, rapid Eurasian watermilfoil growth rates were documented (up to 3 cm per day), presumably because of warm summer lake-water temperatures.

Repeated diver inspections during the June-September period and a late survey in October, 1987, indicated that no major Eurasian watermilfoil populations remained, but that fragments of this plant had become widely distributed around the lake perimeter. The 1987 observations indicated that there was potential for rapid development of nuisance population of Eurasian watermilfoil throughout the recreationally important shorelines of Christina Lake. A similar intensive control program has been recommended in 1988.

### **Champion Lake**

Eurasian watermilfoil was first found in Champion Lake (near Trail) in 1980, and a spot treatment using 2,4-D (BEE) in 1981 was combined with bottom barrier applications in 1982 (Newroth 1986a). This small lake (located in a Provincial park) has been maintained annually with scuba diver surveys and hand removal of the few remaining plants. Control efforts usually require three divers for three or four days to maintain a low level of infestation. In 1987, less Eurasian watermilfoil was found than in earlier years, since sources of viable fragments have been gradually reduced after the initial control projects. This success was due to the integration of herbicide and bottom barrier applications at initial stages of infestation, and the persistence and regularity of subsequent annual intensive control projects, combined with salutary physical characteristics (small lake size, absence of upstream sources of infestation, and high lake-water transparency).

### **Swan Lake**

Eurasian watermilfoil is not known in Swan Lake, but nuisance conditions due to surfacing populations of *Myriophyllum exalbescens* Fern. and *Potamogeton* spp. led to a cost-shared harvesting demonstration project in 1987. Swan Lake is an important recreational resource to the local community, and the harvesting trial was intended to test this technology for control of native aquatic plants in more northerly lakes. Unfortunately, heavy rains and high lake-water levels made harvesting difficult and reduced use of lakeshore recreation facilities adjacent to the harvested area in 1987. Therefore, this trial was not performed under optimum conditions, which may result in local dissatisfaction.

## **OTHER PREVENTIVE EFFORTS AND INVESTIGATIONS**

Ministry staff provide technical advice, monitoring and administration of cost-shared control programs, and investigate a wide variety of complaints about aquatic plant nuisances. In 1987, there were over 20 inquiries on Eurasian watermilfoil and about 40

inquiries related to other species of aquatic plants. For example, *Ceratophyllum* was identified as a problem in an effluent storage reservoir, bottom barrier treatments in public beach areas (Burnaby Lake) were evaluated, and annual surveillance surveys were made in about 30 lakes to check for possible new infestations of Eurasian watermilfoil. Public information materials (boater warning cards at border crossings) were distributed, and some new roadside signs were added to over 300 warning signs discouraging boaters from spreading Eurasian watermilfoil. The movement of large, trailered boats is considered the main mechanism for spread, but movement of aquatic plants by the public for use in private ponds and by contractors moving aquatic plant control equipment also have caused spread of Eurasian watermilfoil (Newroth 1987). Measures to help reduce the opportunities for further spread within British Columbia are being considered.

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# Highlights of the Potomac River and Chesapeake Bay

by  
H. Glenn Earhart\*

The Baltimore District completed the second year of mechanical harvesting as part of its Aquatic Plant Control Program (APCP) in the Potomac River.

The tabulation below illustrates the densities for the Potomac River project area. However, hydrilla has been located in other areas throughout the Chesapeake Bay including the Susquehanna River watershed. In the spring of 1987, the State of Maryland requested the Corps of Engineers to evaluate the potential for an APCP for the entire Chesapeake Bay. A reconnaissance level report was completed in the summer of 1987 indicating that a Federal program was justified for the entire Chesapeake Bay. The Corps of Engineers initiated the procedures to develop the Design Memorandum (DM) at 100 percent Federal cost. However, the Water Resource Development Act of 1986 was implemented which required cost-sharing of the DM. The DM for Chesapeake Bay APCP has been temporarily delayed until Maryland makes a decision on contributing the required local funding.

## Hydrilla Densities for 1982-1987

1982—	10 A—100% Hydrilla
1984—	600 A—100% Hydrilla
1985—	1900 A—100% Hydrilla
1986—	3600 A— 75% Hydrilla
1987—	Estimated 4,200 A—80% Hydrilla
1995—	Estimated 36,000 A—Hydrilla

Our harvesting program got a late start in FY 86 due to the delay in signing the Local Cooperation Agreement (LCA) until late July. In FY 86 we harvested 36 acres (A) out of a proposed 99 A at 16 sites. Densities were measured and averaged between 20 tons/acre (T/A) in Alexandria Waterfront to 100 T/A at Piscataway Creek. Other problems encountered besides the extraordinary densities were problems with transferring plants from the harvester to the shore conveyor due to tidal ranges of up to 2.5 ft. As a result, the harvester or high-speed transport could not get close enough to shore on low water to reach the shore or trailer conveyor.

Our FY 87 program was substantially more successful. We harvested all of the sites prepared in the annual work plan twice as well as some new sites. We started harvesting on 22 June 1987. The first site harvested was in Piscataway Creek which has densities averaging 20 T/A. Cumulatively in 1987, we harvested 52A at 27 sites. Several of the sites proposed in the annual work plan did not require a second cutting. In 1987, densities ranged from a low of 5 T/A at Mt. Vernon area to 39 T/A in the Broad Creek area. We initiated the second cutting 54 days after the first cutting at Piscataway Creek and encountered densities averaging 32 T/A in the same area that 54 days previously was 20 T/A. Our original program hypothesis for the extraordinary densities experiences in FY

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86 was due to our late start. We now feel that may not be the case. We think the Potomac River has ideal growing conditions for hydrilla.

As far as spreading of hydrilla, in FY 86, the hydrilla did not go beyond Marshall Hall and limited itself to the 6-ft depth contour. In FY 87, hydrilla was found in the Indian Head area of Maryland and was commonly found in 8 ft of water this past summer.

Contractually, this past summer was the first year of a three-year requirement contract issued to Friends of the Waterfront, a new company in the harvesting business. The contract provides various pieces of equipment not limited to 21 harvesters, high-speed transport trucks, and several workboats. Our program has several unique requirements. We have numerous sites that rarely exceed harvesting more than 3 A per site. More importantly, suitable unloading sites with satisfactory water depths, access to main roads, and within close proximity to the harvesting sites are at a premium. The contract requires a harvesting rate of 2 A per day. Consequently, a lot of our time is spent mobilizing and demobilizing, transporting, transferring, and unloading harvested plants.

A unique program problem that is occurring is the significant volume of floating hydrilla mats. Hundreds of acres of hydrilla is senescencing within a two- or three-week period and floating down the Potomac River. Mats of hydrilla are collecting around daymarkers making them obscure for boaters. Also, it is not unusual to have mats greater than an acre in size floating down the river. Even the largest boats must make allowances when going up the Potomac and discovering a floating mat in their path.

Our total program in FY 1987 was \$261,000. The Federal contribution was \$130,500 with Maryland contributing \$63,100, Virginia \$58,900, and District of Columbia \$8,500. Actual cost in FY 87 averaged \$1,200/A in Maryland and \$980/A in Virginia. Supervision and inspection costs were about \$300/day. Also included in the program costs were the fixed costs of shoreline surveys, annual work plan development, public information, and transfer. Also, since the Potomac River incorporates Maryland, Virginia, and District of Columbia, the Metropolitan Washington Council of Governments acts as a program coordinator for all three interests and is responsible for easements, rights-of-way, and program coordination.

# Report on Division/District Working Session

by  
William C. Zattau\*

Twenty-nine individuals, representing the Office, Chief of Engineers (OCE), Corps Divisions and Districts, and other Federal, State, and local agencies participated in the second annual Operations Working Session. The session was chaired by Bill Zattau of the Aquatic Plant Control Operations Support Center (APCOSC). Carl Brown, OCE, provided opening comments and challenged the group with several topics for consideration. Topics discussed included:

- How effective are the Corps Districts (or field elements) in communicating with each other? Participants noted that there is a lack of information transfer between Districts and that potentially useful information lies dormant at the field or District level and is rarely circulated. It was mentioned that novel treatment combinations or modifications to existing application methods are often discovered during routine operational activities as are combinations which fail to work. In the future, such field experiments and discoveries will be forwarded to the APCOSC which will be responsible for getting the word out through the APCOSC Information Exchange Bulletin.
- How efficient are operations personnel in implementing newly developed technology? It was suggested that implementation would be enhanced by publication of more user manuals (Instruction Reports) by the Waterways Experiment Station (WES) such as that written in 1981 for the alligatorweed flea beetle. (According to Dr. Al Cofrancesco, WES, a user manual is being written for the waterhyacinth weevils). A participant stated that it would be helpful if WES issued quarterly updates on research in progress, possibly through the WES Information Exchange Bulletin. Such a bulletin would speed information dissemination to field elements. It was suggested that technical transfer efficiency would also be enhanced by increased participation at this meeting by more Corps operational personnel and more State cooperators.
- How effective is the Aquatic Plant Control Research Program (APCRP) in providing new technology to operations personnel? Several participants suggested that publication of supplemental, field-oriented manuals to accompany the Technical Reports would promote technical transfer to the operational elements. Release of incremental information, prior to completion of the entire study, would also be helpful. It was pointed out that a major function of APCRP Review Meeting was to provide such information to the field.
- Should a task force be established to study the effectiveness of the APCRP? Carl Brown, OCE, brought up the subject of whether an Operations Task Force should be established for this purpose. After some discussion, the concept was marginally approved. The direction of the Task Force and chairperson will be determined. Some attendees felt that this working session serves as a defacto Task Force. A participant noted that such a Task Force should take the form of the one recently established to rewrite ER 1130-2-413.

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- Should the working session be continued as an annual affair? All participants were in favor of continuation. The APCOSC was selected to serve as continuing chair for the breakout session and to collect topics for discussion.
- Format of Formal Division/District Presentations. There was little discussion on this topic, so it was assumed that the format initiated this year will continue.
- Electronic Bulletin Board. A participant suggested that the APCOSC investigate establishing an electronic bulletin board for use between the Center, WES, District, and field offices in order to increase communication. This suggestion is being investigated by the Center.

In conclusion, the consensus was that this session should continue on an annual basis and that attendance by more Federal, State, and local cooperators should be encouraged. Since there was no concurrent Research Working Session this year, some attendees had hoped that some of the researchers would have participated in our session. Topics for discussion for next year's Working Session should be forwarded to the APCOSC prior to the APCRP meeting to ensure inclusion in the agenda.

# Autogenic Changes in Sediment Nutrient Availability Effected by Rooted Aquatic Macrophytes

by

J. W. Barko\*, R. L. Chen\*, R. M. Smart\*, and D. G. McFarland\*

## ABSTRACT

Changes in the vertical distribution of sediment redox potential and nutrient availability were examined during the growth of freshwater macrophytes in a greenhouse facility. *Sagittaria latifolia*, an emergent macrophyte species with an extensive root system, markedly affected sediment redox, resulting in a change from reduced to oxidized conditions within six weeks. In contrast, *Hydrilla verticillata*, a submersed macrophyte species with a relatively minor root system, did not appreciably alter sediment redox. Nutrient uptake by both species substantially reduced concentrations of nitrogen and phosphorus in sediments. Decreased concentrations of sediment nutrients appear to have resulted primarily from nutrient uptake by macrophytes rather than from change in sediment redox potential.

## INTRODUCTION

Rooted aquatic macrophytes rely primarily on sediment as a source of Nitrogen (N) and phosphorus (P) (Nichols and Keeney 1976, Barko and Smart 1980). Availability of these and other sediment nutrients to submersed macrophytes depends on physical and chemical characteristics of the sediment and microbial activity in the rhizosphere (Barko and Smart 1986; Barko, Adams, and Clesceri 1986; Gunnison and Barko 1988). These factors, in turn, are affected by the development of macrophyte roots in the sediment profile (Barko et al. 1988).

Aquatic macrophyte roots can influence sediment oxidation-reduction potential (redox) by transporting oxygen produced in shoots to sediment (Tessenow and Baines 1978; Carpenter, Eleser, and Olson 1983; Jaynes and Carpenter 1986). Sediment oxygen demand and the magnitude of oxygen release by plant roots may influence nutrient availability in the sediment. Solubility of many metals in sediment is controlled by sediment redox (Gambrell et al. 1977), sediment pH (Gotoh and Patrick 1974), and metal complex formation (Lindsay 1979). Changes in sediment redox and related factors potentially influence macrophyte growth in aquatic ecosystems by affecting the availability of nutrients. This study was designed to assess the effects of freshwater macrophyte growth on sediment redox and nutrient dynamics under controlled greenhouse conditions.

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## METHODS AND MATERIALS

*Hydrilla verticillata*, a submersed macrophyte, and *Sagittaria latifolia*, an emergent macrophyte, were grown separately in a greenhouse facility (described in Barko and Smart 1981) on fine-textured inorganic sediments obtained from Lake Washington (Washington) and Brown's Lake (Mississippi). These sediments support excellent macrophyte growth in the laboratory and have been used in several previous investigations (e.g. Barko and Smart 1986). The solution in which the plants were grown contained major cations and anions, but lacked N, P, and micronutrients (cf. Smart and Barko 1985). Sediment in this investigation provided the only source of N, P, and micronutrients for macrophyte uptake.

Growth experiments were conducted in large (1200-l) fiberglass tanks under partially controlled environmental conditions. The two species were grown under different light regimes at  $25^{\circ} \pm 1^{\circ}$  C water temperature. Tanks containing unvegetated sediments and sediments planted with *Sagittaria* were exposed to full sunlight within the greenhouse (ca.  $1500 \mu\text{E}/\text{m}^2/\text{s}$  at midday). A 33 percent reduction in sunlight was imposed on tanks containing *Hydrilla* by covering with neutral density shade fabric.

Each species was planted in eighteen 1.5-l containers per sediment type. These containers were filled with sediment to a depth of about 10 cm. Nine additional containers of each sediment type were maintained as unvegetated controls. At two-week intervals, three replicate containers of each species-sediment combination and unvegetated controls were removed from culture tanks for physical, chemical, and dry weight biomass determinations.

Sediment redox profiles were measured using a millivolt meter, with platinum electrodes coupled to a calomel reference electrode. Platinum electrodes were constructed and standardized according to procedures described in Chen and Keeney (1974). Redox profiles were measured in replicate containers with a motor-driven assembly that advanced a platinum electrode at a rate of about two cm/h vertically through the sediment. Strip chart recorders provided a vertically continuous record of sediment redox. In order to assess lateral variability in redox potential, additional determinations were made at fixed depths within a single container per treatment. Five measurements were made at each of two depths (3 and 7 cm) below the water-sediment interface.

Following determination of sediment redox, aboveground biomass was clipped at the sediment surface, oven-dried at  $80^{\circ}\text{C}$  to constant mass and weighed. The sediment from these containers was then sectioned horizontally into two equal sections about 5 cm thick with respect to depth (i.e., upper and lower sediment strata). Three sediment cores (2.54 cm in diameter) were obtained from each section for chemical analyses. The remaining sediment in each container was used for "root" mass determination (which included all belowground structures). One core sample from each sediment container was centrifuged at 10,000 rpm for 20 min to separate interstitial water from sediment. The remaining cores were extracted chemically either with 1 N NaCl (Bremner 1965) or with a dilute acid-fluoride solution (Olson and Dean 1965) to determine concentrations of exchangeable ammonium and available phosphorus, respectively. Hereafter, these fractions are referred to as extractable N and P.

Concentrations of total N and total P in macrophyte shoots were determined following

digestion of plant tissue samples in a mixture of sulfuric acid and hydrogen peroxide (Allen et al. 1974). Analyses of N (as ammonium) and P (as orthophosphate) were performed colorimetrically using Technicon AutoAnalyzer II procedures (Ballinger 1979). Analyses of iron (Fe) in sediment interstitial water were performed by atomic absorption spectrophotometry. Statistical analyses of data were conducted using the Statistical Analysis System (SAS). Analyses were restricted to determinations of means and associated standard deviations, with attention to trends over the six-week study period.

## RESULTS

### Biomass production

*Hydrilla* biomass increased during six weeks from less than 0.2 to approximately 20 g/sediment container on both Lake Washington and Brown's Lake sediments (Figure 1). Shoot biomass comprised most of the total biomass (roots plus shoots) produced in this species; root biomass comprised less than two percent of total plant biomass. Changes in total biomass of *Sagittaria* were also pronounced; biomass in this species increased from 10.2 g/container to approximately 60 g/container on both Brown's Lake and Lake Washington sediments. By the end of the study, root biomass of *Sagittaria* comprised approximately 30 percent of total biomass.

### Redox potential

Redox potential at the sediment-water interface of both sediments (as exemplified in Figure 2) ranged from +250 to +300 mv throughout the experiment. On unvegetated sediments redox decreased sharply from +250 at the sediment surface to -250 mv one cm below the surface; redox remained low (-250 to -300 mv) with increasing sediment depth. A more pronounced decline in redox with depth was measured typically in Lake Washington sediment than in Brown's Lake sediment. Growth of *Hydrilla*, with minimal root mass, did not appreciably affect the vertical distribution of sediment redox over a six-week period of growth (Figure 2). However, growth of *Sagittaria*, with a relatively robust root system, resulted in substantial oxidation of both Washington and Brown's Lake sediments after about four weeks of growth (Figure 2, Table 1).

Only minor lateral variations were observed in redox potential in unvegetated sediments (Table 1). Variations in sediment redox potential due to *Hydrilla* growth were likewise minor. In contrast, growth of *Sagittaria* resulted in relatively large lateral variations in sediment redox potential; these variations increased with both time (weeks) and sediment depth, as sediments became increasingly oxidized.

### Nutrients in interstitial water

Interstitial water N concentrations in both upper and lower strata of unvegetated sediments remained relatively constant throughout the experiment; however, in the lower stratum, N concentrations exceeded those in the upper stratum (Figure 3). Beyond about two weeks of growth, considerable amounts of dissolved N were depleted from the interstitial water of sediments vegetated with both macrophyte species. Initial decreases in dissolved N were greater in sediments vegetated with *Sagittaria* than with *Hydrilla*.

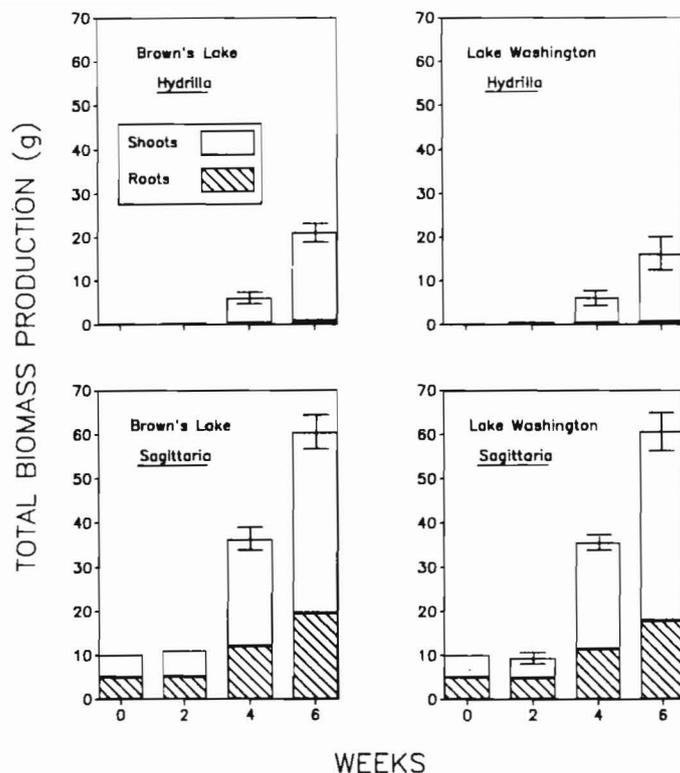


Figure 1. Total dry weight biomass, shoots plus "roots," in *Hydrilla* and *Sagittaria* on Brown's Lake and Lake Washington sediments over an experimental period of six weeks. Vertical bars represent means (n=3) with associated standard deviations

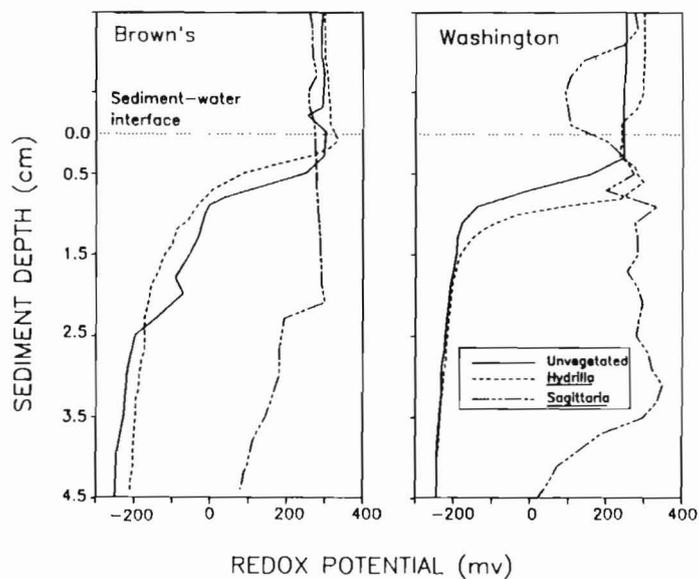


Figure 2. Comparisons of redox potential in unvegetated and vegetated Brown's Lake and Lake Washington sediments after six weeks of *Hydrilla* and *Sagittaria* growth. Vertical profiles of sediment redox provided here are typical of those determined beyond about four weeks of macrophyte growth

**Table 1**  
**Determinations of Redox Potential in Brown's Lake and Lake Washington Sediments at Fixed Depths (3 and 7 cm) Measured During the Growth of Aquatic Macrophytes. Values are Means with Associated Standard Deviations (n=5 for Each Depth)**

<i>Week</i>	<i>Depth</i>	<i>Redox Potential (mv) by Treatment and Sediment</i>		
		<i>Unvegetated</i>	<i>Hydrilla</i>	<i>Sagittaria</i>
<b>Brown's Lake</b>				
2	3	-289 ± 3.5	-297 ± 7.8	-262 ± 10.4
	7	-331 ± 9.3	-342 ± 17.1	-299 ± 8.6
4	3	-340 ± 4.2	-304 ± 6.7	-247 ± 13.3
	7	-337 ± 4.8	-314 ± 5.4	-216 ± 29.1
6	3	-330 ± 8.4	-311 ± 8.5	-177 ± 37.3
	7	-342 ± 10.5	-322 ± 5.6	-134 ± 46.5
<b>Lake Washington</b>				
2	3	-278 ± 11.9	-278 ± 11.2	-214 ± 18.6
	7	-337 ± 22.8	-342 ± 22.9	-247 ± 16.6
4	3	-308 ± 5.1	-273 ± 7.9	-117 ± 13.7
	7	-309 ± 5.1	-282 ± 8.2	-208 ± 25.1
6	3	-328 ± 12.9	-296 ± 21.9	-54 ± 50.3
	7	-348 ± 15.3	-319 ± 16.2	-114 ± 56.4

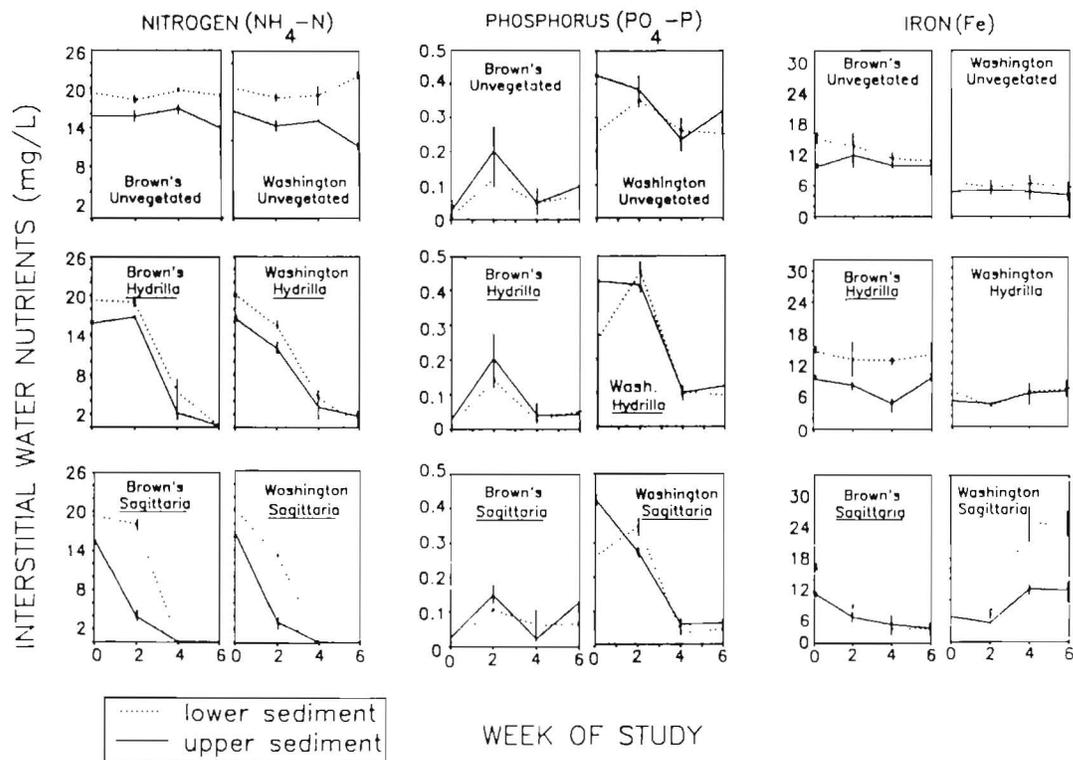
By the end of the study, concentrations of interstitial water N in sediments were essentially exhausted by both species.

Concentrations of dissolved P in the interstitial water of Brown's Lake sediment fluctuated during the experiment, and appeared to be unaffected by the growth of either *Sagittaria* or *Hydrilla* (Figure 3). In contrast, consistent declines in dissolved P concentrations in the interstitial water of both strata occurred in vegetated Lake Washington sediment. In Lake Washington sediment, interstitial water P concentration was reduced from about 0.45 to < 0.10 mg/l by the growth of both species over a six-week period.

Dissolved Fe in the interstitial water of unvegetated sediments and in sediments vegetated with *Hydrilla* remained essentially unchanged during this study (Figure 3). However, in containers vegetated with *Sagittaria*, dissolved Fe declined in Brown's Lake sediment and increased in Lake Washington sediment over the study period. Differences in Fe concentrations between upper and lower sediment strata were minor except in Brown's Lake sediment vegetated with *Hydrilla* and in Washington sediment vegetated with *Sagittaria*, where Fe concentrations were consistently greater in the lower stratum.

### **Extractable sediment nutrients**

Differences in concentrations of extractable N and P between upper and lower sediment strata were statistically insignificant. Therefore, concentration data were pooled across strata (Figure 4). Changes in concentrations of extractable N and P in



**Figure 3.** Changes in concentrations of N, P, and Fe in the interstitial water of vegetated (*Hydrilla* or *Sagittaria*) and unvegetated Brown's Lake and Lake Washington sediments over an experimental period of six weeks. Concentration data are provided for both upper and lower sediment strata. Values are means (n=3) with associated standard deviations

unvegetated sediments were minor throughout the experiment. However, substantial declines in concentrations of extractable N occurred in all vegetated sediments. In contrast, declines in extractable P concentrations, resulting from plant growth were minor, except in Lake Washington sediment vegetated with *Sagittaria*. A minor increase in extractable P occurred between four and six weeks on Lake Washington sediment vegetated with *Sagittaria*. A minor increase in extractable P occurred between four and six weeks on Lake Washington sediment vegetated with *Hydrilla*.

### Distribution of nutrient between macrophytes and sediments

During the investigation, N accumulation in shoots of *Hydrilla* and *Sagittaria* accounted for a progressively increasing portion of measured (plant plus extractable

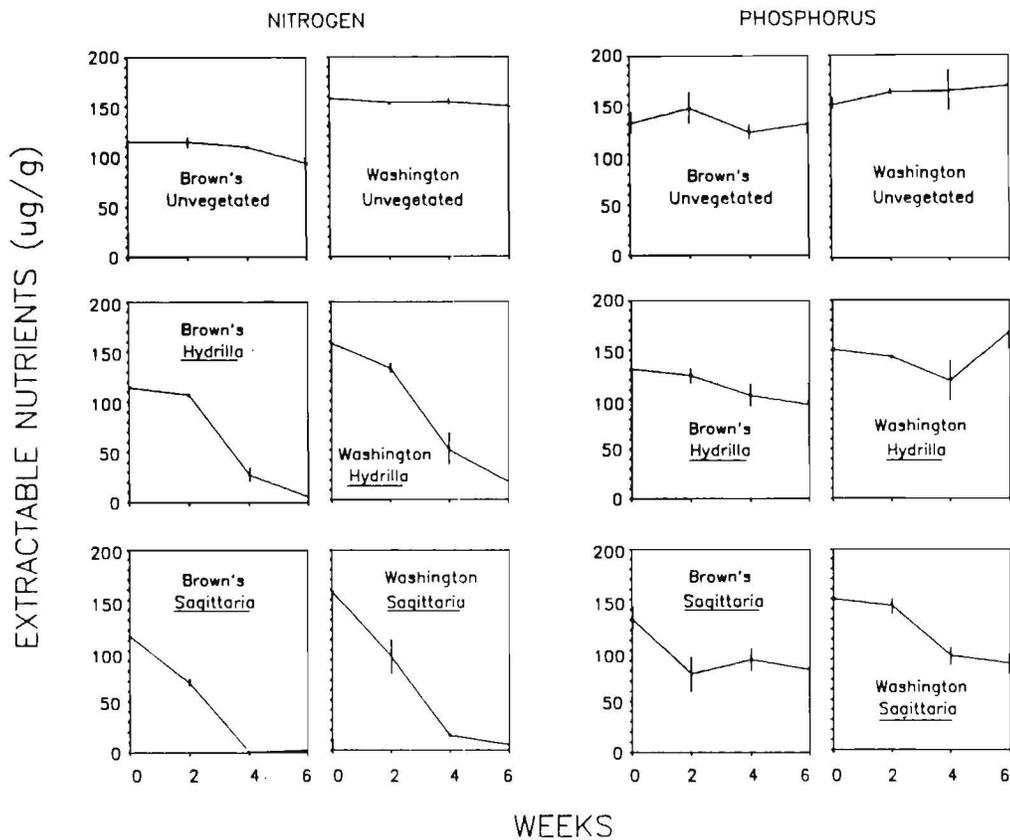
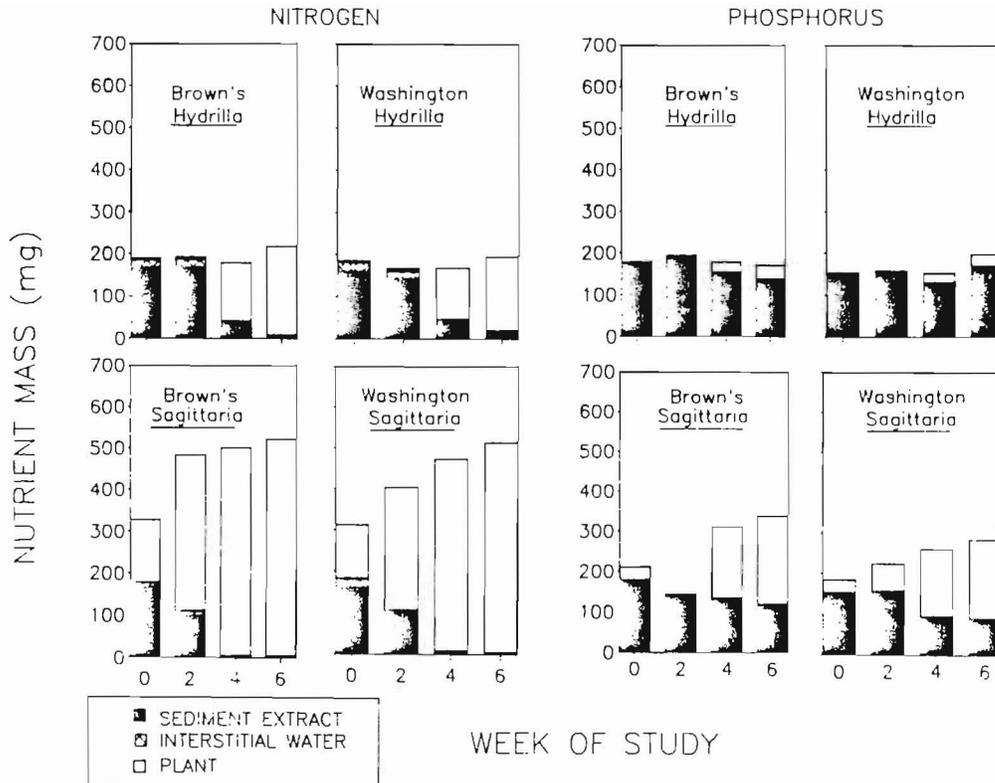


Figure 4. Changes in concentrations of N and P in vegetated (*Hydrilla* or *Sagittaria*) and unvegetated Brown's Lake and Lake Washington sediments over an experimental period of six weeks. Values are means (n=6) with associated standard deviations

sediment) N pools (Figure 5). By six weeks, the N content in shoots of both species individually comprised greater than 90 percent of measured N pools. Accumulation of P in shoots, as a portion of measured P pools, was quite large in *Sagittaria*, but relatively minor in *Hydrilla*. By six weeks, the P content of *Sagittaria* shoots comprised greater than 70 percent of measured P pools, while the P content of *Hydrilla* shoots comprised greater than 20 percent of measured P pools. With N and P in *Sagittaria*, but only N in *Hydrilla*, contents in shoots at six weeks exceeded 100 percent of respective extractable contents of unvegetated sediments. This result suggests sources of nutrient supply (within sediment) additional to those measured by sediment extraction.

## DISCUSSION

Total biomass production in both species, expressed on the basis of sediment surface area (approximately 1.4 kg/m<sup>2</sup> for *Hydrilla* and 4.2 kg/m<sup>2</sup> for *Sagittaria*), was about



**Figure 5. Changes in the distribution of N and P mass between macrophytes and sediments over a six-week growth period. Height of vertical bars represents the sum of extractable nutrient mass (corrected for interstitial water) in sediment and respective nutrient mass in macrophyte shoots**

three times greater than observed in natural environments. Thus, the effects of aquatic vegetation on sediment chemistry reported here probably exceed effects occurring in most natural habitats.

An increase in redox potential of vegetated sediments by *Sagittaria* in the absence of any redox increase in unvegetated sediments suggests that oxygen was transported from shoots to roots and diffused into the sediment. Net transport of oxygen to sediments differs among aquatic macrophyte species (Sand-Jensen, Prahl, and Stockholm 1982), and may be related to the extent of lacunae development (Sculthorpe 1967). A pronounced increase in the depth of the oxidized region of sediments vegetated with the emergent macrophyte, *Sagittaria*, in contrast to negligible effects of the submersed macrophyte, *Hydrilla*, indicates that macrophyte growth form has an important influence on sediment redox status. In part, greater oxidation of sediments by *Sagittaria* may have reflected its greater root mass.

Decreases in extractable N concentrations coincided with increases in N contained in plant shoots, suggesting that changes in extractable N resulted primarily from plant uptake. However, upward diffusion of ammonium and/or oxidation of sediments by macrophytes, facilitating nitrification-denitrification reactions, may have accounted for

some losses of sediment N. Certainly changes in redox potential from reduced to oxidized conditions in sediments vegetated with *Sagittaria* could have established conditions favorable for losses of ammonium-N (Reddy and Patrick 1984).

Sediment oxidation by macrophytes can enhance sediment P retention (Jaynes and Carpenter 1986). Changes in sediment redox potential in combination with reductions in interstitial water P concentration due to plant uptake may greatly affect the equilibrium between sediment interstitial water and sediment, thus altering release rate of P from sediment into the overlying water. Desorption of P from solid phase pools in sediments into the interstitial water may influence P uptake rates of aquatic macrophytes. On Lake Washington sediment, plants had access initially to much greater concentrations of interstitial water P than in Brown's Lake sediment. Nevertheless, most of the P taken up by plants from either sediment in this study appears to have been derived from solid phase extractable pools as also demonstrated by Barko et al. (1988).

Local precipitation of ferric oxides on surfaces of *Spartina* roots was reported by Mendelsohn and Postek (1982). In contrast, we found an increase in dissolved Fe with increasing redox potential in Lake Washington sediment vegetated with *Sagittaria*. Increased dissolved Fe during the growth of *Halodule* in Fe-rich, anaerobic marine sediment was also reported by Pulich (1982). Accumulation of dissolved Fe in Lake Washington sediment planted with *Sagittaria* may have been caused by the formation of metal-organic complexes resulting in dissolution of Fe in vegetated sediments. Alternatively, changes in pH (underdetermined) may have caused increased solubility of Fe in the rhizosphere (Berthelin and Boymond 1978).

In general, far less attention has been given to effects of aquatic macrophyte growth on sediment composition than to effects of sediment composition on macrophyte growth. From this investigation and that of Barko et al. (1988) it is apparent that aquatic macrophytes, even with minor root systems, can promote significant reductions in concentrations of sediment nutrients. Reduced growth has been shown to result from progressive nutrient deficiency imparted by prior nutrient uptake (Barko et al. 1988).

Based upon results of this laboratory study and others referenced herein, it appears that nutrient uptake from sediment by submersed aquatic macrophytes may at times exceed nutrient replenishment in littoral zones. Sedimentation is likely to be the dominant mechanism of nutrient replenishment in most aquatic systems. Therefore, it is reasonable to postulate that reductions in sediment loadings may eventually result in decreased productivity of rooted submersed aquatic vegetation. It is of course also possible that other mechanisms of nutrient replenishment (e.g., diffusion, advection, mineralization, fixation, bioturbation, etc.) in littoral sediments are also operational in maintaining macrophyte productivity. These mechanisms, within the context of aquatic macrophyte nutrition, have been investigated in some detail in marine systems, but have been largely ignored in freshwater systems. The sustained vigor of rooted submersed macrophyte communities will depend, among other factors, on the balance between nutrient losses and gains in littoral sediments. In this regard, it is important to better understand mechanisms affecting this balance.

This investigation reinforces results of earlier studies, conducted in our laboratory, which have indicated strong relationships between sediment composition and aquatic

macrophyte growth. It is now apparent that sediment composition is as much a product of macrophyte growth as it is a delimiter of macrophyte growth. Future investigations of the feasibility of lessening sediment nutrient availability by chemical means, or perhaps by selective macrophyte harvesting and replanting, will be valuable in broadening the scope of aquatic plant management in freshwater systems.

## ACKNOWLEDGEMENTS

Thanks are extended to A. Howell, V.L. Sorgenfrei, J. Conley, and J. Gould of the US Army Engineer Waterways Experiment Station for assistance in performance of greenhouse experiments and chemical analyses. We are grateful to R.R. Twilley and W.D. Taylor for helpful technical suggestions and assistance. Thanks also to M. Emerson and E.C. Rogers for clerical assistance in manuscript preparation. Financial support was provided by the Aquatic Plant Control Research Program, US Army Engineer Waterways Experiment Station.

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# The Value of Submersed Aquatic Plants for Macroinvertebrates

by

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## INTRODUCTION

Aquatic plants influence physical and chemical conditions in lakes, ponds, and rivers, as well as the density and distribution of fishes and macroinvertebrates. Aquatic insects, flatworms, bryozoans, sponges, snails, and *Hydra* use plant stems and leaves for support. When aquatic insects emerge, they frequently rest on exposed leaves and stems before taking flight. Freshwater snails lay eggs on stems, and juvenile and adult snails graze on attached diatoms and other algae (periphyton) that grow submersed plants. The beneficial effects of plants are frequently noted; for example, Bardach (1964) reported that densities of macroinvertebrates were 60 times greater in vegetated as compared with nonvegetated sections of streams.

Although aquatic macrophytes comprise an integral part of freshwater systems, their densities should not become excessive. Colle and Shireman (1980) reported that condition indices of harvestable-sized largemouth bass declined when *Hydrilla* density exceeded 30 percent coverage. Plant control is often needed in lakes, ponds, and rivers when recreation, navigation, or water supply purposes are adversely affected by over abundant vegetation. However, reservoir managers, planners, and engineers can achieve project purposes without negatively affecting habitat value.

The purpose of this paper is to summarize major findings of research on the value of aquatic plants for macroinvertebrates. Field sites are at Eau Galle Reservoir, Wisconsin; at Lake Seminole, Florida; and at a borrow pit near the Mississippi River in Louisiana. The work at Eau Galle Reservoir, initiated in the late summer of 1986, began as an investigation of benthic macroinvertebrates at vegetated and nonvegetated sites. The major findings of this work are summarized in Miller, Beckett, and Blancher (1987). In 1987, the work was expanded to include an investigation of macroinvertebrates associated with *Ceratophyllum* and *Potamogeton*. In the summer of 1987, a similar set of studies was initiated at Lake Seminole, Florida. Sediment samples were collected at vegetated (*Hydrilla* and *Potamogeton*) and nonvegetated sites, and macroinvertebrates on live plants were collected and identified. In 1987, an investigation of macroinvertebrate colonization of artificial plants was begun at Eau Galle Reservoir and in a borrow pit in Louisiana. Artificial plants were placed in the water for a 4- to 6-week colonization period. The purpose of this work was to compare macroinvertebrate community composition and density on live and artificial plants that had nearly identical physical structure.

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An analysis of the effects of aquatic plants on distribution and species composition of fishes at Lake Seminole and Guntersville Lake, Alabama, also part of this program, is summarized by Killgore et al. (1988). Barko et al. (1988) discuss the effects of aquatic plants on water chemistry.

## STUDY AREAS

Eau Galle Reservoir is located about 80 km east of St. Paul, Minnesota. The dominant aquatic macrophytes are *Ceratophyllum demersum* and *Potamogeton pectinatus*, and minor species include *P. nodosus*, *P. foliosus*, and *Najas flexilis* (Filbin and Barko 1985). The surface area is 0.6 km<sup>2</sup>; maximum and mean depth of 9.0 and 3.2 m, respectively (Kennedy 1985). A description of the limnology of Eau Galle Reservoir is in Barko et al. (1984) and Kennedy (1985). Study sites were located in the east cove in water less than 1 m deep (see Miller, Beckett, and Blancher 1987 for a more complete description of these study areas).

Lake Seminole is a 37,500-acre eutrophic lake located on the common border of Florida, Georgia, and Alabama. The lake was created by the closure of the Jim Woodruff Dam on the Apalachicola River downstream of the confluence of the Flint and Chattahoochee rivers. The lake is operated for navigation, electric power generation, and recreation by the US Army Engineer District, Mobile. Sediment samples for macroinvertebrates were collected in a vegetated and nonvegetated site in a section of the lake known as Fishpond Drain that was near Sealey's Island. Live *Potamogeton* and *Hydrilla* (for macroinvertebrates) were also obtained.

The borrow pit is located approximately 10 km south of Delta, Louisiana, on the riverside of the main line levee. It is approximately 0.5 km from the river (at normal stage). The total surface area of the borrow pit is approximately 0.2 km<sup>2</sup>; maximum depth during the study was approximately 3 m.

## METHODS

Individual stems of dominant live plants were collected at all three locations. This collection was done by carefully isolating a plant with a dip net. The plant and any dislodged invertebrates were washed into a jar or plastic bag, preserved, and returned to the laboratory. Macroinvertebrates were individually picked from the plants with the aid of a dissecting microscope.

Plastic *Ceratophyllum* and *Vallisneria* (total stem length 45.7 cm) were obtained from Aquatic Supply House for the field studies. The *Ceratophyllum* is structurally more complex than the latter species. Sixteen artificial plants (each with four stems) were secured to a 0.25-m<sup>2</sup> piece of hardware cloth to create a density of 64 stems/0.25 m<sup>2</sup>. Two pieces of hardware cloth per plant type (i.e. 32 artificial *Ceratophyllum* and 32 *Vallisneria*) were placed in the borrow pit and in Eau Galle Reservoir. At the borrow pit plants were placed at two sites that were 2 m from shore in water that was 1 m deep. Live *Ceratophyllum* covered approximately 90 percent of the littoral substrate. At Eau Galle Reservoir, plants were placed at two sites that were 1-2 m from shore in water 0.5 m deep. Live *Ceratophyllum* covered 60-80 percent of the substrate of the area where artificial plants were placed.

## RESULTS AND DISCUSSION

### Studies in the borrow pit: artificial plants

Significantly greater numbers of macroinvertebrate taxa and individuals were found on live as compared with plastic *Ceratophyllum* in the borrow pit (Table 1). The principal reason for this is probably the result of good availability; i.e., live plants appeared to support a greater growth of periphyton than did the artificial plants. Periphyton is an important food source for snails and other grazers. In addition, it is likely that certain grazers (snails for example) feed on live tissue as well as attached periphyton. Similar numbers of taxa were found on plastic *Vallisneria* and *Ceratophyllum*. However, plastic *Vallisneria* supported about half the number of macroinvertebrates as did artificial *Ceratophyllum*. The latter species, which has many small leaves and branches, provides more sites for macroinvertebrate, and more surface area for periphyton growth than does the comparatively simple *Vallisneria*.

Table 1.  
Mean Numbers of Macroinvertebrate Taxa and Individuals Collected on Live and Artificial Plants in a Borrow Pit in Louisiana, July, 1987.\*

	<i>Taxa</i>	<i>Individuals</i>
Live <i>Ceratophyllum</i>	21.8 (4.4) <sup>a</sup>	267.8 (9.4) <sup>a</sup>
Plastic <i>Ceratophyllum</i>	17.6 (3.5) <sup>b</sup>	159.4 (70.4) <sup>b</sup>
Plastic <i>Vallisneria</i>	18.0 (3.7) <sup>b</sup>	8.38 (20.2) <sup>c</sup>

\*Based on total stem length of 10 cm. Means with the same superscript are not significantly different,  $p > 0.05$ . Standard deviations are in parentheses.

The macroinvertebrate fauna on plastic and live *Ceratophyllum* consisted principally of midges (7 species), mayflies (1 species) and snails (2 species, Figure 1). Total numbers of mayflies and snails were significantly greater on living as compared with artificial plants ( $p < 0.05$ ). This increase was probably due to greater amounts of periphyton on the live as compared to artificial plants. Greater numbers of mayflies were on plastic *Ceratophyllum* as compared with plastic *Vallisneria*, illustrating the potential importance of structural complexity which provides refuge from predators.

Live and plastic plants were colonized principally by periphyton feeders and fewer numbers of collectors and predators (Figure 2). These findings illustrate the value of plants in providing structure; i.e., they are colonized by periphyton. Macroinvertebrates that collect detritus (for example, the caddisflies) were not common.

### Studies at Lake Seminole

Three species of aquatic plants, *Hydrilla verticillata*, *Nymphaea odorata*, and *Potamogeton* sp., were collected at Lake Seminole and examined for invertebrates. Chironomids (diptera) and oligochaetes (naidids) were common-to-abundant on all three plant species; each macrophyte was dominated by a different chironomid taxon. *Psectrocladius* sp. was the most common chironomid on *Hydrilla*, whereas *Ablabesmyia peleensis* and *Thienemanniella* nr. *fusca* were the most abundant chironomids on *Nymphaea* and *Potamogeton*, respectively. Ceratopogonid (diptera) larvae were present

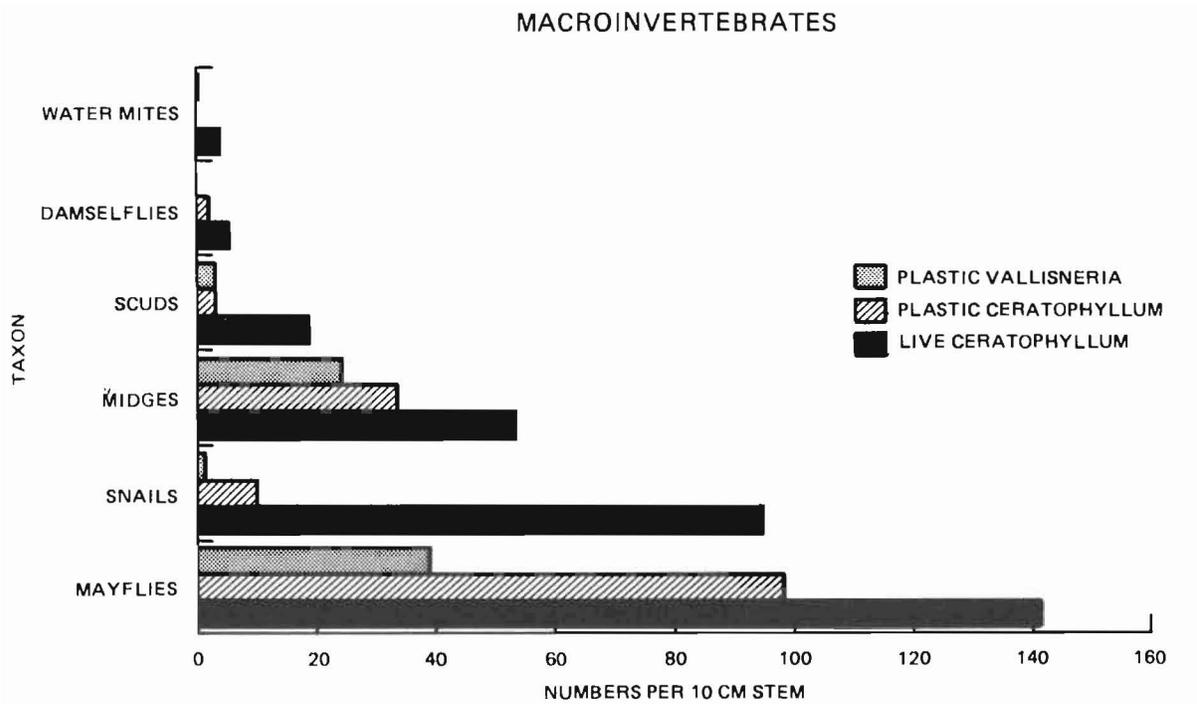


Figure 1. Macroinvertebrates which colonized plastic *Vallisneria*, and live and plastic *Ceratophyllum*

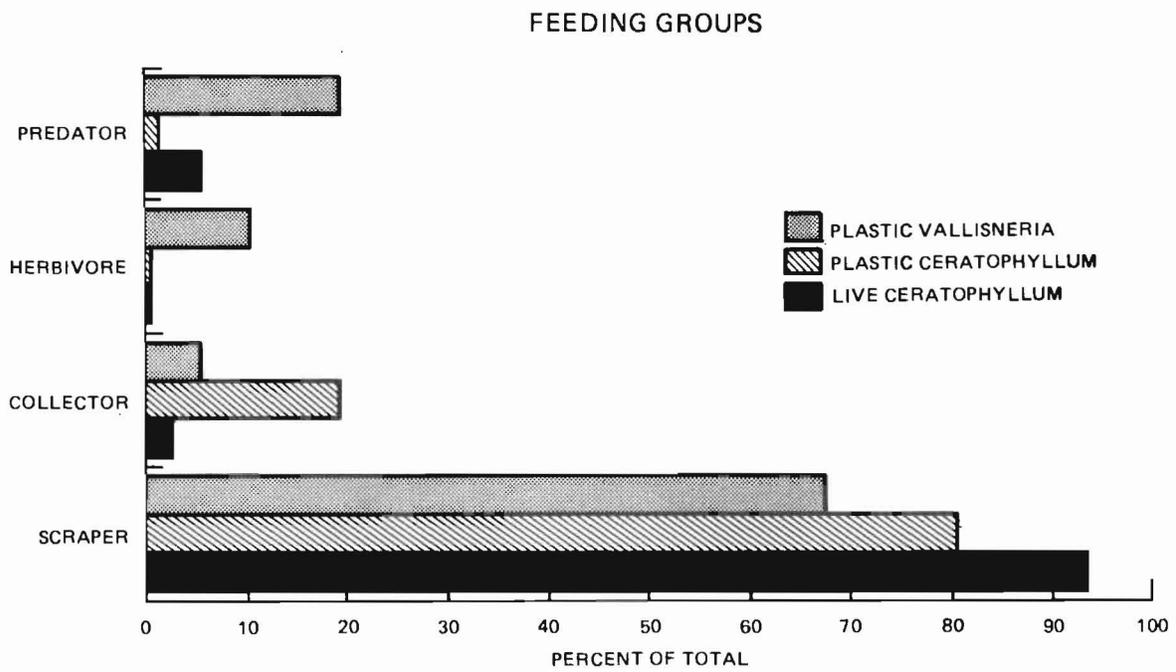


Figure 2. Macroinvertebrate feeding groups found on plastic *Vallisneria* and live and plastic *Ceratophyllum*

on all three plant species. Ninety-five percent of the naidids on *Hydrilla* belonged to a single species, *Allonais pectinata*. *Allonais pectinata*, *Nais variabilis*, and *Pristina leidy* were all common on *Nymphaea* and *Potamogeton*. Larval and adult beetles were collected from both *Hydrilla* and *Nymphaea*. Although the micro-caddisfly *Oxyethira* were present on *Hydrilla* and *Nymphaea*, it was significantly more abundant on *Potamogeton*.

*Hydrilla* densities at the study site in Lake Seminole were estimated at 380 g/m<sup>2</sup>. A single stem of *Hydrilla* weighed about 0.25 g and supported approximately 155 oligochaetes and chironomids (which typically constituted the majority of the macroinvertebrate fauna). It was estimated that each square meter of surface water surface could support up to 200,000 oligochaetes and chironomids. It should be apparent that *Hydrilla* provides substrate for an extensive food base that can be used by terrestrial organisms such as amphibians and birds, as well as fishes and other invertebrates.

### Studies at Eau Galle Reservoir

*Potamogeton nodosus* is a common aquatic plant throughout North America and possesses both floating and submersed leaves. At Eau Galle Reservoir, a diverse assemblage of invertebrates was associated with this species; 53 and 52 invertebrate taxa were collected in June and August, 1987. Composition of the fauna changed markedly between the June and August sampling dates. In June, *Paratanytarsus* sp. and *Thienemanniella* nr. *fusca* were the most common chironomids on the *Potamogeton*, whereas the burrowing larvae *Polypedilum illinoense* and *Endochironomus* sp. were the most abundant chironomid on this macrophyte in August. Likewise, in June the dominant naidid worms on the *Potamogeton* were *Nais pardalis* and *Stylaria lacustris*, whereas in August *Pristina leidy* and the predacious *Chaetogaster diaphanus* were the most common.

*Ceratophyllum demersum* has a worldwide distribution and is the dominant macrophyte in Eau Galle Reservoir. There is a diverse epiphytic fauna on this species which includes *Hydra*, snails, flatworms, amphipods, ostracods, water mites, mayflies, damselflies, caddisflies, lepidopterans, hemipterans, and large numbers of chironomids and oligochaetes. A fairly diverse assemblage of caddisflies were found; six species were present, and *Leptocerus americanus* was present in especially high numbers. *Stylaria lacustris* was the most common worm collected on *Ceratophyllum*; *Nais pardalis* was also present in large numbers.

## SUMMARY

Numerous studies have shown the value of stems and leaves of aquatic plants for macroinvertebrates (Gerking 1962, Nichols 1974, Killgore 1979, Pernak 1971, Minshall 1984, Keast 1984, Ball and Hayne 1952). Most macroinvertebrates do not live free in the water column but require substrate on which they permanently attach or crawl about in search of food. Many macroinvertebrates associated with plants are grazing on periphyton which can colonize both natural and artificial surfaces.

By providing substrate, aquatic vegetation is indirectly responsible for a dense and

diverse macroinvertebrate fauna. These organisms provide food for larval and adult fishes, and ultimately, terrestrial species. Removal of large amounts of vegetation would locally reduce the macroinvertebrate fauna. A well-designed plant management program must balance water supply or recreational needs with the habitat value of aquatic systems.

### Future studies

Studies on the habitat value of aquatic plants during the past two years have illustrated the importance of periphyton as a food source. Studies will be conducted to measure the amount of periphyton on artificial and live plants. Information on periphyton content of various types of plants will improve our understanding of the food base needed by grazing macroinvertebrates.

Artificial plants will be placed in water bodies that are void of vegetation. Results can be contrasted with data obtained at sites with natural vegetation. These studies will enable an analysis of the value of physical structure versus biological property of live plants for macroinvertebrates. It is possible that artificial plants could be used to improve habitat where conditions are not suitable for live vegetation.

Fish larvae are dependent on aquatic plants. In the summer of 1988, larval fishes will be collected at vegetated and nonvegetated sites to investigate the effects of various species of aquatic plants on densities and community composition of immature fishes.

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# Use of Modified Popnets to Estimate Fish Density in Eurasian Watermilfoil

by

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## INTRODUCTION

The density of fish in submersed aquatic vegetation (SAV) is poorly understood because of the lack of effective techniques for sampling fish in these structurally complex habitats. Recently, Serafy, Harrell, and McInish (1988) presented a popnet system capable of sampling fish populations in SAV. Using this system, Killgore, Morgan, and Hurley (1987) estimated fish density values ranging from 18,000 to 100,000 fish per hectare in *Hydrilla* beds at the Potomac River. Although the popnet system described by Serafy, Harrell, and McInish (1988) can be used to obtain estimates of fish density in dense SAV, it is limited to sampling only shallow (< 1.5 m) waters.

A number of modifications have been made to the popnet system in order to deploy these nets in deeper, submerged aquatic vegetation habitats. These nets were subsequently used to evaluate the density and standing crop of fish in Eurasian watermilfoil at Lake Guntersville, Alabama. The purpose of this paper is to describe the popnet modifications including the setting, tripping, and fishing of a popnet by boat, and summarize the fish abundance data collected at Lake Guntersville.

## METHODS

### Description of the popnet

*Net construction.* The net design is essentially the same as proposed by Serafy, Harrell, and McInish (1988) and Killgore, Morgan, and Hurley (1987). The top and bottom of the net is open and attached to a PVC frame (Figure 1). The upper frame (float line) was filled with a buoyant foam, and 1.9-cm rebar was inserted in the lower frame (lead line) to provide sufficient weight to hold the net on the bottom. A number of different depth nets (Figure 1b) varying from 122 to 275 cm were used depending on the water depth selected to set the net and the vegetation type. A major modification was made to the release mechanism in order to set and pop the nets in deeper water from a small boat (Figure 1a). A pin-key system was designed consisting of a 20-cm-long,

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10-mm-diam steel pin which was fastened to the bottom frame. A set of two pins was put on opposite sides of each bottom frame, and corresponding holes were drilled in the upper frame to match up with the pin location. Each pin has a small hole drilled in the end in order to receive a smaller pin (key) which holds the float and lead lines together and acts as the trigger release when pulled from the larger pin. During the setting of nets from the boats, the larger pin is run through the hole in the top frame and the key is inserted through the hole. The key is fabricated from high-strength steel with a loop on one end for attaching a line. Each pair of pins is attached by a line to a large float wrapped with reflecting tape. A small anchor is attached to the float for maintaining float position.

**Setting.** Two boats are required for setting the popnet in deep water. Usually, six nets were assembled onshore and placed on a large boat (>5 m). Once a site had been identified, a second boat was moved up to the bow of the boat loaded with the nets. The net was pinned and used to push the boats away from each other. The net was lowered into the water by holding on to the upper frame and simultaneously released. The man in the bow of each boat handled this step of the procedure, while a second man in each boat was responsible for handling the lines attached to the float and keys. During the setting process, care was always taken to make sure that the excess net streamed towards the outside of the frame in order to reduce possible fouling problems. After the net was dropped, the boats were backed away from the net set, and once the key lines had been extended, the floats and anchor lines were dropped.

The time to set a net was usually 15-30 min depending on the water depth and site selection. Usually, nets were set during midday and allowed to equilibrate until after dusk. After sampling in the evening, the nets were picked up the next day and repositioned for additional sampling in the same general area or moved to other areas. Two men in each boat could easily lift the nets and stack them for transfer back to the shore or to other sampling areas.

**Net popping.** The nets were usually released approximately one to two hr after dark. Net popping was effected by using two boats, each quietly approaching the set popnet from opposite directions. Reflecting tape on the floats facilitated spotting of the net-set at night. The floats were retrieved from the water, and the keys holding the float line to the lead line were simultaneously pulled. Depending on water depth and density of plants, the nets took 3-6 sec to reach the water surface.

**Removing fish from nets.** After the nets had been popped, each boat was positioned on opposite sides of the upper frame and anchored (Figure 1c). Bongi straps were used to hold the upper frame to the side of each boat. A modified seine (Figure 1c and d) was used to remove fish. A weighted piece of PVC tubing was attached to one end of the seine, and braided rope was fixed to the end of this tubing. Using the ropes, the seine was vertically lowered from one boat and pulled across the bottom of the sample plot to the second boat. In the case of dense vegetation, the first pull broke up much of the vegetation which was removed from the net after fish were returned to the net. The Zippin depletion method (Platts, Megahan, and Minshall 1983) using three removals was employed for the estimation of numbers of fish. Past studies on the Potomac River indicated that three depletions were sufficient to estimate fish density with a low standard error. Fish biomass was the sum weight of fishes collected in all removals. Approximately 30 min was required to pop and sample each net.

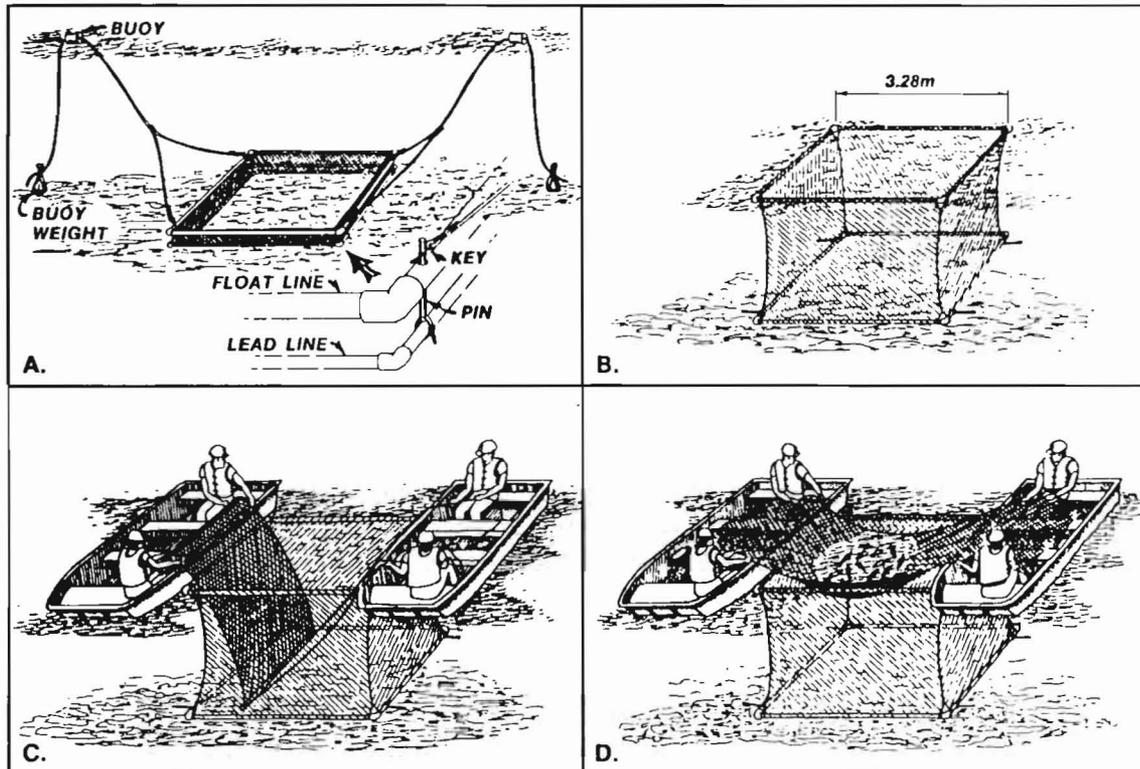


Figure 1. Schematic of the popnet system: A-popnet set on the bottom with the float and lead line attached with a pin/key system; B-popnet fully extended subsequent to release of the floatline; C-two boats positioned on opposite sides of the popnet with the seine lowered to the bottom preparing to remove the fish; and D-fish captured with the seine after being pulled through the popnet

### Field application of popnets

Popnets were used in July and October 1987 to estimate fish density and biomass in a gradient of SAV densities at Lake Guntersville, a run-of-the-river reservoir on the Tennessee River in Northern Alabama. In July, 11 nets were deployed in milfoil beds at Conners Island near river mile 356, and 3 nets were placed in areas with *Chara* at Spring Creek. In October, a total of 10 nets were placed at Mud Creek cove near river mile 394 either in dense milfoil beds or near the edge (abrupt decrease in plant density) of channels created by the application of 2,4-D by TVA personnel. All nets were set in the afternoon and tripped shortly after sundown. Three replicate plant biomass measurements were made at each net. All fish collected were identified, measured, and weighed. Duncan's Multiple Range Test (SAS Institute, Inc. 1985) was used to determine if significant differences ( $P < 0.05$ ) existed in fish density and biomass between the different levels of plant density.

## RESULTS

A total of 11 species were collected with popnets during the study with an average number of 4 species per net (Table 1). The bluegill was the dominant species (85 percent) collected at all sites. Average lengths of fish were similar between nets regardless of plant density and were generally less than 50 mm long.

## RESULTS

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**Table 1**  
Percent Occurrence and Mean ( $\pm$ SD) Lengths of Fish Species Collected with Popnets in or around Eurasian Watermilfoil at Lake Guntersville in July and October, 1987

<i>Species</i>	<i>N</i>	<i>Percent Occurrence</i>	<i>Mean Length, mm (<math>\pm</math>SD)</i>
<i>Lepomis macrochirus</i>	1,739	85.7	34.9 $\pm$ 13.4
<i>Labidesthes sicculus</i>	104	5.1	41.1 $\pm$ 11.8
<i>Lepomis microlophus</i>	80	3.9	42.2 $\pm$ 36.8
<i>Lepomis gulosus</i>	44	2.2	34.2 $\pm$ 5.4
<i>Lepomis megalotis</i>	36	1.8	28.3 $\pm$ 6.9
<i>Micropterus salmoides</i>	12	0.6	93.2 $\pm$ 30.6
<i>Dorosoma cepedianum</i>	6	0.2	32.0 $\pm$ 6.7
<i>Notemigonus crysoleucas</i>	3	0.1	90.0 $\pm$ 48.6
<i>Pomoxis nigromaculatus</i>	3	0.1	103.7 $\pm$ 54.9
<i>Ictalurus melas</i>	1		31.0
<i>Lepomis cyanellus</i>	1		46.0

Data on fish density (number/10 sq m) and biomass (g/10 sq m) were grouped by date (July and October) according to three relative levels of milfoil density (dry weight): low density (20-600 g/sq m), medium density (600-1,000 g/sq m), and high density (>1,000 g/sq m). There was no significant difference in fish density between areas of low, medium, and high milfoil density in either July or October (Table 2). However, fish biomass was significantly higher in areas with low milfoil density during the July sampling period.

**Table 2**  
Summary of the Density and Biomass of Fishes Collected with Popnets in Eurasian Watermilfoil at Lake Guntersville, 1987

<i>Plant Density</i>	<i>N</i>	<i>Fish Density (No./10 sq m)</i>	<i>Fish Biomass (g/10 sq m)</i>
<b>Connors Island-July</b>			
Low	3	236.0 $\pm$ 29.6	370.3 $\pm$ 89.3*
Medium	3	182.3 $\pm$ 32.5	123.1 $\pm$ 29.9
High	2	255.0 $\pm$ 24.0	149.1 $\pm$ 138.4
<b>Mud Creek Cove-October</b>			
Low (treated)	6	66.8 $\pm$ 22.4	63.8 $\pm$ 42.5
Medium	2	121.4 $\pm$ 84.1	92.8 $\pm$ 6.5
High	2	47.0 $\pm$ 9.9	80.2 $\pm$ 29.4

\*P<0.05

In July, popnets set in *Chara* (3 nets) and in milfoil densities less than 20-g/sq m dry weight (1 net) represented areas with sparse SAV and were evaluated separately. Although *Chara* was relatively dense (10- to 475-g/sq m dry weight), it was growing

along the bottom and occupied little of the vertical water column. Fish density ( $87.3 \pm 27.1$ ) and biomass ( $48.4 \pm 15.7$ ) estimates at these sites were substantially lower than the more heavily vegetated areas during the July sampling period.

## DISCUSSION

There are various quantitative methods for sampling fish in vegetated areas including dropnets (Freeman, Greening, and Oliver 1984), electroshocking (Killgore, Morgan, and Hurley 1987), block rotenone (Shireman, Colle, and DuRant 1981), and popnets. The efficiency of electroshocking generally decreases as plant density increases because of the difficulty in locating and collecting stunned fish in dense vegetation (Killgore, Morgan, and Hurley 1987). Shireman, Colle, and DuRant (1987) showed that sampling effectiveness of rotenone applied within small (0.08 ha) blocknets was as accurate as those from large-area (0.41 ha) samples and can be replicated with less manpower requirements. However, pickup efficiency of dead fish also decreases in dense plant beds and can bias the results.

Enclosure traps, such as dropnets and popnets, may have the least source of bias in estimating the density of fish residing in dense plant beds because all fish within the net can be collected or accurately estimated. This technique can also be easily replicated. However, fish biomass estimates may be underestimated because larger fish, such as harvestable-size largemouth bass, can escape a rising (or falling) net more easily than smaller fish, as well as larger fish being more scarce (Jacobsen and Kushlan 1987). Therefore, several techniques may be necessary to sample the entire fish assemblage associated with SAV. Popnets can be used to estimate the density of smaller, more permanent fish residing in dense SAV, while electroshocking can be employed to sample the larger, more mobile fish.

The abundance of fish in milfoil beds at Lake Guntersville follows a basic pattern found in many vegetated habitats; that is, the fish assemblage is dominated by sunfish (*Lepomis*) less than 50 mm in length. Small sunfish may be restricted to the milfoil beds by predation pressure (Gotceitas and Colgan 1987) where the foraging efficiency of larger predators is decreased. However, the results of this study indicate that fish density may not necessarily decrease as plant biomass increases. Fish density estimates taken in areas of dense milfoil ( $>1,000\text{-g/sq m}$  dry weight) were similar to estimates collected in areas with lower plant biomass. These estimates may be due to the canopy formation by milfoil that allows fish easy access to the underlying water column for feeding while maintaining a relatively high degree of protection from predators.

Establishing predictive relationships between SAV and fish abundance is an important step in the successful management of problem aquatic plant populations. Future studies will focus on the quantification of variables other than plant biomass that may influence fish distribution and abundance. Included are spatial variables such as distance of plant bed from unvegetated deeper water, as well as plant species composition, plant morphology (degree of canopy formation near the water surface), and volume of water occupied by the plants.

## ACKNOWLEDGMENTS

The tests described and the resulting data presented herein were obtained from research conducted under the Aquatic Plant Control Research Program of the US Army Engineer Waterways Experiment Station. Many individuals from the Waterways Experiment Station, Tennessee Valley Authority, and Alabama State Game and Fish Commission assisted in modification of the popnet system, collection of the data, and interpretation of results. We thank Leon Bates, Kenneth Conley, Frank Ferguson, Bobby Grinstead, Haywood Gwinner, Jerry Hooper, Bill Host (Pin-Key idea), Philip Kilpatrick, Larry Neal, and David Sample.

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# Effects of Winter Drawdown on Submersed Aquatic Plants in Eau Galle Reservoir, Wisconsin

by  
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## INTRODUCTION

Drawdown has been used in efforts to manage nuisance aquatic plant populations (and other biota) in several waterways (see review by Cooke 1980). In many cases, significant positive results have been achieved, if only temporarily (e.g., Beard 1973, Manning and Sanders 1975, Nichols 1975, Tarver 1980). Generally, biomass is much reduced after drawdown, and there is a shift in dominant species. However, frequently the former dominant populations soon recover, or their replacements develop undesirable standing crops (Eggler and Moore 1961; Harris and Marshall 1963; Tarver 1980; Tazik, Kodrich, and Moore 1982).

Most documented drawdowns for management purposes have been relatively extreme, exposing large areas of lake bottom for long periods of time, six months to over a year, and may even have been repeated in successive years (Beard 1973, Nichols 1975, Goldsby and Sanders 1977). Such drastic measures of plant control, while relatively inexpensive and environmentally innocuous, obviate the multiple-use goals of most actively-managed waterways.

This study reports the effects of a brief lowering of water level in Eau Galle Reservoir, a small flood-control impoundment (0.6 km<sup>2</sup>) in west-central Wisconsin (Pierce and St. Croix Cos.). While this drawdown was for maintenance of the reservoir's outfall structure rather than for plant control, dramatic and desirable changes in submersed plant populations were induced in spite of the small change in water level and brevity of exposure.

## METHODS

### Eau Galle Reservoir

The reservoir has a maximum depth of 9 m, mean depth of 3.2 m, perimeter of 4.0 km, and a shoreline development ratio of 1.5 (Figure 1). Water quality is quite typical of north temperate eutrophic hardwater lakes: average pH is 8.3, alkalinity 164 mg·ℓ<sup>-1</sup> CaCO<sub>3</sub>, conductivity 319 uS·cm<sup>-1</sup>, 1.8 mg·ℓ<sup>-1</sup> total N, and 0.12 mg·ℓ<sup>-1</sup> total P (Kennedy 1985, Kennedy and Gunkel 1988).

There is a wide variety of aquatic plants colonizing the reservoir's littoral zone and adjacent wetland areas. Submersed macrophytes are abundant throughout the shallow regions of the reservoir and periodically have become exceedingly dense in

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the protected bays. Filbin and Barko (1985) monitored the monthly biomass and species composition of submersed macrophytes in Eau Galle Reservoir in 1981 and 1982 by harvesting plants along transects in three littoral areas.

### Macrophyte sampling

Sampling of macrophytes similar to that conducted prior to the drawdown in the winter of 1984-85 was performed during the summer growing seasons of 1985 and 1986 along two of the same transects (A and C in Figure 1) and a new one (E in Figure 1). In the present study, each of three transects extending from the shore toward open water across littoral zone that had been exposed during the drawdown was sampled every 6-8 weeks in the summer, four times in 1985, and three times in 1986. Sampling sites were established at 25-m intervals on the longer Transects A and C and at shorter intervals on Transect E. The transect lines were identical for all seven samplings, but the actual sampling sites were offset 1-2 m each time so that no location was harvested more than once in either year. Three replicate samples were taken at approximately 1.5-m intervals on a line perpendicular to and on each side of the transect line. A 0.5-m square metal quadrat was dropped for each of the six replicate samples at each site; water depth was measured near the center of the quadrat; and all plant biomass above the sediment surface (i.e. standing crop) was collected by hand and put into a plastic bag for return to the laboratory. Quadrats located in water deeper than approximately 75 cm were not sampled.

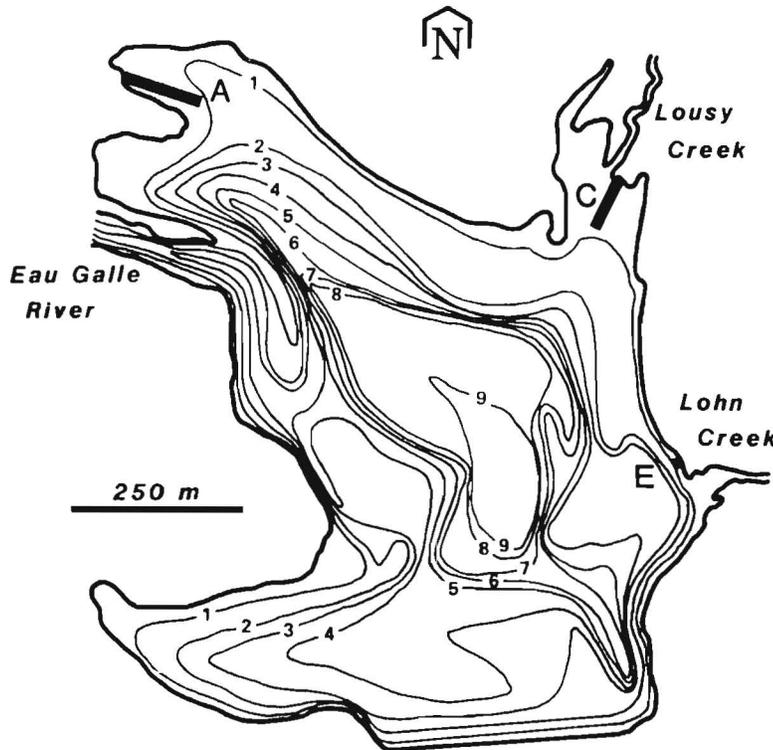


Figure 1. Morphometric map of Eau Galle Reservoir with depth contours (1-m intervals) and plant sampling transects (A, C, E) indicated

The contents of each bag were washed and sorted according to plant species within 24 hr. Tissue was weighed after drying first in air and then in a forced draft oven at 105°C for 12-24 hr. Dried plants were ground in a Wiley mill to pass through a 1-mm mesh, subsampled, and combusted at 550°C for 3 hr for gravimetric determination of inorganic materials (ash, encrusting CaCO<sub>3</sub>, sediment residue, etc.) associated with the biomass. Data reported here represent organic weight of harvested tissue. Data were analyzed using a computerized statistical package (SAS Institute, Inc. 1985).

## RESULTS

### Extent of drawdown

Pool elevation of Eau Galle Reservoir varied during the drawdown (Figure 2) as did air temperature, so there was a wide range of extent to which surface sediments in the shallow littoral zone were exposed to freezing and desiccation (Figure 3). The lowest surface elevation of Eau Galle Reservoir during the winter of 1984-85 was on November 29, 1984, at 285.3 m above MSL, exposing a band of normally submerged sediments varying in width up to several meters (cf. Figure 1). This degree of exposure (i.e., surface elevation < 286.5 m MSL, more than 1 m below normal level) lasted only 7 days. In contrast, only sediments higher than 286.0-m MSL were exposed for over half of the total duration of reduced water levels (October 10, 1984, through February 23, 1985). Furthermore, the lowest air temperatures during the drawdown occurred on days when surface elevation was only about 0.5 m below normal. Thus, the most significant consequences of drawdown on submersed macrophytes susceptible to frost and drying were expected only in a fairly shallow area in the littoral zone much less than 1 m vertically below normal pool elevation.

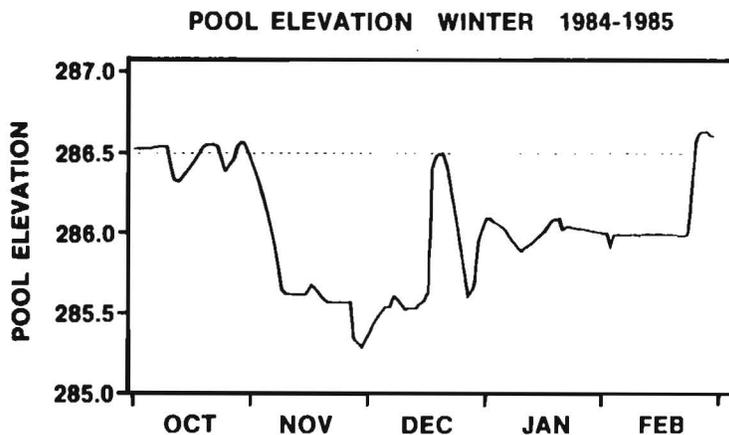


Figure 2. Pool surface elevation of Eau Galle Reservoir during drawdown in winter 1984-85, as meters above mean sea level (MSL). Surface level normally is maintained at approximately 286.5-m MSL by a morning glory outlet structure

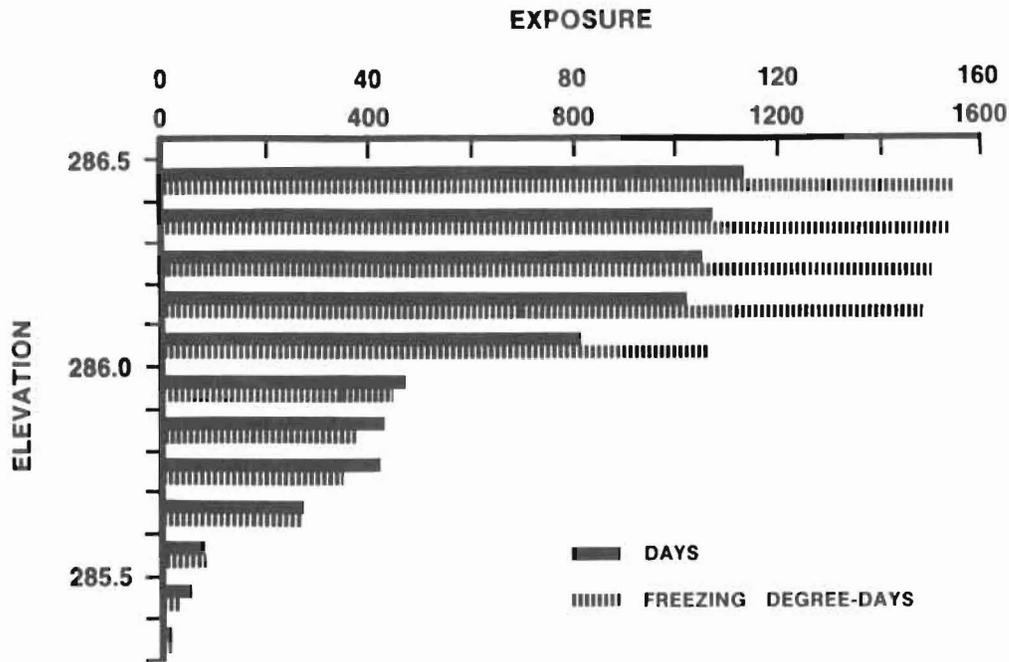


Figure 3. Exposure of littoral sediments to desiccation and freezing during draw-down in days (upper abscissa) and freezing degree-days (lower abscissa) by 0.1-m strata below normal pool elevation

### Effects on community composition

Overall species composition of the communities of submersed macrophytes was not dramatically altered by exposure during drawdown (Table 1). No species were excluded; the two newly found species may have been present before the drawdown. There was much variation in the number of macrophyte species found on the three transects, especially early in the growing season and during the first summer after drawdown (Table 2). More species were encountered in the second summer after exposure. Similarly, the distribution of all species, measured in terms of the proportion of sampled quadrats which contained each species, increased from the summer of 1985 to the summer of 1986 (Table 3). The three rarest species, *Elodea canadensis*, *Heteranthera dubia*, and *Potamogeton pectinatus*, in the season immediately after winter exposure increased their frequency of occurrence more than did the other species by the second growing season in 1986. *Potamogeton nodosus*, one of the dominant species before and after the drawdown, increased its frequency of occurrence less than did all the other species from the first to the second growing season after drawdown.

The Shannon diversity index, incorporating both the number of species found on each transect and the relative abundance of each species, likewise increased from 1985 to 1986 (Table 4). Again, there was considerable variability between transects on any sampling date and among dates within a transect location, but the general trend of increasing diversity from the first to the second season is clear. However, in spite of increasing diversity values from 1985 to 1986, there was also an increase in biomass of nearly all species, and especially for a few species, in the second year.

**Table 1**  
**Species of Submersed Aquatic Macrophytes Encountered**  
**in Transect Sampling (+) and Elsewhere (o) in Eau Galle**  
**Reservoir Before and After Drawdown During Winter of**  
**1984-1985. Data from 1981 Are for Portions of Transects**  
**With Depth <0.8 m Only (Filbin and Barko 1985)**

<i>Species</i>	<i>1981</i>	<i>1985</i>	<i>1986</i>
<i>Potamogeton nodosus</i> Poir.	+	+	+
<i>Potamogeton foliosus</i> Raf.	+	+	+
<i>Potamogeton pectinatus</i> L.	+	+	+
<i>Najas flexilis</i> (Willd.) Rostk. & Schmidt	o	+	+
<i>Elodea canadensis</i> Michx.		+	+
<i>Heteranthera dubia</i> (Jacq.) MacM.		+	+
<i>Ceratophyllum demersum</i> L.	+	+	+

**Table 2**  
**Number of Species Encountered at Each**  
**Time of Sampling on All Transects**

<i>Year</i>	<i>Month</i>	<i>Transect</i>			<i>Mean</i>
		<i>A</i>	<i>C</i>	<i>E</i>	
1985	Jun	5	4	5	4.7
	Jul	6	6	7	6.3
	Aug	3	7	7	5.7
	Sep	6	3	7	5.3
	Mean	5.0	5.0	6.5	5.5
1986	Jun	6	6	7	6.3
	Jul	7	7	7	7.0
	Sep	7	7	7	7.0
	Mean	6.7	6.7	7.0	6.8

**Table 3**  
**Percentage of Sampled Quadrats over All Tran-**  
**sects That Contained Each Species, in Order of**  
**Increasing Change from 1985 to 1986**

<i>Species</i>	<i>1985</i>	<i>1986</i>	<i>Percent Change</i>
<i>Potamogeton nodosus</i>	42.3	54.3	28.3
<i>Najas flexilis</i>	18.8	27.4	45.6
<i>Potamogeton foliosus</i>	25.9	41.5	60.2
<i>Ceratophyllum demersum</i>	58.3	94.2	61.4
<i>Elodea canadensis</i>	17.8	35.4	99.2
<i>Heteranthera dubia</i>	15.4	47.5	207.9
<i>Potamogeton pectinatus</i>	3.7	13.0	252.3

**Table 4**  
**Shannon Diversity Index (D) of Each Transect**  
**at Every Sampling Time (Dmax = 0.85)**

Year	Month	Transect			Mean
		A	C	E	
1985	Jun	0.29	0.20	0.28	0.26
	Jul	0.18	0.46	0.59	0.41
	Aug	0.04	0.35	0.37	0.25
	Sep	0.30	0.31	0.21	0.27
	Mean	0.20	0.33	0.36	0.30
1986	Jun	0.37	0.50	0.43	0.43
	Jul	0.26	0.49	0.61	0.45
	Sep	0.25	0.28	0.43	0.32
	Mean	0.29	0.42	0.49	0.40

### Effects on abundance

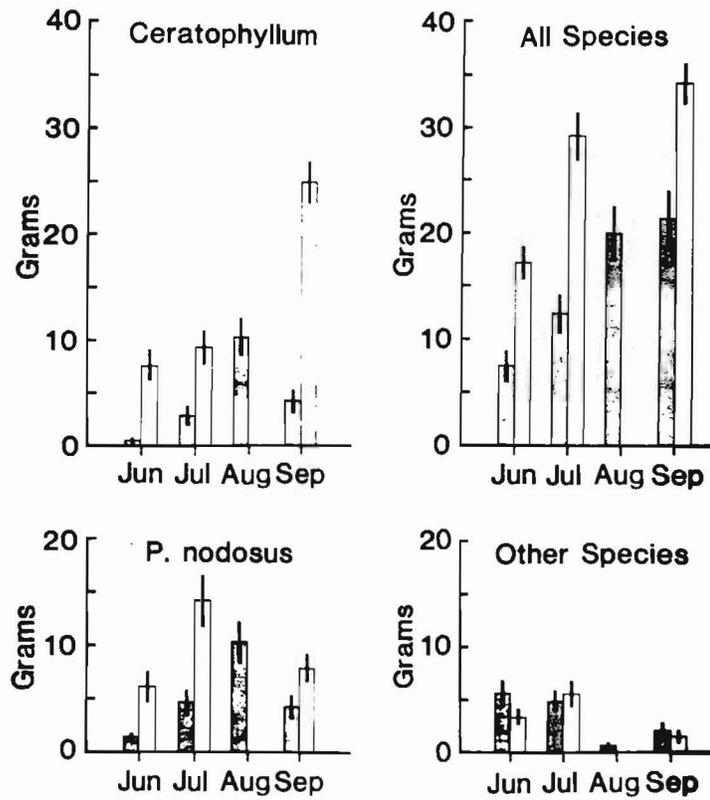
While species composition did not change in response to winter exposure, the relative abundance of each species and the total standing crop did change significantly between 1981 and 1985-86, presumably largely due to effects of exposure during the winter of 1984-85. The extent of colonization of shallow waters by dense beds of submersed macrophytes increased successively in both years after drawdown as well, as determined from aerial photographs. Densely colonized areas were expansive in several regions in 1981 and 1982, virtually excluding recreational visitors from many parts of the reservoir. After drawdown, colonized areas in those same regions were only a fraction as large as before drawdown.

Although areas colonized by submersed macrophytes were significantly smaller after winter exposure, standing crop densities within those smaller areas were substantially less than previously measured densities only in the summer of 1985, immediately after exposure, and not for all species (*Potamogeton nodosus*) as noted in Table 5. By the end of the summer of 1986, standing crop of all species combined was similar to or greater than measurements made in 1981. However, species responded differently. *Ceratophyllum*, while varying greatly between transects, was less abundant for most of each summer after drawdown and reached predrawdown densities only in early fall. *Potamogeton nodosus* increased its density in both years subsequent to drawdown after being virtually absent from previous samples. Species that were sampled before the drawdown (e.g. *Potamogeton pectinatus*) were measured in lower quantities after exposure; several species that were harvested in the postdrawdown samplings were not sampled previously.

Biomass of nearly all species increased significantly from 1985 to 1986 over all months sampled (Figure 4, Table 5). In both years after drawdown, macrophyte biomass was dominated by *Ceratophyllum demersum* and *Potamogeton nodosus*. Early in the summer of 1985, all other species together comprised a majority of the standing crop harvested, but their contribution to peak standing crop by the end of the season was small. The following year, *Ceratophyllum demersum* and *Potamogeton nodosus* increased their dominance with dramatically greater biomass at all sampling times while the rare species showed only similar or even lower standing crops than in the previous year.

**Table 5**  
**Comparison of Biomass (Ash-Free Dry Weight,  $g \cdot m^{-2}$ ) from Areas Common to Transects Sampled Before and After Drawdown in Winter of 1985-86**

Transect	Date	Species			Total
		<i>P. nodosus</i>	<i>Ceratophyllum</i>	Others	
A	Jul 81	0	78.8	1.8	80.6
	Jul 85	26.0	0.7	2.0	28.7
	Jul 86	70.3	18.1	1.8	90.2
C	Jul 81	0	42.6	63.4	106.0
	Jul 85	7.3	13.1	23.9	44.3
	Jul 86	50.0	62.9	23.9	136.8
A	Oct 81	24.4	126.8	13.5	164.7
	Sep 85	25.9	12.2	0.5	38.7
	Sep 86	29.0	102.2	1.4	132.6
C	Oct 81	0	107.1	0	107.1
	Sep 85	-	65.3	24.8	90.1
	Sep 86	24.9	104.9	4.5	134.3



**Figure 4. Average biomass (g ash-free dry weight per quadrat) over all transects at any one sampling time for various species categories comparing standing crop in 1985 (shaded) with that in 1986 (open). Error bars show standard error of the means, n varies from 69 to 78 (see text for further explanation)**

Not all species increased in standing crop from 1985 to the summer of 1986. *Potamogeton foliosus* decreased significantly in abundance from the summer immediately after drawdown to one year later. Other less common species had biomass estimates with great enough variability that their smaller increases or decreases in abundance from 1985 to 1986 were not statistically significant. The overall effect of recovery after drawdown on standing crop of rarer species was therefore negligible (Figure 4).

Trends and variability over sampling time, transect, and distance from shore (i.e. site) of standing crop of *Ceratophyllum demersum*, *Potamogeton nodosus*, and other species combined can be seen in Figure 5. In Transect A (Figure 5), *Potamogeton nodosus* dominated the biomass throughout the first summer, even though it was clearly limited to nearshore Sites 1 and 2. It was plentiful early in both summers and increased its abundance to midsummer. By the middle of the season in 1986, *Ceratophyllum demersum* became more abundant, especially at sites farther from shore and usually concomitantly deeper. Note that in the first summer after drawdown, there was little biomass harvested from the sites more distant from shore; relatively large standing crops of *Ceratophyllum* were collected at Site 4 in September and of other species adjacent to shore during the first sampling in June. In contrast, by 1986 *Ceratophyllum* had increased in biomass at all sites, though only at the end of the growing season, and other species were collected only in relatively small quantities.

Transect C (Figure 5) was similar to Transect A in that *P. nodosus* was abundant in the first summer and confined to the sampling sites nearest shore. *Ceratophyllum demersum* was present at deeper sites in late summer of 1985 and was collected in much greater quantities and at more sites in 1986. In contrast to the situation in Transect A, other species made up the largest portions of biomass at the deeper sites in Transect C, especially early in the growing seasons before those sites were dominated by *Ceratophyllum*.

Transect E (Figure 5) had a similar relative abundance of "other" species at the deeper sites. However, this transect was unique in that sites closest to shore did not always support the greatest standing crop of *Potamogeton nodosus*, and in many samples there were no representatives of this species. This species seemed to persist in its abundance later in the season on this transect than it did on the other transects. In addition, here *Ceratophyllum* was more abundant in 1985 earlier in the season and at the shallowest sites nearshore, than it was on the other transects.

## DISCUSSION

The macrophytes of Eau Galle Reservoir clearly were different in their susceptibility to winter exposure and their ability to recover from it, as noted by other investigators (see Cooke et al. 1986). *Potamogeton nodosus*, normally limited in range to shallow nearshore sites, might be expected to easily tolerate the frequent exposure to freezing and desiccation that is part of natural habitat variability resulting from fluctuating water levels. It is, therefore, not surprising to see that *Potamogeton nodosus* populations were apparently not severely reduced in biomass as a result of drawdown. Similarly, the increase in frequency of occurrence of this species in successive years after winter

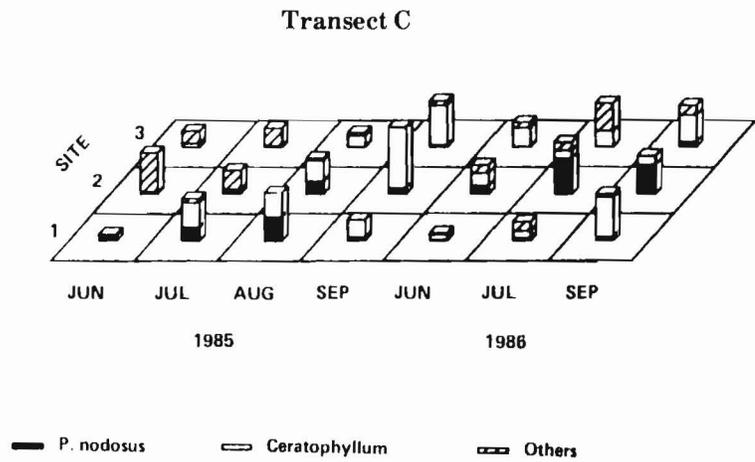
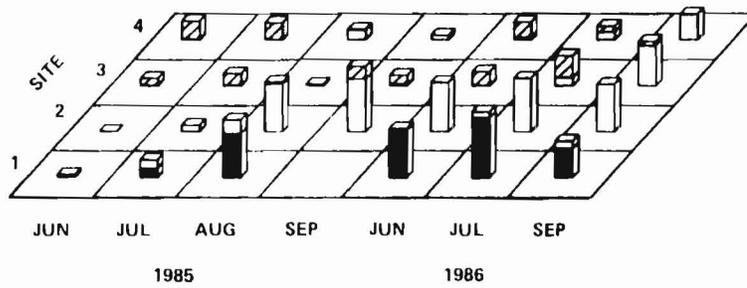
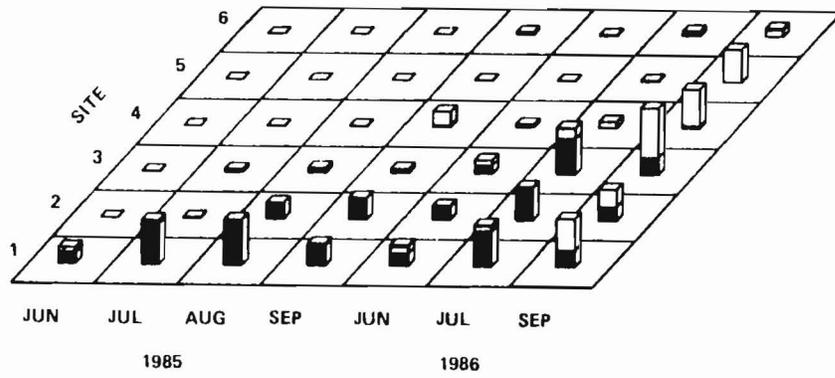


Figure 5. Relative total biomass of various species categories at each sampling site after drawdown on Transect A, Transect C, and Transect E

exposure was slight, implying either that it was poorly adapted to recover from any effects of exposure or alternatively that the population had not actually been excluded from many of the sampling sites, and thus there was little recovery to be made. The former of these possibilities seems unlikely given that *Potamogeton nodosus* was still the second most abundant species after drawdown.

On the other hand, the dominant species, *Ceratophyllum demersum*, was severely diminished in both standing crop and frequency of occurrence after winter exposure. This species has been shown to be particularly sensitive to cold exposure, and its growth is often at least temporarily retarded after reduced water levels (e.g. Beard 1973, Nichols 1975). The impact of freezing and desiccation on the nearshore populations of *Ceratophyllum* has been dramatically evident in the early summer of each year since drawdown in the form of a band of open water between shore and rapidly growing dense beds of *Ceratophyllum* whose plants have already reached the water surface by mid-June. The shoreward edge of the thriving *Ceratophyllum* beds falls approximately on the 1.5-m-depth contour, generally delimiting littoral sediments which were not exposed. Recovery of this species by increasing biomass and expanding distribution may take several growing seasons.

Effects of winter exposure on rarer species in Eau Galle Reservoir are more difficult to predict because of lacking detailed estimates of biomass previous to drawdown. Whether these species were absent from the reservoir in areas previously sampled or simply missed is not known. If they were absent in 1981-82, did they colonize the reservoir before or after the drawdown and its resulting changes to the extant macrophyte community? Measurements from the summers subsequent to drawdown show that these species dominate early in the season, under bright and cool conditions, but they decline by late summer, perhaps as a result of being shaded out by encroaching *Ceratophyllum demersum*. Such conditions of brightness during late spring were suspected by Peltier and Welch (1970) to cause especially prolific growth of *Najas* spp. in years of atypical transparency and cloudlessness. Shading by dominant plants later in the growing season may impose similar constraints to biomass development of the rarer spring species of submersed littoral plants as it does to ground plants in a deciduous forest.

Competitive exclusion may also explain both the appearance of these new species only after winter exposure drastically cut back *Ceratophyllum demersum* and also the decline of some of them (e.g. *Potamogeton foliosus*) in the second year after drawdown as *Ceratophyllum* populations recovered. Similar observations have been made in other waters subjected to externally-induced decimations of dominant species. For example, in another Wisconsin lake, Beard (1973) also observed marked reduction of growth of *Ceratophyllum* (and other species) after winter drawdown and immediate recolonization by *Najas flexilis*, *Megalodonta beckii*, and *Potamogeton diversifolius*. Harvesting and siltation likewise cause shifts in dominant species and greater species diversity (Ham, Wright, and Berrie 1981; Purohit, Singh, and Upreti 1986).

Less common species may be limited to special habitats in the littoral zone, specifically sites near the mouths of cold, spring-fed streams where bottom temperatures were much lower than those of both overlying water and of bottom waters away from stream inlets (e.g. Transect A). Rarer species were always more frequent, and *Ceratophyllum* less dominant, on Transects C and E (Figure 5) at the deeper sites where cold stream

underflow was easily detectable during plant collection. Thus, stream mouths may be refugia for cold-water hearty species, from both excessively high temperatures in summer and freezing in winter.

## CONCLUSIONS

Winter exposure affects communities and populations of aquatic macrophytes as fire and frost affect terrestrial plant communities. Submersed plant productivity usually is maximal under the best conditions of light, i.e. near the water surface, which is precisely the stratum most likely to be severely perturbed by changes in water level. Zones of maximal productivity will be established in shallow waters and marsh with fluctuating water levels (e.g., Robel 1962, Ager and Kerce 1970). When such zones are exposed, differential susceptibility to extreme environmental conditions suddenly and markedly shift species dominance. Differential rates of recovery from damage of the extreme conditions alter competitive advantages, allowing more species to survive, at least until strong dominance by a few species is reestablished. In the meantime, standing crop is reduced and both community and habitat diversity are increased. Such changes undoubtedly affect other organisms in the littoral zone of lakes and reservoirs. For example, fish populations are likely to be more diverse and faster growing when the macrophyte beds they inhabit have a greater array of species and provide increased complexity of microhabitat (e.g. Judd and Taub 1973). Thus, even brief, partial drawdowns of appropriate reservoirs may provide many benefits for management without many of the disadvantages of more drastic manipulations of water levels.

## ACKNOWLEDGEMENTS

The authors appreciate the contributions of several persons in this research. P. Bradley, B. Fulton, Y. Hartz, W. James, R. Kuta, K. Mueller, B. Nelson, S. Nelson, and R. Olewinski, Jr., participated in field sampling and laboratory analyses at the Eau Galle Laboratory in Wisconsin. J. Conley, A. Helmuth, and E. Henderson ashed the tissue in Mississippi. Dr. Donald H. Les, University of Wisconsin—Milwaukee, confirmed the plant identifications. R. Gunkel and J. Carroll assisted with data processing.

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# **Influence of Submersed Macrophytes on Sedimentation Rates In a North-Temperate Reservoir**

by

William F. James\* and John W. Barko\*

## **ABSTRACT**

Sedimentation rates were determined for the vegetated and adjacent nonvegetated areas of Eau Galle Reservoir, Wisconsin, to examine influences of submersed macrophytes on their sedimentary environment. Sedimentation rates were two times greater in the vegetated areas of the lake. Surficial sediments in these areas exhibited a high moisture content and low bulk density, suggesting deposition of predominately fine-grained particles with a high nutrient content. In contrast, sediment in the adjacent nonvegetated area exhibited a low moisture content and high bulk density, characteristics typically associated with poorly sorted sediments located in erosional environments. Submersed macrophytes in this reservoir appear to be reducing erosional forces and/or enhancing sedimentation in the littoral zone.

## **INTRODUCTION**

The filling of a lake basin with sediment is important to the expansion of aquatic macrophytes. Sediment accumulation results in a reduction in water volume and, therefore, a larger area of colonizable sediment exposed to conditions favorable for macrophyte growth. While the watershed is an important source of sediment to a lake basin, the macrophyte community may also play an important role in enhancing sedimentation rates in the littoral zone. Carpenter (1981) demonstrated that macrophytes are an important link in a positive feedback system that accelerates the accretion of colonizable sediment. Macrophyte decomposition products are also an important source to littoral sediments (Godshalk and Wetzel 1977). Macrophytes stabilize their sedimentary environment by reducing turbulence that can resuspend and remove fine particles from the littoral sediments (Madsen and Warncke 1983). Thus, macrophytes can be influential in accelerating the rate of sedimentation within the littoral zone and promoting the expansion of their colonizable area as a lake ages.

Since littoral areas generally occur in turbulent environments, sediment accretion can be negated by erosional forces that act to remove fine particulate sediments to deeper areas of the lake. Hakanson (1977) proposed that sedimentation rates should be low in erosional, high-energy environments such as the littoral zone. Sediments in erosional zones are typically composed of coarse, sandy materials and exhibit a moisture content of 40 to 50 percent. Macrophyte growth can be diminished under these circumstances (Barko and Smart 1986) because sandy sediments are nutritionally poor substrates.

A study was conducted at Eau Galle Reservoir, Wisconsin, to determine net

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sedimentation rates in the vegetated and nonvegetated areas. Variations in the physical characteristics of the surficial sediment were also examined. The purpose of this study was to examine the role macrophytes play in stabilizing their sedimentary environment, promoting sediment accretion, and accelerating their growth potential.

## STUDY SITE AND METHODS

Eau Galle Lake is a small Corps of Engineers impoundment located in west-central Wisconsin. Initially flooded in 1969, the lake is small (0.62 km<sup>2</sup>), circular, and has a mean and maximum depth of 3.2 and 9 m, respectively. The sediment surface area is sinusoidal in shape; therefore, over 60 percent of the sediment surface area is exposed to depths less than 4 m (Figure 1). Steep depth gradients surround the deepest area of the lake between the 4- and 6-m depths. The Eau Galle River provides over 90 percent of the lake's water income and drains a predominately agricultural watershed (166 km<sup>2</sup>). Littoral vegetation, mostly submerged, is extensive around the perimeter of the lake, with densities reaching 500-g/m<sup>2</sup> dry weight in some areas. A more detailed description of the macrophyte vegetation is provided by Filbin and Barko (1985) and Godshalk, Barko, and James (in preparation).

Sediment cores were collected along transects that radiated from the center of the lake to the shoreline (Figure 1). One core was taken at each 50-m interval; additional samples were collected within the zone of steep depth gradients. A core was also collected within 10 m of the shoreline. A Wildco KB Sediment Core Sampler (Wildco Wildlife Supply Co.), provided with a PVC core liner (5.08-cm OD, 4.45-cm ID, and either 50.8 cm or 91.4 cm in length), was used to collect samples. The sampler was dropped from the lake's surface to collect intact sediment samples at depths greater than 3 m. A hand-held attachment was used to collect core samples in shallow areas of the lake. The depth of sample collection was recorded for each station.

Each core sample was sectioned at 5-cm intervals until parent material was reached. Parent material was defined as those soils present before the reservoir was inundated. This material consisted of hard clays, sands, pebbles, and roots and was characterized as having a percent moisture content of < 30 percent. The sediment above the parent materials was that which had settled during the lake's existence. Each section was weighed immediately, then dried to a constant dry weight at 105°C. Sedimentation rates (kg/m<sup>2</sup>/year) were calculated as the mass of sediment dry weight above parent material divided by the area of the core tube and 17 years (reservoir age). The percent moisture content and bulk density of the surficial core section (0-5 cm) were determined according to Hakanson (1977) and Barko and Smart (1986).

## RESULTS

Pronounced net sedimentation rate variations were apparent within the lake (Figure 2). Net sedimentation rates were greatest in the deep, central area of the lake (> 6 m) and declined with both decreasing depth and decreasing distance from the shoreline. An area of steep sedimentation gradients was observed between the 4- and

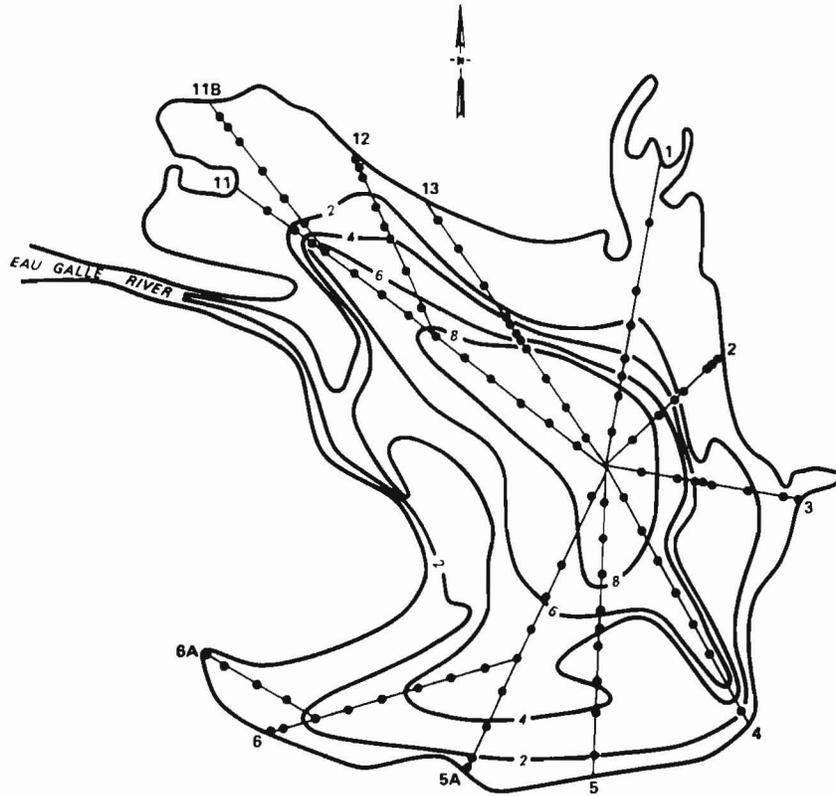


Figure 1. Morphometric map of Eau Galle Lake with transect and sampling locations

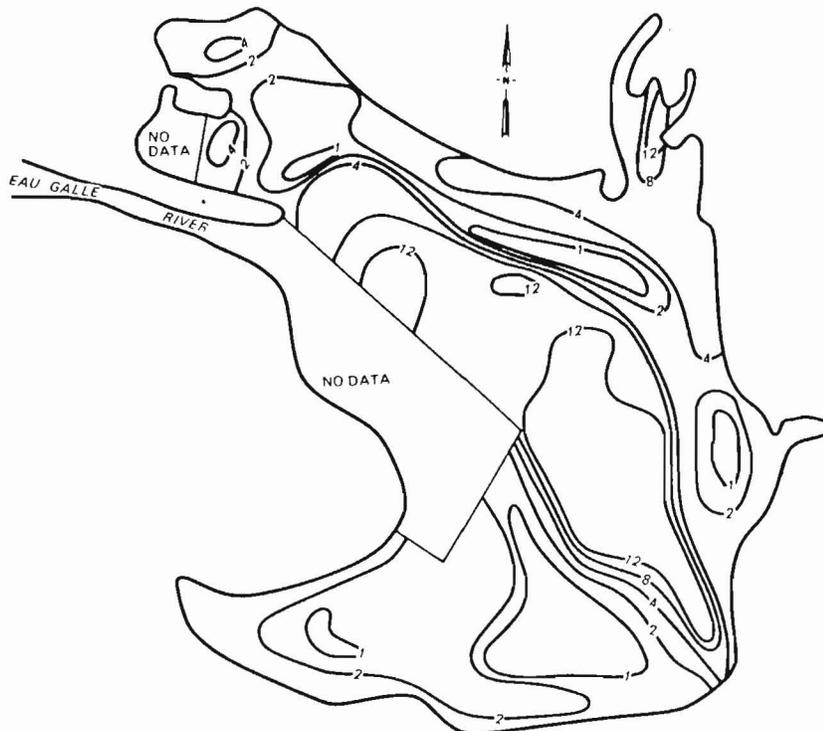
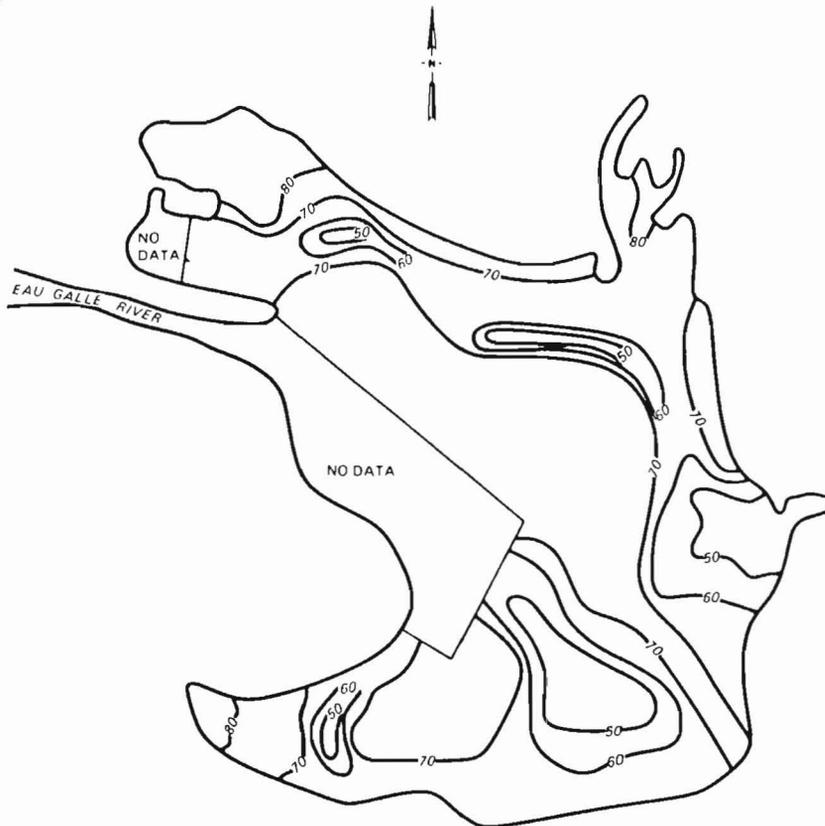


Figure 2. Contour map of spatial variations in the net sedimentation rate. Each contour represents a sedimentation rate in units of  $\text{kg/m}^2/\text{year}$

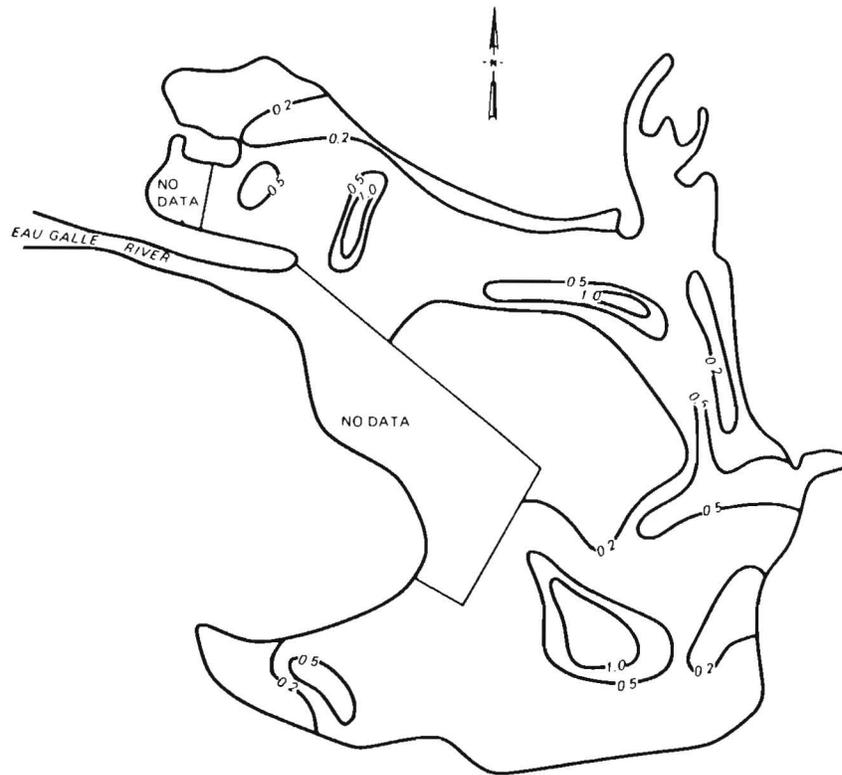
6-m depths. Within shallow, nonvegetated areas (between the 1- and 4-m depths) sedimentation rates were relatively low. Within the vegetated littoral zone between the shoreline and the 2-m depth, however, elevated sedimentation rates occurred.

Percent moisture content and bulk density of surficial sediments exhibited similar spatial patterns and were inversely related ( $r^2 = 0.91$ ,  $n = 77$ ; Figures 3 and 4). Percent moisture content was highest ( $> 70$  percent) in the deepest area of the lake and was associated with maximum sedimentation rates (Figure 3). Percent moisture content declined to values of ca. 50 percent in the shallow, nonvegetated sediments. Percent moisture content then increased to values ranging from 70 to 80 percent in vegetated sediments located near the shoreline. Bulk density was low in the deep and vegetated shoreline areas of the lake and highest in the nonvegetated, shallow sediments (Figure 4).



**Figure 3. Contour map of spatial variations in the percent moisture content of the surficial sediments (0-5 cm). Each contour represents a moisture content in units of percent**

Four functionally and spatially distinct depositional categories could be defined from these observations: an accumulation zone, a transitional zone, an erosional zone, and a littoral zone (Figure 5). Mean sedimentation rates were highest in the zone of accumulation where sediment, as suggested by the high mean percent moisture content and low bulk density, consisted of fine-grained particles. The transitional zone, located along the steep depth gradients, was characterized as having a moderate mean sedimentation rate. Sediments in this region had a mean percent moisture content and bulk density of 69 percent and 0.34 g/ml, respectively. The mean sedimentation rate in



**Figure 4. Contour map of spatial variations in the bulk density of the surficial sediments (0-5 cm). Each contour represents a bulk density in units of g/ml**

the erosional zone was lowest. Sediment in this region consisted of larger particles (sands) as indicated by a high mean bulk density (0.73 g/ml) and low mean percent moisture content (50 percent). The littoral zone exhibited an elevated mean sedimentation rate compared with the adjacent erosional zone. This zone occurred within vegetated regions of the lake. Sediments here displayed a much higher mean percent moisture content (70 percent) than that of the erosional zone because of the occurrence of finer sediments. Overall, mean sedimentation rates, percent moisture content, and bulk density within each zone indicated that the sedimentary environment of the littoral zone was markedly different than that in the nonvegetated erosional zone.

## DISCUSSION

The accumulation of sediment within a lake basin is an important consideration in aquatic plant control and management. As a lake ages, productivity may shift in importance from phytoplankton to macrophytes because of a decrease in water storage capacity, expansion of colonizable sediments for macrophyte growth, and encroachment of emergent macrophyte species. Littoral vegetation may become increasingly more important in stabilizing their sedimentary environment and promoting sedimentation (Carpenter 1981). Since these ideas have received little attention, it was the purpose of this study to examine spatial variations in the sedimentation rate and the composition of the sediment within vegetated and nonvegetated areas of Eau Galle Lake.

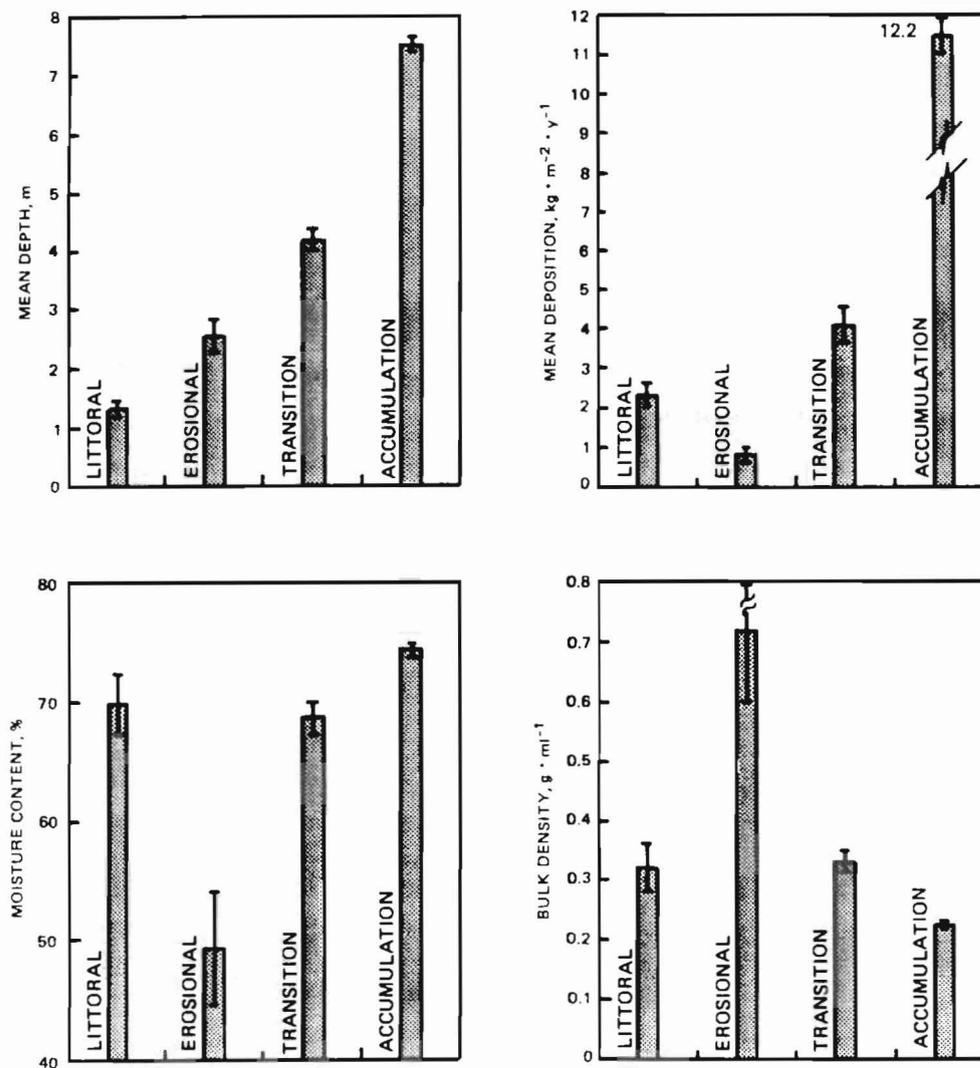


Figure 5. Means and standard errors for sedimentation rate, percent moisture content, and bulk density within zones of accumulation, transition, erosion, and littoral

The most striking pattern observed in this investigation was the delineation of distinct depositional zones within the lake. These zones were separated physically (i.e., with respect to water column depth and distance from shoreline) and by sediment composition. Hakanson (1977) found similar depositional patterns in Lake Vanern. He indicated that sedimentation was influenced by basin morphometry and water movements that create erosional and depositional environments. Sediments in the erosional zone of Lake Vanern were found to be poorly sorted, exhibiting a percent moisture content ranging from 40 to 50 percent. Sediment in the depositional zone consisted of fine-grained particles and had a percent moisture content of 60 to 75 percent.

Although sediment in Eau Galle Lake is potentially subjected to erosional forces at depths of less than 3 m (Gunkel, Gaugush, and Kennedy 1984), vegetated areas exhibited markedly higher sedimentation rates than nonvegetated areas. These differences strongly suggest that macrophytes are decreasing erosion and/or enhancing sedimen-

tation in the littoral zone. The percent moisture content and bulk density of the sediments within the vegetated region reflected the occurrence of fine particles which would not be expected to remain in an erosional environment (Hakanson 1977).

Macrophytes appear to play an important role in retaining fine particulate material in the littoral zone. This role is of ecological significance to plant growth in a potentially erosional environment. Dense macrophyte beds have been shown to effectively reduce current velocities and erosional forces that would otherwise act to resuspend fine particulate material (Madsen and Warncke 1983). As a consequence, macrophytes accelerate the deposition of fine particulate material (Gregg and Rose 1982).

Differences in percent moisture and bulk density of the surficial sediments also indicated that the sediment composition was favorable for macrophyte growth in the littoral zone and unfavorable for growth in the nonvegetated erosional zone. Barko and Smart (1986) found that sediment composition strongly affects macrophyte growth. Sediments, exhibiting a high percent moisture content, a bulk density  $< 0.9$  g/l, and a low organic material content, appear to provide conditions that are favorable for nutrient uptake by rooted submerged plants. They found that macrophyte growth can be restricted on sandy sediments exhibiting a bulk density  $> 0.9$  g/l.

Macrophytes appear to play an important role in modifying their sedimentary environment to promote their growth as lakes age. Macrophytes enhance sedimentation and decrease the erosional aspect of the shallow sediments by reducing turbulent resuspension of particles. Thus, macrophytes appear to be an important component in a positive feedback system that accelerates the eutrophication process.

## ACKNOWLEDGMENTS

The authors appreciate the assistance of R. Kuta and Y. Hartz during the conduct of this research.

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# A Survey of the Aquatic Plant Community of Devils Lake, Wisconsin

by  
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## INTRODUCTION

Devils Lake is a 373 acre (151 ha), moderately softwater, mesotrophic, stratified, seepage lake with a maximum depth of 47 ft (14.5 m) located in southcentral Wisconsin about 50 miles (80 km) northwest of Madison and 200 miles (320 km) southeast of the Eau Galle Reservoir. The lake is quite unique to Wisconsin with topography unlike that found anywhere else in the Upper Midwest with towering 500-ft (150 m) quartzite bluffs on the east and west shores. The watershed is small, 2.65 square miles (6.86 km<sup>2</sup>), and is about 95 percent forested, lying entirely within the boundaries of a state park.

Historically, the lake has a reputation for its outstanding water quality (Secchi disc readings are typically greater than 6 m during much of the summer) and aesthetic appearance (Lillie and Mason 1986). The lake is the center attraction for over a million park visitors each year. However, the recent appearance of large mats of filamentous algae and dense concentrations of phytoplankton during the fall turnover period generated numerous complaints and created alarm among both the public and park management staff. As a result, the Wisconsin Department of Natural Resources (WDNR) initiated investigations to search for factors contributing to the apparent decline in water quality. External nutrient loadings from the small forested watershed were minimal. A major suspect at first was the park's wastewater treatment system which consisted of a two-celled lagoon situated in the terminal moraine at the southeast end of the lake. The bottoms of the lagoons were about 35 ft (11 m) above the surface of the lake, so there was some concern that effluent might seep back into the lake. However, investigations revealed a steep hydrologic gradient away from the lake. The lagoon has since been abandoned, and wastewater is now discharged to a nearby municipal wastewater treatment facility. The remaining five private cottages on the lake were likewise evaluated as possible sources of nutrients and ruled out. Direct inputs of nutrients from swimmers, boaters, fishermen, and scuba divers were estimated and deemed inconsequential. With the major external nutrient sources ruled out, we began to examine internal sources. In addition to increases in phytoplankton, changes in the size and structure of the lake's weed beds had occurred. Large beds of milfoil created localized problems where previously no visible macrophyte beds existed. These subjective observations, together with the knowledge that milfoil may actively transport nutrients from the sediments to the overlying waters (Barko and Smart 1979, Landers 1982, and Smith and Adams 1986), prompted further attention on the macrophyte community. Consequently, and in conjunction with a larger interdisciplinary limnological investigation, an extensive macrophyte survey of Devils Lake was conducted on

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July 30-August 1, 1984 (Lillie 1986). Specific objects were: (a) document the composition, density, and distribution of plants; (b) make historical comparisons where possible; and (c) estimate the possible impact of any observed changes in the macrophyte community upon the lake's water quality.

Samples were collected via scuba within 0.1 m<sup>2</sup> quadrats at 5-m intervals from shore to a water depth of 9 m (virtually no plants existed beyond this depth) along 28 transects spaced 200 m apart about the perimeter of the lake. Presence/absence of each species and gross density of total plants at each quadrat were recorded. All plants within quadrats exceeding 50 percent visual coverage were collected for biomass determinations (stems and shoots; no roots except for the small spikerush, *Eleocharis acicularis*). A representative subsampling of less dense and sparsely vegetated quadrats was also completed. Samples were labeled and bagged in the field, returned to the laboratory where they were sorted by species, and bagged again for drying. Dry weights were determined after drying at 106°C for 48 hr. Voucher specimens were prepared and taxonomy was verified by staff of the University of Wisconsin Herbarium and, in the case of pondweeds, by Dr. S.G. Smith of the University of Wisconsin—Whitewater. Data for three distinctly separated shoreline areas, designated as North, Inlet, and Southeast Beach, were analyzed separately. The Southeast Beach area is an area of extensive recreational use with two sandy beaches. The North shore has extensive beach areas and a well-developed boat landing with access via a small dredged bay. The Inlet area is less intensively developed with one boat landing and limited shoreline access.

## RESULTS

The total standing crop was estimated at 66 acres (27 ha) representing 18 percent of the entire lake bottom with an estimated total dry weight biomass of 51,000 kg or about 56 tons (Table 1). Average stand densities ranged from 160 to 183 g/m<sup>2</sup>, comparable to densities reported from other productive Wisconsin lakes (Lillie and Mason 1986). Approximately half of the total plant biomass was distributed within 1.5 to 3 m. Plants growing in shallower water were exposed to severe water level fluctuations, greater wind and wave exposure, and human disturbance. The plant community consisted of 16 species (Table 2). *Potamogeton robbinsii* was by far the dominant plant accounting for almost half of the total plant biomass. *Myriophyllum spicatum* and *Elodea canadensis* were next in order of importance. We were unable to distinguish between *Potamogeton amplifolius* and *Potamogeton illinoensis* in the field, so these two pondweeds were lumped together. *Nitella flexilis* and the filamentous *Cladophora* sp. were physically intertwined and impossible to separate and thus were also lumped together.

Based on visual observations by divers, the distribution of dense stands of milfoil was quite restricted and confined primarily to 2-3 m (Figure 1). Only a very few scattered stems extended out to depths of 9 m. Distributions of *P. robbinsii* and *E. canadensis* were almost identical with rather broad ranges from 1.5 to 4.5 m. However, it is important to note that while the depth distributions of the three major species overlapped, they generally were distributed in relatively monotypic stands adjacent to one another. A whole cluster of less significant plants was located in relatively shallow, disturbed areas.

**Table 1**  
**Standing Crop of Aquatic Macrophyte Biomass and Coverage**  
**of Dense Plant Stands at 0-9 m in Devils Lake**

<i>Location</i>	<i>Total Plant Biomass kilograms (dry wt)</i>	<i>Dense Plant Stands acres</i>
Inlet	14,700	14
Southeast	13,000	18
North	18,400	28
Other	4,900	6
Total	51,000	66*

\*Approximately 7 acres of milfoil.

**Table 2**  
**Community Composition of Dominant Macrophytes of Devils Lake**

<i>Taxa</i>	<i>Percent Absolute Frequency Occurrence</i>	<i>Percent Total Biomass dry wt</i>	<i>Importance* Value</i>
<i>Potamogeton robbinsii</i>	40	46	0.33
<i>Myriophyllum spicatum</i>	35	20	0.20
<i>Elodea canadensis</i>	36	11	0.14
<i>P. illinoensis and amplifolius</i>	18	5	0.07
<i>Ceratophyllum demersum</i>	16	4	0.06
<i>Nitella flexilis and Cladophora</i>	18	5	0.06
<i>Eleocharis acicularis</i>	2	1	0.01
Others/mixed	-	8	-

\*Based on average of relative biomass and relative frequency of occurrence.

The distribution of milfoil was confined primarily to three distinct, narrow beds, each located directly offshore from areas of relatively high recreational use (Figure 2). The milfoil formed dense contiguous stands (averaging 180 g/m<sup>2</sup>) 25-50 m wide and up to 300 m long. The three beds totaled about 7 acres (2.8 ha) and represented only 2 percent of the total lake surface area, or 21 percent of the lake bed at the 1.5-3 m depth.

A cross-sectional diagram of the Southeast Beach bed (Figure 3) revealed the following pattern in plant distribution: from shore to 2 m very little vegetation existed. The milfoil bed began abruptly at 2 m and continued to 3 m where the bottom slope increased sharply. Beyond this, *P. robbinsii* formed a dense mat which continued to about 6 m after which a mixture of *Nitella* and *Cladophora* dominated. The pattern was similar in the North shore bed, but it was reversed in the Inlet area bed where *P. robbinsii* and a mixture of other native species were located on the inshore side of the milfoil bed.

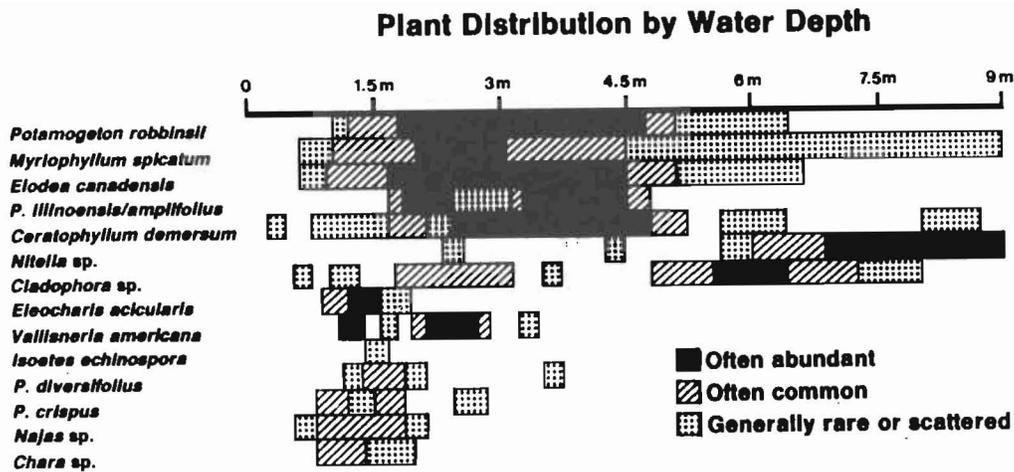


Figure 1. Distribution of macrophytes by depth in Devils Lake, Wisconsin

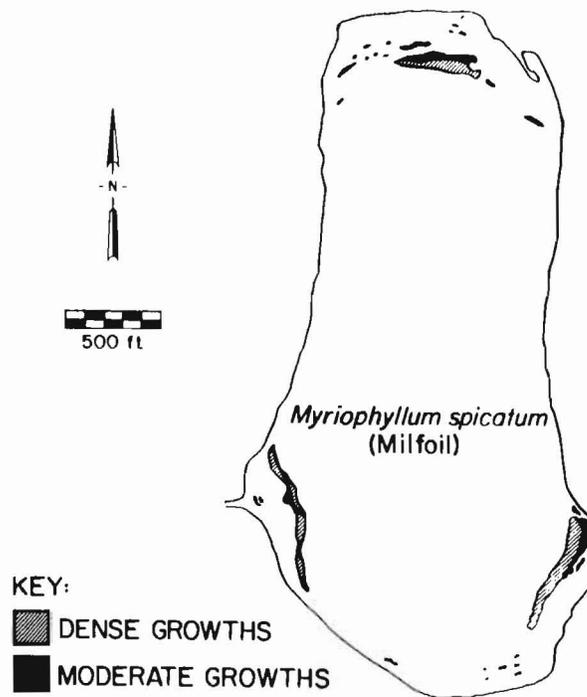


Figure 2. Distribution of *Myriophyllum spicatum* in Devils Lake, Wisconsin

*Myriophyllum spicatum* is a relative newcomer to Devils Lake. An earlier study utilizing similar sampling methodologies in Devils Lake (Baker 1975) described milfoil distribution as "very scattered at 1.2-4.5-m depths contributing little to the population of the total community." This condition does not exist today with average biomass of 180 g/m<sup>2</sup> forming a solid wall of plants. The introduction and expansion of milfoil appears to have occurred at the expense of, or coincidental with, a reduction in *Elodea*. Whether the milfoil aggressively displaced *Elodea*, or simply replaced *Elodea* as it declined due to some other cause, is open to question. The dominant *P. robbinsii* is a low-growing form,

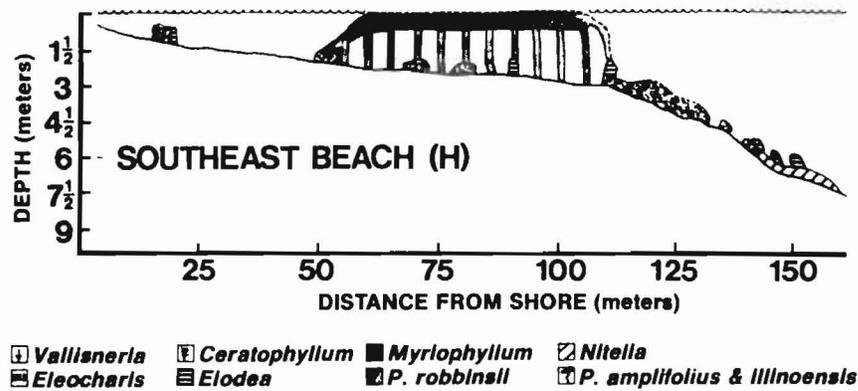


Figure 3. Horizontal profile of macrophyte community along the Southeast Beach region of Devils Lake

which rarely, if ever, exceeds 1 m in height. Although extremely abundant, it creates no problem, but rather serves as a relatively rich habitat for invertebrates. Its distribution in Devils Lake appears to have been unaffected by the invasion of milfoil.

### MANAGEMENTS CONSIDERATIONS

Other than *P. amplifolius* and *P. illinoensis*, *M. spicatum* is the only macrophyte in Devils Lake whose stems reach the surface. As such, the plant creates a severe, but localized, nuisance to swimmers, boaters, and wind-surfers. Additionally, possible phosphorus loading to the lake via uptake of phosphorus from sediment and subsequent release by *M. spicatum*, based on studies by Prentki (1979) on nearby Lake Wingra, could account for as much as 40-60 percent of the annual observed summer increase in phosphorus mass in the lake. Hence, even though the milfoil is presently confined to only 2 percent of the total lake surface area, some form of control may be warranted.

Development of a rational management strategy for milfoil in Devils Lake calls for more detailed knowledge of the life history dynamics of the plant and its ecological significance to other biota. Most recently (1987), the WDNR conducted more extensive studies in Devils Lake, including additional macrophyte surveys, detailed fishery and invertebrate surveys, and with the assistance of the Waterways Experiment Station, surveys of the physical and chemical composition of bottom sediments. While a preliminary analysis of the sediment data failed to reveal any significant correlations between milfoil distribution and sediment parameters on a lake-wide basis, it did document that the situation in Devils Lake is quite unusual. The sediments underlying the milfoil beds are predominantly sand (70 percent to be exact, and 50 percent coarse sand) (Table 3). The low organic content (2 percent) and high sediment density (1.27 g/ml) represent poor growing conditions (Barko and Smart 1986) that are not compatible with the high milfoil densities found in this lake. Further examination of the data, with possible additional work, will aid in a better understanding of factors affecting the unusual distribution of milfoil beds in the lake. In turn, an effective management control plan (if needed) might be developed in the future.

**Table 3**  
**Summary of Sediment Characteristics of Littoral Area of Devils Lake\***

<i>Physical Parameter</i>	<i>Values</i>	<i>Mean ± 1 SE (N = 77)</i>
Soil Density:	g dry wt/ml wt vol.	1.27 + 0.04
Organic content	% dry wt	2.06 + 0.24
Moisture content	% dry wt	29.23 + 1.55
Total Sand content	% dry wt	70.19 + 2.56
Silt content	% dry wt	27.87 + 2.41
Clay content	% dry wt	1.94 + 0.17

\*Data analyzed and provided by US Army Engineer Waterways Experiment Station, Environmental Laboratory, Vicksburg, Miss.

## ACKNOWLEDGMENTS

Assistance in field studies by G. Wegner, G. Quinn, P. Garrison, P. Muntz, D. Marshall, R. Masnado, G. Fannucchi, T. Brasino, D. Soltis, S. Fruhwirth, and D. Stahl is gratefully acknowledged. Sediment analysis was performed by the US Army Engineer Waterways Experiment Station, Environmental Laboratory, Vicksburg, Miss. Editorial reviews by J. Mason and J. Barko, and drafting by R. Burton area also greatly appreciated. Some information presented here were taken from Lillie (1985) and Lillie and Mason (1986).

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# Effects of Water Chemistry on Aquatic Plant Species: A Summary of the Effects of Inorganic Carbon Supply

by  
R. Michael Smart\*

## BACKGROUND

It has long been realized that the chemical composition of water can affect the growth and distribution of submersed aquatic plants. However, since information on the effects of specific water chemistry parameters on submersed aquatic plants is lacking, it is difficult to ascribe differences in growth and distribution to specific water chemistry parameters. As a consequence, our ability to predict the likelihood of excessive growth of submersed aquatic vegetation in particular water bodies is hindered.

## OBJECTIVES AND APPROACH

The objective of this work unit is to determine the influence of specific water chemistry parameters on the growth and potential distribution of submersed aquatic plant species. This article summarizes effects of inorganic carbon supply on the growth and photosynthesis of submersed aquatic plants.

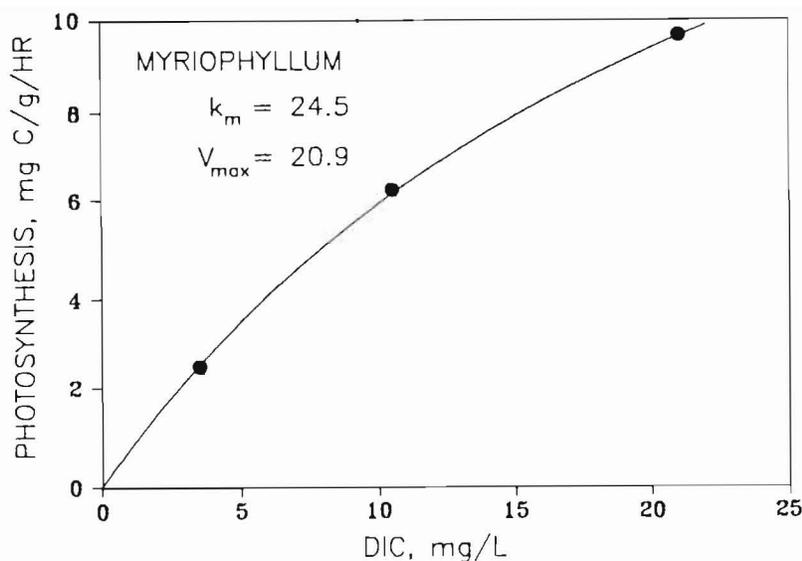
## RESEARCH SUMMARY

### Photosynthesis and growth

Previous studies of photosynthesis and plant growth (Smart and Barko 1985, 1986) indicated that the water chemistry parameter of primary concern is dissolved inorganic carbon (DIC). Photosynthesis in submersed aquatic plants is largely dependent on the uptake of bicarbonate ions from solution. Photosynthetic carbon uptake follows first order kinetics with respect to DIC over a wide range of DIC concentrations and pH values (Titus and Stone 1982, Smart and Barko 1986). Data obtained for *M. spicatum* (Smart and Barko 1986) illustrate the relationship between photosynthetic rate and solution DIC concentration (Figure 1). The half-saturation constant for photosynthesis in *M. spicatum* is 24.5-mg DIC/l, meaning that photosynthesis in this species is operating at half its maximal rate at a DIC level of 24.5 mg/l. Photosynthesis will thus be proportional to DIC concentrations near or below this level of DIC. Since most natural freshwaters exhibit DIC concentrations of this magnitude (Hutchinson 1975), photosynthesis in most natural systems is potentially limited by inorganic carbon.

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**Figure 1. Photosynthesis and kinetic constants for *M. spicatum* in relation to dissolved inorganic carbon (adapted from Smart and Barko 1986)**

Photosynthesis provides the energy necessary for plant growth; thus there should be a close coupling between photosynthetic rates and plant growth; Since photosynthesis is proportional to solution DIC, one would thus expect a close relationship between plant growth and DIC. However, the growth of *M. spicatum* (Figure 2) and other species of submersed aquatic plants has been repeatedly shown to differ from that expected on the basis of the relationship between photosynthesis and solution DIC. Since photosynthetic rates have been used as indicators of plant growth potential (Adams, Guilizzoni, and Adams 1978), it is important to resolve the disparity between the responses of growth and photosynthesis to solution DIC. This area has been the subject of continuing research within the Water Chemistry work unit.

### DIC depletion

One possible reason for the disparity between the responses of growth and photosynthesis is that submersed aquatic plants rapidly deplete concentrations of DIC during growth. This rapid depletion of DIC makes it difficult to conduct controlled studies of the growth responses of these plants to different levels of DIC (Smart and Barko 1986). After only a few weeks' growth, DIC levels can be depleted to less than half of their original concentration, as exemplified by the rapid decline in solution DIC during the growth of *Hydrilla verticillata* (Figure 3). Even rapid aeration with CO<sub>2</sub>-enriched air does not completely prevent the depletion of solution DIC (Smart and Barko 1987). Obviously, submersed aquatic plants have a very high demand for DIC, and the potential for carbon limitation of growth is quite high.

The high demand for DIC and the resultant rapid depletion of DIC observed during laboratory growth experiments suggests that similar declines in DIC may also occur in the field. For example, a field population with a biomass level of 250-g dry mass/m<sup>2</sup>, photosynthesizing at an average rate of 5-mg-C/g dry mass/hr, has a daily carbon

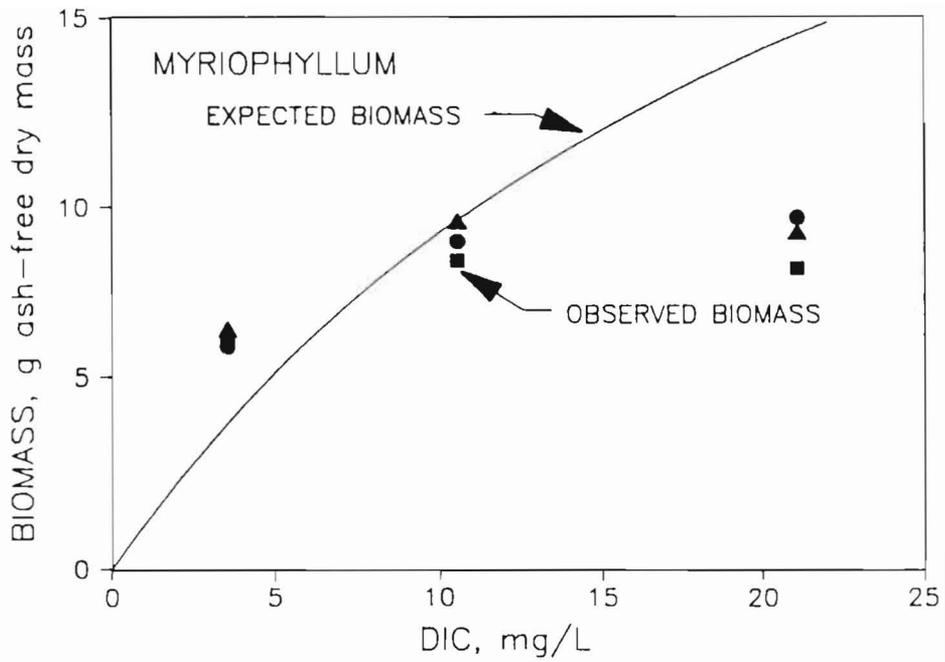


Figure 2. Biomass response (data points) and expected biomass response (line) of *M. spicatum* in relation to dissolved inorganic carbon (adapted from Smart and Barko 1986)

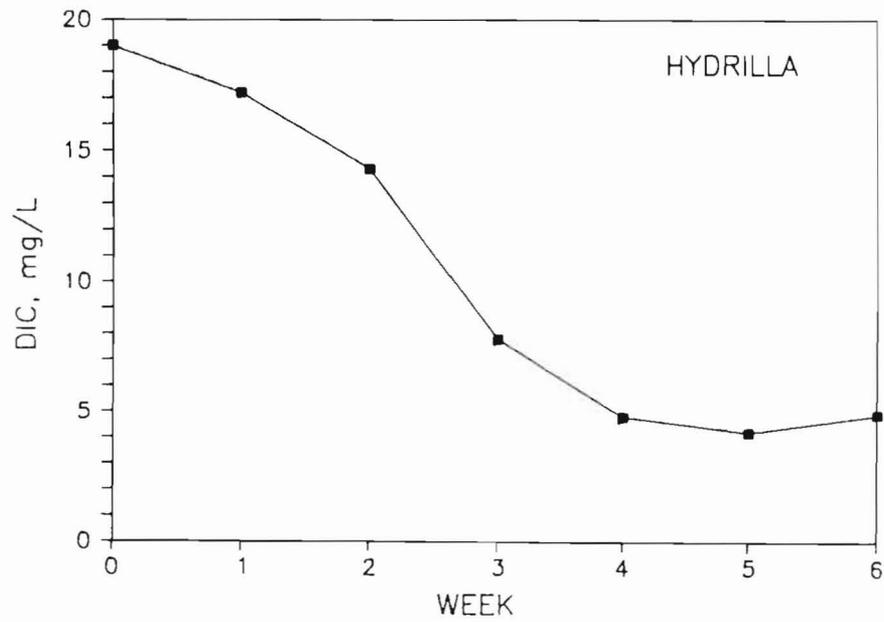
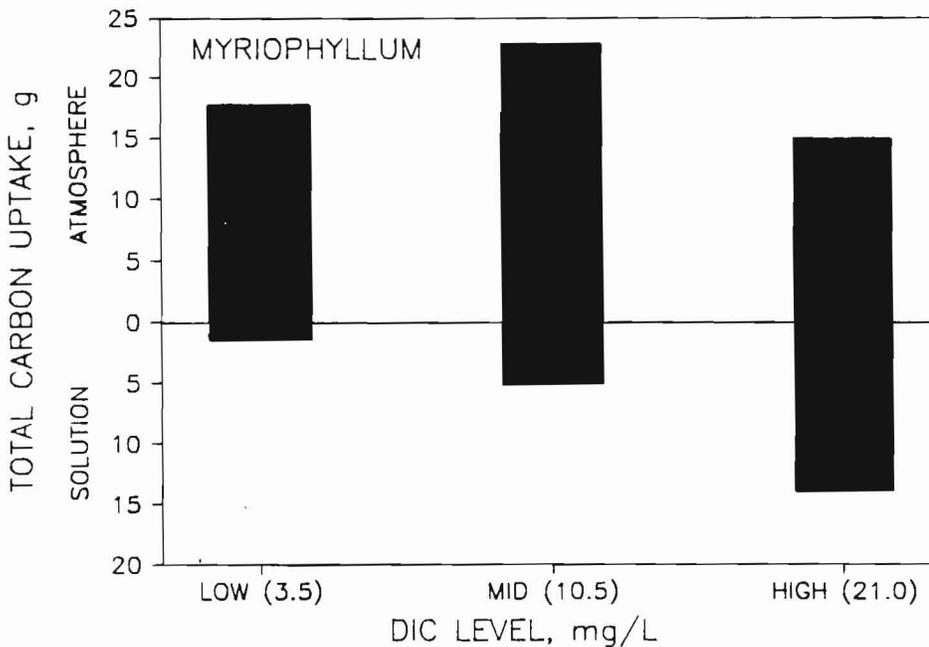


Figure 3. Dissolved inorganic carbon (DIC) concentrations during an experiment involving the growth of *Hydrilla* (adapted from Smart and Barko 1988)

requirement of about 17.5-g DIC/day. In a water column 2 m deep with an average level of DIC (20 mg/l), the total quantity of carbon in the water column is only about 40-g DIC/m<sup>2</sup>. Thus, the water column contains only a few days' supply of available inorganic carbon, and DIC will become depleted unless additional carbon supplies become available.

### DIC regeneration

In fact, there are a number of potential sources for supplying or regenerating inorganic carbon. These include: atmospheric exchange, sediment respiration, water column respiration, and advection. In our greenhouse studies, the only significant source of carbon for replenishment of solution DIC is atmospheric exchange (Smart and Barko 1984). A significant proportion of the inorganic carbon used by submersed aquatic plants in greenhouse studies reported here is typically derived from atmospheric exchange (Figure 4). Atmospheric carbon supply is also unrelated to solution DIC level; thus atmospheric CO<sub>2</sub> exchange moderates the effects of solution DIC on the growth of submersed aquatic plants (Smart and Barko 1986).



**Figure 4. Inorganic carbon supplied from the atmosphere and from solution in relation to solution DIC concentration during an experiment involving the growth of *M. spicatum* (adapted from Smart and Barko 1986)**

Under field conditions the other sources of inorganic carbon supply to the DIC pool may augment that supplied by atmospheric CO<sub>2</sub> exchange. Preliminary investigation of the inorganic carbon budget of Eau Galle Reservoir, a hard water system in Wisconsin, suggests that atmospheric exchange, sediment respiration, and water column respiration are of approximately equal importance in the regeneration of DIC removed by the photosynthetic activities of submersed aquatic plants and algae.

The importance of advection in supplying additional DIC is unknown and will be investigated during FY 88.

### Growth limitation

Given adequate temperature and irradiance regimes, the most likely plant growth-limiting factors are inorganic carbon and nitrogen availability. While sediment nitrogen is potentially limiting (Barko and Smart 1986), most fine-textured inorganic sediments initially contain adequate nitrogen to support nuisance levels of submersed plant biomass (Smart and Barko 1987). Similarly, even waters of low DIC concentration can support the nuisance growth of submersed aquatic plants via atmospheric CO<sub>2</sub> exchange. The intensity of an aquatic plant problem may, however, depend on both the availability of inorganic carbon for fueling photosynthesis and sediment nitrogen for providing additional plant growth. For example, the growth of *Egeria* was only slightly stimulated by an increase in the supply of inorganic carbon (Figure 5). This minimal response to inorganic carbon resulted from the co-occurrence of nitrogen and carbon limitation at a similar level of biomass production. Similarly, *Egeria* exhibited only marginal response to increased nitrogen availability due to co-limitation by inorganic carbon supply. The growth of *Egeria* was thus dependent on the supply of both nitrogen and carbon.

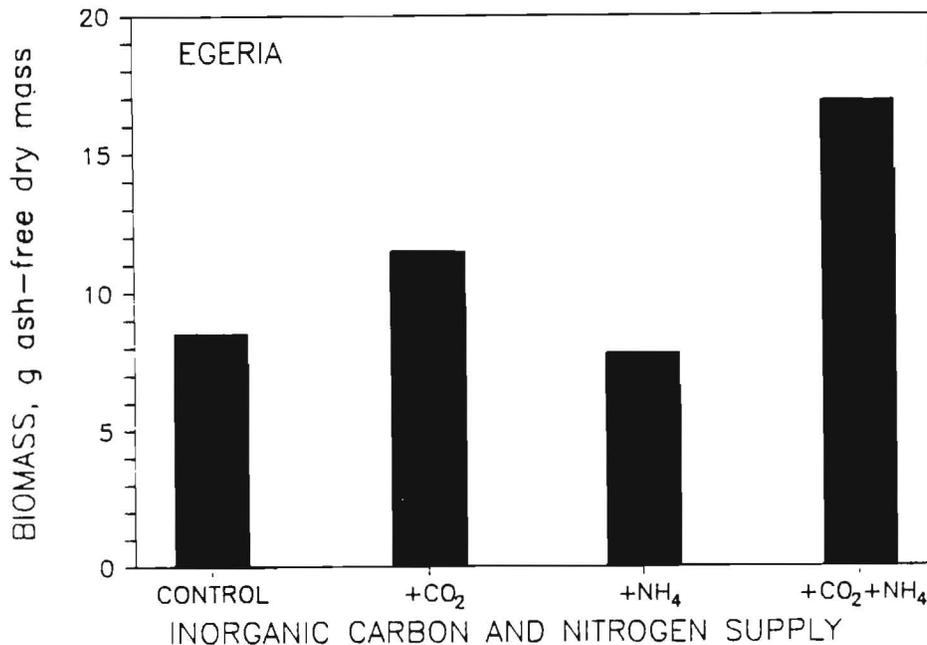


Figure 5. Biomass response of *Egeria* in relation to airstream CO<sub>2</sub> supply and sediment nitrogen level (adapted from Smart and Barko 1988)

## DISCUSSION

In spite of the potential importance of inorganic carbon as a growth-limiting factor, there is unlikely to be a simple relationship between solution DIC and biomass production. The reason is that inorganic carbon availability is only loosely related to water chemistry. While waters of higher alkalinity generally contain a greater quantity of readily available carbon, it is the rate of regeneration of this carbon that is of primary importance. Actively growing submersed macrophyte populations are capable of depleting DIC levels in a matter of days or weeks, and continued growth of these populations depends on the carbon regenerating capacity of the system. The potential of a water body for supporting problem level populations is thus more dependent on the recycling of carbon than on water chemistry *per se*. While biomass production may be proportional to inorganic carbon supply, the supply of carbon is dependent on rates of water column respiration, sediment respiration, atmospheric exchange, and advection in addition to the chemical composition of the water.

Another factor complicating the responses of submersed aquatic plant populations to water chemistry and inorganic carbon availability is the occurrence of nitrogen limitation. Under the higher rates of submersed aquatic plant growth resulting from increases in inorganic carbon supply, the supply of nitrogen often becomes growth-limiting. Biomass production and the intensity of submersed aquatic plant infestations may thus be dependent on both nitrogen availability and inorganic carbon supply.

## ONGOING AND FUTURE RESEARCH

Extrapolation of these laboratory results to field conditions will require the conduct of both descriptive and manipulative field experiments. Rates of replenishment of DIC from atmospheric, water column, and sedimentary sources should be quantified under diverse field conditions. This effort is currently being conducted in part at Eau Galle Reservoir in Wisconsin.

The relative importance of carbon and nitrogen limitation and possible interactions between these factors will be reevaluated under environmental conditions more closely approximating those occurring in the field. This evaluation is currently being conducted.

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# Field Evaluation of Selected Herbicides

by  
Howard E. Westerdahl\*

There are two areas within the Chemical Control Technology area requiring large-scale field evaluations: first, cooperation with industry on field testing for new herbicide and expanded use registrations; and second, evaluation of new herbicide application techniques to specific types of aquatic systems. Federal and state regulatory agencies often require additional testing to obtain, maintain, and expand herbicide registrations. Moreover, as existing patent rights for registered herbicides near expiration, chemical companies consider retracting a herbicide registration if profits and/or market are marginal and additional testing is required by regulatory agencies. Often government interest in a herbicide and willingness to cooperate with chemical companies to obtain additional environmental data can be sufficient justification for chemical companies to refrain from retracting a registration. Cooperative efforts to date, involving the Waterways Experiment Station, have been field testing to provide information on herbicide fate and persistence in specific aquatic environments. This information fulfills some of the herbicide testing requirements for registration as well as greatly increases our understanding of a herbicide's performance under operational conditions. Tests of this magnitude require an Experimental Use Permit (EUP) from the US Environmental Protection Agency (USEPA), and state approval, prior to field application.

Results from other work units in the Chemical Control Technology area involving evaluation of new controlled-release herbicide formulations and application techniques under laboratory and small-scale (<0.05 ha) field tests may require an EUP prior to their evaluation under operational conditions.

The EUP data requirements and sampling frequency depend on the herbicide being tested, the intended use of the information by the registrant, i.e. research or registration purposes, and target sites for chemical use. Table 1 is a list of parameters considered important for describing environmental fate and dispersion of herbicide formulations. Additional parameters may be required if a new application technique is tested. The

Table 1  
List of Parameters for Environmental Fate and Dispersion Studies

<i>Herbicide Residue</i>	<i>Water Quality</i>	<i>Nontarget Effects</i>
Water	Temperature	Zooplankton
Sediment	Dissolved solids	Benthic organisms*
Target plant	pH	
Crayfish	Dissolved oxygen	
Clams	Nitrogen forms*	
Game fish	Phosphorus forms*	
Rough fish		

\*If the USEPA or state regulatory agency requires these data.

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concurrence of the USEPA Registration Branch on the necessity for an EUP is important if the data are eventually to be used to support registration.

During FY 86 and 87, a field evaluation of the herbicide Garlon 3A (triclopyr) was conducted in Lake Seminole, Georgia, by the Corps of Engineers (CE) and in Banks Lake, Washington, by the Bureau of Reclamation (BOR), respectively. Following analysis of the data, a report will be completed during FY 88 describing the fate of Garlon 3A in Lake Seminole. A similar report will be prepared for Banks Lake in FY 89 by the BOR. These reports will be made available to the USEPA by the respective Federal agencies during their review of Dow Chemical Corporation's request for a Garlon 3A aquatic registration. Similar investigations using a new plant growth regulator, Mariner (bensulfuron methyl), and the herbicide Casoron 10G (dichlobenil) are planned for FY 88 and 89, respectively.

# Determination of Weak Points in the Life Cycle Of Selected Nuisance Aquatic Plants

by

Kien T. Luu\* and George J. Pesacreta\*

## INTRODUCTION

In 1985, an interdisciplinary group of scientists from the Waterways Experiment Station, other Federal agencies, universities, and the private sector met to consider the feasibility of an integrated approach to aquatic plant control. One recommendation proposed from that gathering was that a better understanding of aquatic macrophyte growth cycles and identification of physiological weak points in those cycles are needed to improve the effectiveness of present control techniques. A weak point is defined as a period during the growth cycle when a plant is least likely to recover from a control method. Once weak points are identified, they must be marked, or tagged, using growth cycle events and/or morphological characteristics; thereby, enabling operations personnel to identify the optimal time for applying appropriate control strategies.

Based on a number of studies involving terrestrial plant species, carbohydrate allocation, which describes the distribution of photosynthetically produced sugars in various plant tissues, has been used to identify physiological weak points in the life cycle of plants. Major weak points, based on carbohydrate allocation, in a plant's growth cycle include: initial spring growth, when reserve carbohydrates from storage organs are the primary source of energy; onset of flowering, when vegetative growth slows as carbohydrates are shunted to energy-demanding sexual reproductive tissues; and onset of dormancy, when carbohydrates are translocated to storage structures.

Carbohydrates can be divided into two groups: total structural carbohydrates, which include permanent structural substances such as cellulose, and total nonstructural carbohydrates. Total nonstructural carbohydrates can be separated into two fractions: soluble sugars (glucose, fructose, maltose, sucrose, etc.) which are readily available for metabolism and quantities vary in plant tissues; and reserves (starch and fructosan). These reserve components are typically stored in structures such as stem bases, tubers, turions, rhizomes, and roots.

The relationship between carbohydrate allocation and seasonal growth characteristics has been used to enhance control of terrestrial, perennial plants by keying on carbohydrate-cycling events (Lindscott and McCarthy 1962; Schirman and Buchholtz 1966; Klingman, Ashton, and Noordhoff 1975; McAllister and Haderlie 1985). One approach has been to disrupt the normal source-to-sink translocation of carbohydrates that precedes winter dormancy. For example, mowing of shoots in the fall prevents accumulation of belowground food reserves in perennials by interrupting the translocation of shoot carbohydrates to roots and rhizomes (Klingman 1965). Without adequate reserves, plants are more susceptible to winter injury and/or death, and spring

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growth is diminished. Spring growth, when belowground reserves are low, is also a critical period during which a method, such as repeated mowing, is most effective for controlling perennial species (Klingman, Ashton, and Noordhoff 1975).

As with their terrestrial counterparts, perennial aquatic plants may rely on stored carbohydrate reserves for survival through winter and initiation of spring growth. Moreover, recovery from periods of stress caused by fluctuating water temperatures, drought, nutrient depletion, and turbidity may be dependent on carbohydrate reserves. Linde, Janisch, and Smith (1976) identified a relationship between carbohydrate reserves and growth cycle events in cattails. When the pistillate spike was lime green in color, and the staminate spike appeared dark green, carbohydrates were at their lowest level in the plant. This information allowed the timing of a control strategy to coincide with the color of the pistillate and staminate spikes.

To use weak points effectively in managing other aquatic plants, growth events, morphological characteristics and/or environmental cues must be identified which enable the occurrence or prediction of these life cycle events to initiate a timely control strategy. For example, if flowering in waterhyacinth can be induced in early spring using chemicals, biomass production may not reach problem levels during the growing season. Likewise, during flowering, biological control agents may be more effective in reducing a slow-growing waterhyacinth population. Another example may be the use of timing herbicide applications to prevent translocation of carbohydrates to storage organs during the onset of dormancy. The latter is more difficult since a morphological marker, record of degree-days, photoperiod, water temperature, etc., may be needed to time the application period. It is this type of information that is required to take advantage of a plant's weak points.

The literature on waterhyacinth (*Eichhornia crassipes*), hydrilla (*Hydrilla verticillata*), Eurasian watermilfoil (*Myriophyllum spicatum*), and alligatorweed (*Alternanthera philoxeroides*) was surveyed to determine whether or not weak points had been identified for these plants, and to assess the feasibility of using this information for enhancing control of these plants. Few studies were found which related the weak point in the growth cycle to carbohydrate allocation; therefore, the initial thrust of this research has been to assess carbohydrate allocation patterns in each of the aforementioned plant species and identify weak points based on the relationship between carbohydrate allocation and seasonal growth phases.

Specific laboratory and small-scale outdoor studies have been implemented to identify important environmental factors and seasonal growth characteristics which coincide with minimum carbohydrate reserves in various structures of waterhyacinth and hydrilla. Since considerable information exists on the biology of waterhyacinth, this species was selected for the initial studies. Hydrilla was selected, as the second target species, based on its national-scale, nuisance level. Preliminary results from the waterhyacinth and hydrilla studies are presented below. Upon completion of these studies, similar studies with Eurasian watermilfoil and alligatorweed will be initiated.

# **Part 1. Seasonal Growth Characteristics and Carbohydrate Allocation in Waterhyacinth\***

## **OBJECTIVES**

The objectives of this study are: (a) verify the important morphological and growth characteristics of waterhyacinth; (b) determine seasonal carbohydrate distribution within various plant structures; and (c) based on these results, identify potential weak points in the life cycle of waterhyacinth.

The following report summarizes the progress to date and significant results of this study on growth characteristics and carbohydrate allocation in waterhyacinth.

## **MATERIALS AND METHODS**

Young, waterhyacinth ramets (daughter plants) of similar size were selected and cultured outside in 1,300-l, epoxy-coated, wooden tanks (240 × 76 × 76 cm). A 20 percent Hoagland solution was used (Hoagland and Arnon 1950) as a nutrient source. Water temperature was maintained at 20°-25° C by recirculating water through temperature regulating chillers (Remcor Products Co., Chicago, Illinois). Daily maximum and minimum temperatures of air and water were recorded by self-registering thermometers.

### **Growth characteristics experiment**

Growth rates of waterhyacinth were determined at different seasons, based on weekly increments of fresh weight and plant number. Each single, young ramet of equal age and weight was grown in a separate plastic basket (60l × 42w × 31h cm). These baskets were submerged in each of three tanks filled with nutrient solution. Measurements were taken until plants completely filled the surface area of the baskets. The unexpected flowering of two of the replicates prevented statistical analysis of the data. Therefore, fresh weights and plant numbers were plotted against time to compare growth rates in the three tanks.

### **Carbohydrate allocation experiment**

Ramets of equal size were grown in three tanks for several months before sampling for carbohydrate determination. Open water surface was always maintained, by removal of plants in part of the tanks, to allow room for continuous ramet production. Monthly samples were randomly collected from October 1986 to February 1987 and from June 1987 to July 1988 to measure the seasonal trends of carbohydrates in different plant structures: stembase, root, inflorescence, stolon, membrane, young, mature, and old leaves and petioles (Figure 1).

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\*Prepared by K.T. Luu.

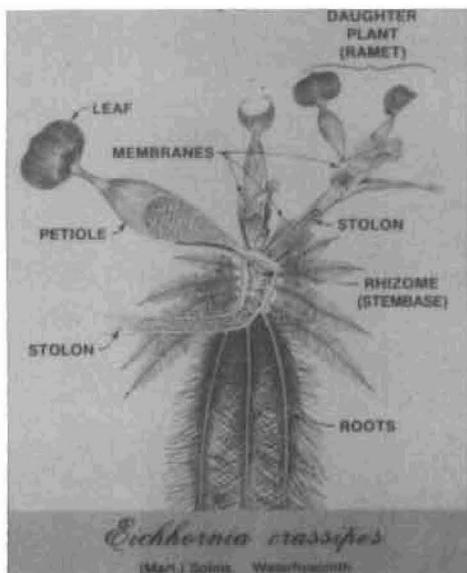


Figure 1. Major plant structures of waterhyacinth: stembase, root, stolon, membrane, leaf, and petiole

In addition, separate samples of blooming and wilted (5 to 7 days old) inflorescences were sampled to determine the change in carbohydrates during and following blooming. Inflorescences were separated into three different parts: rachis, floret, and peduncle (Figure 2).

Plant parts were dried in a forced-air oven at 55° C for 48 hr to a constant dry weight, preceding carbohydrate analyses.

Total nonstructural carbohydrate (TNC) were determined by a modification of the procedure of Swank et al. (1982). Extracts for TNC (starch, hydrolyzed sugars, reducing sugars) were incubated for 15 min at 55° C with one unit of amyloglucosidase (Sigma No. A-3042)/ml to attain complete starch hydrolysis before assaying for reducing sugar (Nelson 1944). Free carbohydrate (hydrolyzed sugars, reducing sugars) was also determined on extracts not incubated with amyloglucosidase.

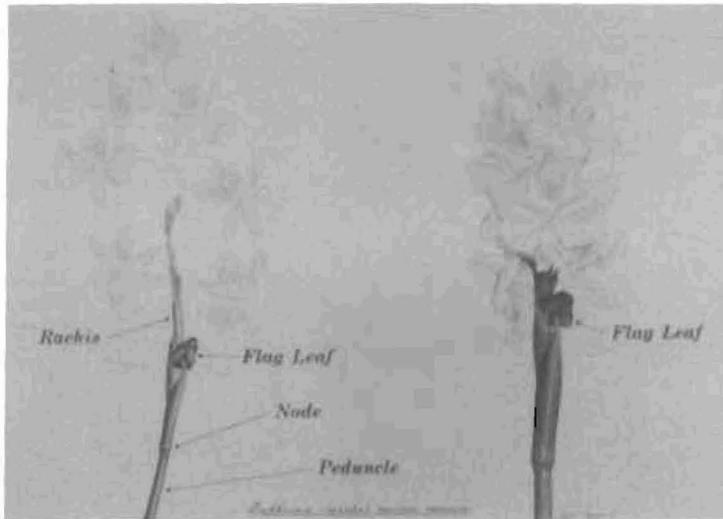
Sugar species of the reserve carbohydrates in waterhyacinth were identified by thin layer chromatography (TLC) based on the methods of Lato et al. (1968), with minor changes (Streeter and Bosler 1976).

A completely randomized design with three replications (three tanks) was used. Treatments were different sampling dates and plant parts. Measured parameters included free carbohydrate, starch, and TNC. The Waller-Duncan test (Steel and Torrie 1980, Smith 1978) was used to separate the effects of treatment means.

## RESULTS AND DISCUSSION

### Growth characteristics

*Flowering versus ramet production.* One month following the initiation of the experiment, the plant in replication 2 (plant 2) flowered (Figure 3). Four weeks later, the plant in replication 3 (plant 3) flowered. The plant in replication 1 (plant 1) never



**Figure 2. Different parts of water-hyacinth inflorescence: peduncle (stalk of inflorescence), rachis (axis carries florets), and florets (colorful flowers)**

produced a flower during the experiment. The cause of flowering in plants 2 and 3 is unknown. One striking characteristic observed is that during the two weeks following flowering, neither plant 2 nor 3 produced additional ramets.

The differences in ramet and biomass production between nonflowered and flowered plants were apparent after week 10 (Figure 3). During weeks 13 and 17, plant 1 (nonflowered) produced over twice the number of ramets and almost doubled in biomass production, as compared to plants 2 and 3 (flowered). Immediately after flowering, waterhyacinths were unable to produce new ramets. This result is in agreement with the findings of Pieterse, Aris, and Butter (1976) who showed that gibberellic acid (GA-3) markedly inhibited ramet production and induced profuse flowering in waterhyacinth. Similarly, studies by Watson (1984), which compared flowering and nonflowering populations, showed that the flowering population took longer to cover the experimental tanks with ramets.

Perhaps, blooming inflorescences are strong energy demanding sinks in waterhyacinths as the plant expends large quantities of energy for the flowering process. In fact, our results have shown that free carbohydrate content in blooming inflorescence was five to six times higher than that of many other plant parts. Results from our work and others, suggest that waterhyacinth is incapable of simultaneously supplying energy for flowering and ramet production.

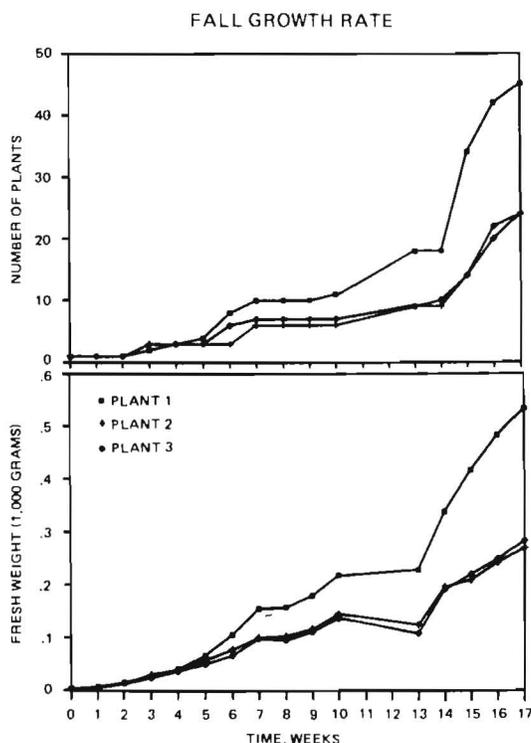


Figure 3. Growth rate expressed as fresh weight and number of plants. Plant 1 (non-flowered), plants 2 and 3 (flowered)

**Membrane structures.** An interesting, and perhaps important structure in waterhyacinth is the membrane wrapped around the petiole base of each leaf and stolon node (Figure 1). This membrane appears to function as a support structure for the attachment of leaf to stem and ramet to stolon.

The membrane consists of a mucilaginous substance, or mucin, which is a heteropolysaccharide composed of D-xylose, L-galactose, and L-arabinose (Anjaneyala, Gowda, and Neelisiddiah 1983). These authors also remarked that the presence of L-galactose is a rare constituent of plant polysaccharides.

As new ramets emerge from buds, they are wrapped entirely in a tube-like membrane filled with this mucin, the physiological role of which is unclear. Since this mucin contains polysaccharides, normally found as constituents of hemicellulose in cell walls, it may relate to insect or disease defense mechanisms of the young ramets.

### Carbohydrate allocation

**Sugar species in reserve carbohydrates.** There are two major types of reserve carbohydrates in plants, starch and fructan. However, results of thin-layer chromatography (TLC) showed that waterhyacinths do not store fructan as a reserve carbohydrate. In fact, starch and sucrose were the main components of carbohydrates in the stembase during the winter. TLC also indicated the presence of fructose and glucose in the stembase. The presence of sucrose, fructose, and glucose indicates that part of the starch and sucrose had been converted into simple sugars for the development of young tissues in response to warm days occurring during November and December of 1986.

Reserve carbohydrates are stored mainly in the stembases of waterhyacinth. During the winter, most of the top growth, especially the tall leaves and petioles, are killed by freezing temperatures. These dead tissues serve to envelop and protect the stembases

from freezing; however, if freezing temperatures persist for several days, many stembases may also die. Overwintering stembases are the sources of young ramets emerging in the spring. Therefore, the reserve carbohydrate in the stembases is a very important source of energy for early spring development of ramets.

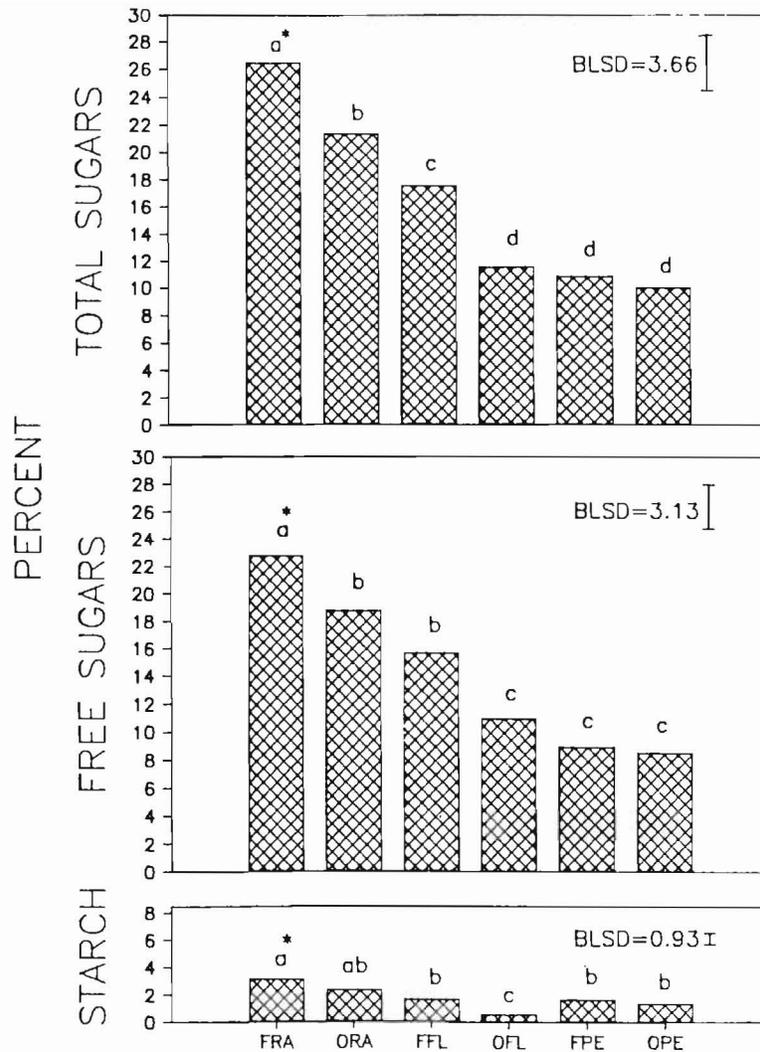
*Carbohydrate levels in selected plant structures.* In the first year study (data not presented) blooming inflorescences contained over 15 percent free carbohydrates and over 2 percent starch. Except for the stembase, blooming inflorescences had five to six times higher free sugar as compared to other plant structures. When the waterhyacinths did not flower, the highest levels of carbohydrates were found in mature leaves (6 percent free carbohydrates) and stembases (7 percent free carbohydrates).

In the second year study (data not presented), highest levels of carbohydrates were found mostly in the stembases and mature leaves (except the samples of June 1987). Mature leaves were found to have the highest level of starch, while stembases often had the highest level of free and total carbohydrates.

The high levels of starch observed in mature leaves were expected, since starch is formed directly from photosynthesis during the day and accumulates in chloroplasts (Salisbury and Ross 1985). In the late afternoon and at night, starch is gradually converted to sucrose for translocation to other plant structures (Hilliard and West 1970, Mason and Maskell 1928). Sucrose from leaves may move down to the stembase where it is converted to starch and stored in the amyloplasts of the stembases (Huber et al. 1985, Wiemken et al. 1986, Salisbury and Ross 1985).

*Carbohydrate distribution among inflorescence structures.* The inability of waterhyacinth to produce ramets two weeks after flowering stimulated our interest in investigating the distribution of energy among the inflorescence structures during the following blooming. Highest carbohydrate levels were found in the blooming rachis (26 percent total carbohydrate). Free carbohydrate and total carbohydrate decreased significantly in the rachis and florets after the inflorescences wilted. However, no change in carbohydrate content was found in the peduncles, even after the inflorescences wilted. This result suggests that the carbohydrate moved from the rachis to the stem via the peduncle, following flower wilt (Figure 4). This movement may act as a mechanism to conserve energy in the plant system, whereby the remaining carbohydrates in the rachis were translocated towards other energy demanding sinks (meristematic tissues of ramet or flower).

*Seasonal carbohydrate levels.* Carbohydrate levels of most plant structures fluctuated, with no clear trend. However, carbohydrate levels in stembases were significantly greater and highest in samples from October 15, 1986 (of the first year study), in comparison to other sampling dates (Figure 5). Under our experimental conditions, October appears to be the time when waterhyacinths build up their carbohydrate reserves in the stembases. This fact is verified by the results of the second year study in which we sampled from June 1987 to July 1988 (data presented only for samples from June to November 1987). The concentration of carbohydrates in the stembases gradually increased beginning in July and attained the highest concentration in October (Figure 6). The reduction of carbohydrate reserve in the stembases, after October, resulted from having several young ramets in the plant samples, and from increase of dead tissue around the rhizomes.



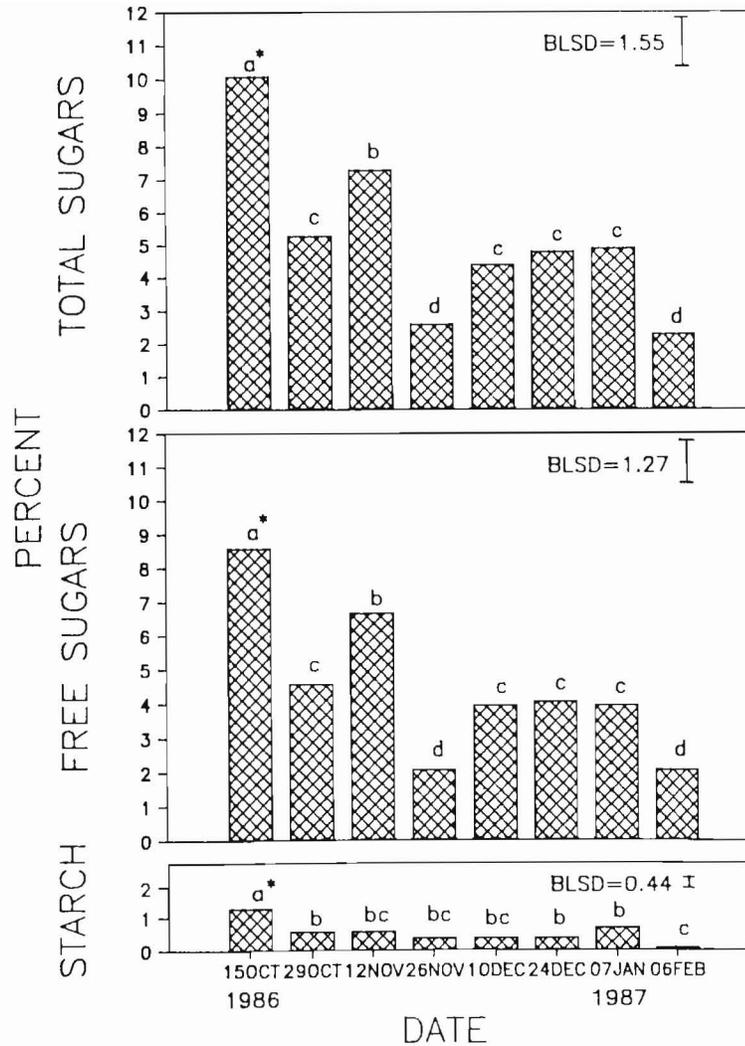
\*Means with the same letter are not significantly different according to Bayesian LSD (K-ratio=100).

Figure 4. Carbohydrate contents in different parts of inflorescence. Flowering rachis (FRA), old rachis (ORA), flowering floret (FFL), old floret (OFL), flowering peduncle (FPE), and old peduncle (OPE)

## CONCLUSIONS

Interim conclusions from the study are:

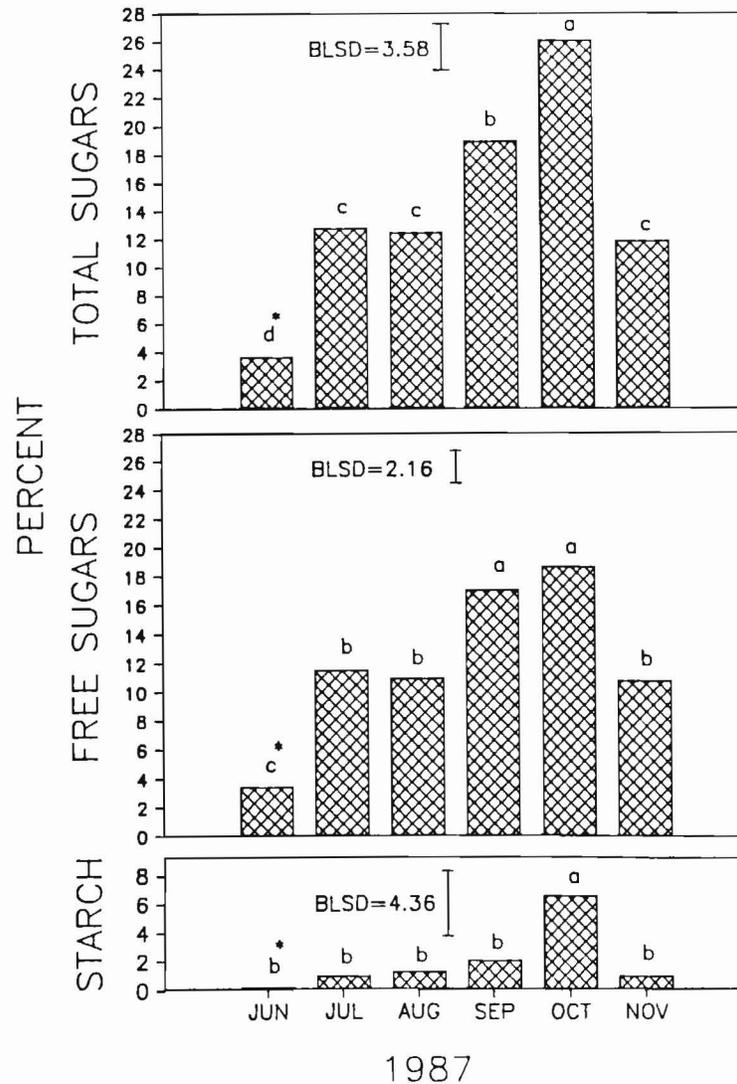
- Carbohydrate levels of most plant structures (except the stembases) fluctuated with no definite trend during pre-winter and winter periods.
- Mature leaves tend to have the highest level of starch, while stembases often have the highest level of free carbohydrates.
- Waterhyacinth stores carbohydrates in the stembases (rhizomes), primarily as starch and sucrose.



\*Means with the same letter are not significantly different according to Bayesian LSD (K-ratio=100)

Figure 5. Carbohydrate contents in stembases at different sampling times during the first year experiment (1986)

- d. Mid-October appears to be the time when waterhyacinths store the maximum carbohydrate reserve in the stembases.
- e. Blooming inflorescences have the highest total carbohydrate concentration compared to many other plant structures, with the exception of the stembases at maximum peak of carbohydrate storage.
- f. Flowering curtails the ability of waterhyacinth to produce young ramets and growth in general.
- g. During blooming, the inflorescence is a strong energy demanding sink in the plant, with carbohydrates concentrating in the rachis. Apparently, the remaining carbohydrates in the rachis translocate toward other energy demanding sinks, following flower wilt.



\*Means with the same letter are not significantly different according to Bayesian LSD (K-ratio=100)

Figure 6. Carbohydrate contents in stembases at different sampling times during the second year experiment (1987)

Based on these interim results and conclusions, potential weak points in the life cycle of waterhyacinth include:

- a. The period shortly before mid-October when the plants are actively translocating carbohydrates to the stembases accumulation as an energy reserve.
- b. The period shortly before blooming when inflorescences are actively developing that coincides with the period of slowest ramet production.

### ACKNOWLEDGMENTS

The author wishes to thank Cindy Waddle, Cindy Teeter, and Dave Stuart for their technical assistance in this study.

## Part II. Carbohydrate Allocation and Growth in Monoecious and Dioecious Hydrilla\*

### OBJECTIVES

Studies on carbohydrate allocation during the growth cycle for the troublesome aquatic plant hydrilla are limited. Although starch has been assayed on ungerminated hydrilla tubers (Miller, Garrard, and Haller 1976), starch levels in tubers following germination and other growth periods have not been studied. Hydrilla may be more susceptible to control techniques during a period when starch is depleted in germinating tubers. Information is needed to determine how carbohydrate allocation occurs following tuber germination and over the growth cycle.

The following pilot laboratory study was designed to determine carbohydrate levels in hydrilla plants germinated from tubers. The objective of this study was to compare carbohydrate allocation and growth of monoecious and dioecious hydrilla following tuber germination. Potential weak points in the growth cycle of hydrilla will be identified based on this study and future studies.

### MATERIALS AND METHODS

Monoecious and dioecious hydrilla tubers were allowed to germinate in an incubator with cool-white fluorescent lamps at 30° C under a 16:8-hr L:D photoperiod. Three germinated tubers were planted in 2-l containers filled with sediment from Brown's Lake, Vicksburg, Mississippi, and covered with washed silica sand (5-mm depth). The tubers were placed below the sediment with 1-cm shoots piercing out of the sand. Fifteen containers of each biotype were placed in 1268-l fiberglass tanks (90-cm water depth) at water temperature of 22±1° and 32±1° C. The nutrient solution overlying the plants has been described previously by Barko and Smart (1986). Lighting was supplied by Sunbrella® fixtures with 400-w multivapor and NaI lamps. Plants were grown on a 16:8 L:D photoperiod for four weeks, and switched to an 8:16 L:D photoperiod from weeks 5-8 to promote propagule production.

Three replicates were harvested at 2, 4, 6, and 8 weeks following planting, from each tank, in a completely randomized experimental design for each biotype. The treatments were the harvesting dates, and measured parameters were biomass, free sugars, and starch.

Plant parts (shoot, root, tuber, new tuber, and new turion) were dried at 55° C for 48 hr, cooled in a desiccator, weighed, and ground in a cyclone mill. Free sugar and starch in plant parts were determined in duplicate by a modification of the procedure of Swank et al. (1982). Extracts for total sugars were incubated for 15 min at 55° C with 1 unit of amyloglucosidase (Sigma No. A-3042) to achieve complete starch hydrolysis (Nelson 1944). Free sugar content was also determined on extracts not incubated with amyloglucosidase.

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\*Prepared by G.J. Pesacreta.

The Waller-Duncan test was used to separate the effects of treatment means. The Student t-test was used to compare treatment effects between biotypes for each sample date.

## RESULTS

### Biomass production and development

Shoot biomass from germinated tubers increased with time for the monoecious and dioecious biotypes at both water temperatures (Table 1). Shoot dry weight of monoecious plants was twice that of dioecious plants at 22° C by week 2, but no difference was found during subsequent weeks. At 32° C, initial production rates were similar; however, from the 4- to 8-week period, dioecious hydrilla biomass was still increasing while biomass for the monoecious plants did not change. By week 8, shoot fragmentation occurred in both biotypes at 32° C, but not at 22° C. Root production was similar between biotypes at both temperatures.

Table 1  
Biomass (g dry wt) for Monoecious and Dioecious Hydrilla Plants Grown from Three Tubers at 22° and 32° C. Means Within a Column followed by the Same Letter are not Significantly Different According to the Waller-Duncan Test ( $p \leq 0.05$ )

Week	Monoecious						Dioecious			
	Shoot	Root	Tuber	Shoot Frag.	New Tuber	New Turion	Shoot	Root	Tuber	Shoot Frag.
22° C										
0			.12a*						.24a*	
2	.19a*	.02a	.06b*				.08a*	.02a	.12b*	
4	1.95b	.31b	.04bc*				1.73b	.28bc	.12b*	
6	3.48c	.50c	.03c				3.72c	.46bc	.06c	
8	4.92d	.53c	.03c		.05	.06	6.35d	.69c	.05c	
32° C										
0			.14a*						.22a*	
2	1.65a	.11a	.04b*				1.78a	.14a	.12b*	
4	5.11b	.33b	.05b				4.98b	.38ab	.05c	
6	6.67b	.53c	.03b				7.17c	.54b	.02cd	
8	5.89b*	.59c	.02b	.35	.05		8.63d*	.52b	0d	.27

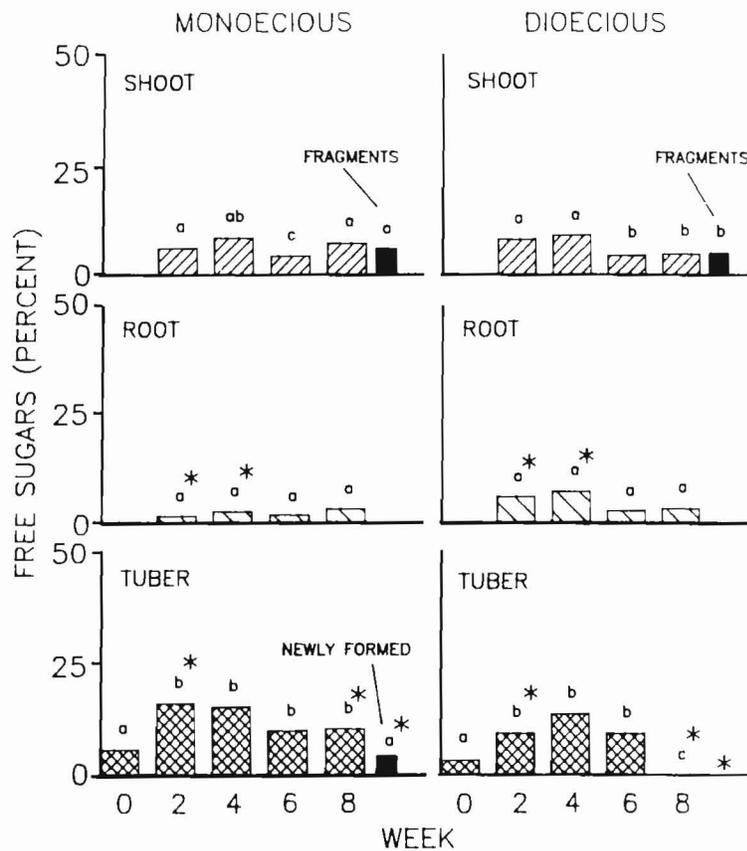
\*Differences between biotypes at each week.

The greatest decrease in tuber mass occurred during the initial two weeks of growth and had declined nearly 75 percent by week 6. By week 8, the dioecious plants at 32° C had completely exhausted their tuber mass. Tuber mass was never completely consumed in the monoecious plants at 32° C and in both biotypes grown at 22° C.

Monoecious plants produced a mean ( $\pm 1$  SE) of 10.7 (1.3) new tubers and 6.5 (0.5) new turions per container at 22° C, and 5.1 (0.6) new tubers per container at 32° C. The dioecious biotype did not produce additional vegetative propagules.

## Carbohydrate allocation

Generally, free sugars were lowest during the initial sampling periods. The free sugar level in germinated tubers was low, but as shoots matured, the amount of free sugars increased in the tubers of both biotypes (Figures 7 and 8). The highest amount of free sugars in tubers were found at weeks 2 and 4. Free sugars from dioecious tubers were completely depleted by week 8 at 32° C. Patterns of free sugars were less apparent for shoots and roots.

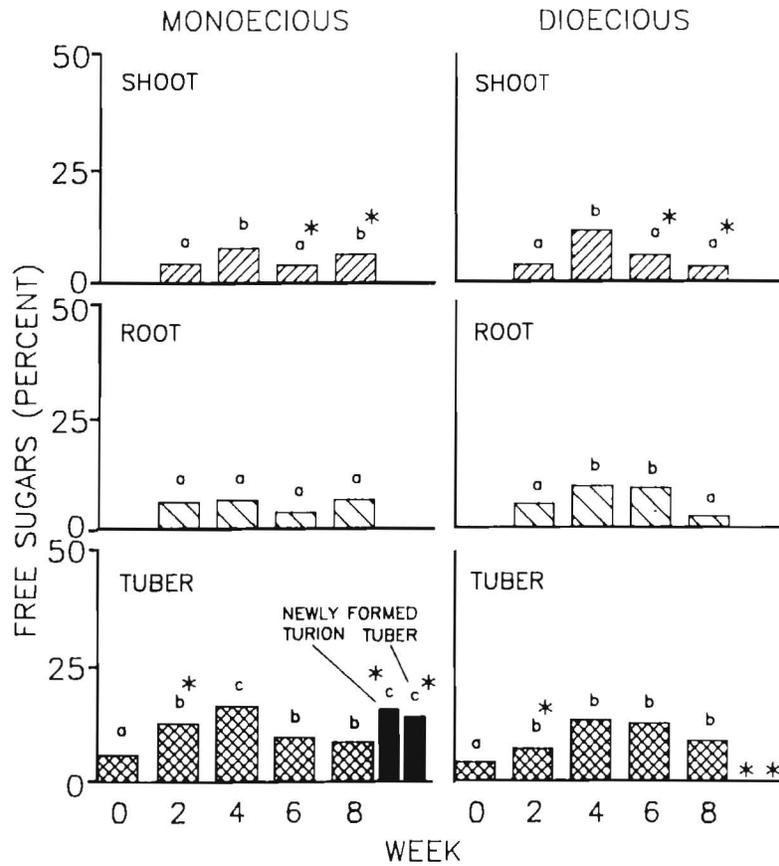


\* Differences between biotypes at each week.

**Figure 7. Free Sugars (percent) for monoecious and dioecious hydrilla plants grown at 22° C water temperature. Means within a column followed by the same letter are not significantly different according to the Waller-Duncan test ( $p \leq 0.05$ )**

Starch trends were similar for shoots and tubers of both hydrilla biotypes. At 22° C and 32° C, the initial concentrations of shoot starch were low, i.e. near 8-10 percent (Figures 9 and 10). Shoot starch levels by week 6 had increased approximately five-fold in all tanks. By week 8, starch levels decreased in shoots of dioecious hydrilla at 32° C. Starch content from recovered shoot fragments was low for both biotypes.

The highest starch levels were found in the freshly planted tubers and newly formed tubers and turions. Starch content of freshly planted tubers was approximately 55-60 percent for both biotypes. The greatest change in tuber starch concentration occurred in all tanks during the first two weeks of development, as



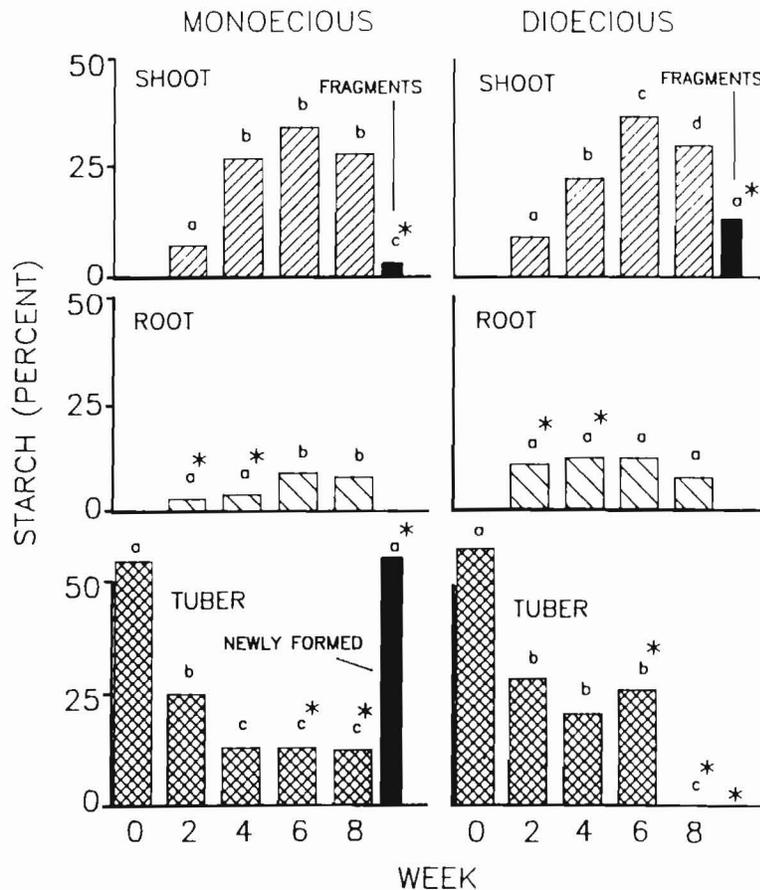
\* Differences between biotypes at each week.

Figure 8. Free sugars (percent) for monoecious and dioecious hydrilla plants grown at 32° C water temperature. Means within a column followed by the same letter are not significantly different according to the Waller-Duncan test ( $p \leq 0.05$ )

tuber starch decreased by at least 50 percent. Following this initial depletion, the decrease in tuber starch concentration was less notable. In dioecious hydrilla, complete depletion of tuber starch reserves occurred by week 8 at 32° C. Newly formed tubers and turions had starch levels similar to freshly planted tubers. Root starch levels increased with time in monoecious hydrilla at 32° C and both biotypes at 22° C. Roots contained lower concentrations of starch than any other plant part.

## DISCUSSION

Starch reserves, in ungerminated monoecious and dioecious tubers were similar to levels reported for dioecious hydrilla by Miller et al. (1976); however, these authors did not report starch levels in germinated tubers. The level of tuber mass and starch depletion following germination in this study demonstrated that starch reserves decrease following initial growth, but some starch does remain. This result differs from studies on *Elodea nuttallii* (Planch.) where starch reserves in underground organs were depleted by 0.4 days following spring growth (Best and Dassen 1987). The authors



\* Differences between biotypes at each week.

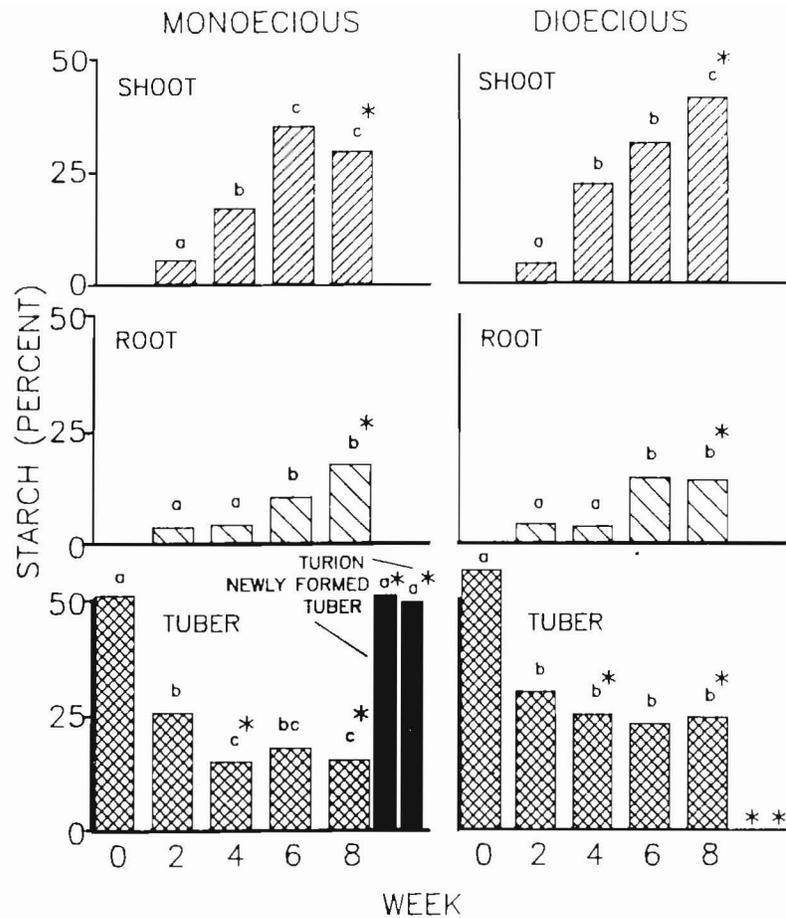
**Figure 9. Starch (percent) for monoecious and dioecious hydrilla plants grown at 22° C water temperature. Means within a column followed by the same letter are not significantly different according to the Waller-Duncan test ( $p \leq 0.05$ )**

speculated that proteins were serving as an alternative energy source maintaining the plant through spring growth and development.

Both hydrilla biotypes accumulated a greater percentage of starch in shoots when exposed to a short photoperiod. Similarly, Haller (1974) found that dioecious hydrilla shoots collected in September contained three times more starch than shoots collected in January. Haller speculated that hydrilla was storing starch in the shoots as food reserves for over-wintering.

Shoot accumulation of starch may precede translocation of carbohydrates to storage organs (e.g. tubers and turions) for over-wintering. Starch levels in the shoots were greatest following exposure to a short photoperiod. Perhaps the shorter photoperiod triggered the accumulation of starch in the shoots. In fact, this phenomenon has been reported by others. Results from two field studies showed that an accumulation of TNC in Eurasian watermilfoil roots occurred as daylength shortened in autumn (Titus and Adams 1979, Kimbel and Carpenter 1981).

Preventing starch storage in shoots may decrease the amount of carbohydrates



\* Differences between biotypes at each week.

Figure 10. Starch (percent) for monoecious and dioecious hydrilla plants grown at 32° C water temperature. Means within a column followed by the same letter are not significantly different according to the Waller-Duncan test ( $p \leq 0.05$ )

available for translocation to tubers, thus reducing the formation of belowground, over-wintering propagules.

## CONCLUSIONS

Dioecious hydrilla tubers, grown at 32° C, completely exhausted tuber carbohydrates by 8 weeks, while tubers of the monoecious biotype did not. Monoecious plants allocated starch into tuber and turion production when exposed to short photoperiods, while both biotypes appear to concentrate carbohydrates in shoots. Differing patterns of starch allocation in monoecious and dioecious hydrilla may be useful in assessing individual survival potential of the biotypes. Based on these preliminary results, a potential weak point in the growth cycle of both hydrilla biotypes occurs after tuber starch is depleted following germination.

## ACKNOWLEDGMENTS

The author wishes to thank Kien Luu for his assistance with the carbohydrate analysis and Cindy Waddle, Cindy Teeter, and Dave Stuart for their technical assistance in this study.

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**COMPUTER-AIDED SIMULATION PROCEDURES  
FOR AQUATIC PLANT CONTROL**

# **Simulation Technology Development An Overview**

**by  
R.M. Stewart\***

## **Background**

Under the Aquatic Plant Control Research Program (APCRP), computer-based simulation models are being developed to provide systematic procedures (Sabol 1985) to allow aquatic plant control managers to ask "what if" questions about a proposed control technique. Some examples of questions are: (a) What level of control would result from applying this control agent at a given rate to a particular aquatic plant infestation? (b) How much will it cost to apply this technique to a particular plant infestation? (c) How long after initial application before control will be realized? and (d) What is the duration of the control? This type information provides a basis for selecting the best control technique(s) to apply in a given operational environment, and for planning the most effective application schedule for the selected technique(s).

The long range goal of this research is to provide aquatic plant managers with computer-aided evaluation procedures for all commonly used aquatic plant control techniques. Simulation procedures developed under the APCRP that are currently available for operational use include HARVEST (Hutto 1982, 1984; Sabol 1983; Sabol and Hutto 1984) and STOCK (Miller and Decell 1984). Research is underway to develop simulation models for various biological and chemical control techniques applicable to various aquatic plants such as waterhyacinth, hydrilla, Eurasian watermilfoil, and waterlettuce. Table 1 lists the biological and chemical control agents that are being considered under the Simulation Model Technology Development research area.

Currently three separate research tasks have been established to support development of these computer-based models. Work being conducted under each task is briefly described in the following sections.

## **PLANT GROWTH SIMULATIONS**

### **Objective**

The objective of this research is to develop personal computer-based plant growth models for waterhyacinth, hydrilla, Eurasian watermilfoil, and waterlettuce. These plant growth models will be structured to function independently or as functional modules within other broad-based simulation models of biological and chemical control techniques.

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\*U.S. Army Engineer Waterways Experiment Station, Vicksburg, Mississippi.

**Table 1**  
**Biological and Chemical Control Agents to be Included in Simulation Models Developed by the Aquatic Plant Control Research Program**

<i>Target Plant</i>	<i>Control Technique Category</i>	<i>Control Agents</i>
Waterhyacinth	Biological	<ul style="list-style-type: none"> <li>• <i>Neochetina eichhorniae</i></li> <li>• <i>Neochetina bruchi</i></li> <li>• <i>Sameodes albigitallis</i></li> </ul>
	Chemical	<ul style="list-style-type: none"> <li>• 2,4-D (DMA)</li> <li>• diquat</li> <li>• glyphosate</li> </ul>
Hydrilla	Biological	<ul style="list-style-type: none"> <li>• White Amur (diploid)</li> <li>• White Amur (triploid)</li> <li>• <i>Bagous affinis</i></li> <li>• <i>Hydrellia</i> sp.</li> </ul>
	Chemical	<ul style="list-style-type: none"> <li>• 2,4-D (BEE)</li> <li>• diquat</li> <li>• endothall</li> <li>• fluridone</li> </ul>
Eurasian watermilfoil	Biological	<ul style="list-style-type: none"> <li>• White Amur (triploid)</li> </ul>
	Chemical	<ul style="list-style-type: none"> <li>• 2,4-D (BEE)</li> <li>• diquat</li> <li>• endothall</li> <li>• fluridone</li> </ul>
Waterlettuce	Biological	<ul style="list-style-type: none"> <li>• <i>Neohydronomus pulchellus</i></li> <li>• <i>Namangana pectinicornis</i></li> </ul>
	Chemical	<ul style="list-style-type: none"> <li>• diquat</li> </ul>

### **FY 1987 accomplishments**

***Validation of waterhyacinth plant growth model.*** The waterhyacinth growth model (Akabay, Wooten, and Howell 1988) has been partly validated with field data collected monthly from April 1986 through December 1987 from four sites in south Florida and three sites in north Florida. Results showed close agreement between simulated and field measured estimates of seasonal changes in the waterhyacinth populations at these sites.

***Development of hydrilla plant growth model.*** A first-generation hydrilla plant growth model was coded in FORTRAN for implementation on a personal computer. The model is more sophisticated than the hydrilla growth routine included in STOCK. The current model calculates daily hydrilla biomass as influenced by temperature and solar radiation within a water column having a surface area of 1 sq m. The water column within this area is subdivided into 0.1-m layers, with a maximum of 40 layers (i.e. 4-m total depth) possible. Maximum time period of the first-generation model is 365 Julian days. This model is described in more detail in the paper by Wooten and Akabay in these proceedings.

### **FY 1988 scheduled work**

***Validation of waterhyacinth plant growth model.*** During FY 88 further validation of the waterhyacinth growth model will be accomplished with additional field data collected from sites in Florida and Texas. This work will provide further evaluation of the applicability of the plant growth model in different geographic regions.

*Validation of hydrilla plant growth model.* The first-generation hydrilla growth model will undergo validation tests using field data collected at numerous sites throughout the United States.

## BIOLOGICAL CONTROL SIMULATIONS

### Objective

The objective of this research is to develop personal computer-based simulation models of the various biological control agents available for aquatic plant control. These models will be composed of two major modules or components. Module I will address responses of the targeted plant population to specified conditions of temperature and solar radiation over a selected time period. This module will be a functional version of the appropriate plant growth model discussed above. Module II will provide simulation responses of the biocontrol agent population under the same environmental conditions and time period. This module will also account for interactions between the biocontrol agent population and the plant population, thereby determining the amount of damage inflicted on the in situ plant population during the simulation period.

### FY 1987 accomplishments

*Validation and modifications of INSECT.* INSECT, Version 1.0 (Akbay, Wooten, and Howell 1988) was partly validated using field data collected during April-December 1986 from sites in north and south Florida. Improvements that have been made to the model based on results of the validation tests were: (a) inclusion of an upper temperature threshold value for *Neochetina* development, (b) improvements to temperature/fecundity relationships for *Neochetina* adults, (c) inclusion of algorithms that allow assignment of "physiological age" to cohorts during the initialization process, and (d) improvements to the relationships for low temperature induced mortality, particularly for immature stages of *Neochetina* during late winter months. Also, we have enhanced the "user-friendliness" in the use of the model. These changes are described in more detail in the paper by Howell, Akbay, and Stewart in this proceedings (pp 154-163).

*Development of HYDAMUR.* A revised stocking rate model for triploid white amur (HYDAMUR) was developed for implementation on a personal computer. Though similar to the original STOCK model, HYDAMUR (Version 1.0) incorporates the revised hydrilla plant growth model described above. HYDAMUR (Version 1.0) is described in greater detail in the paper by Wooten and Akbay in this proceedings (pp 164-170).

### FY 1988 scheduled work

*Further validation of INSECT.* Field data collected in Florida and Texas during 1987 will be used to further validate improved relationships included in INSECT (Version 2.0). By combining the 1986 and 1987 Florida data for particular sites, the model will be evaluated using data for two complete growing seasons.

*Validation of HYDAMUR.* Validation tests (HYDAMUR (Version 1.0)) will be conducted using field data from numerous sites in the United States. Field tests conducted in Florida in recent years with triploid white amur should provide excellent data for these validation efforts.

## CHEMICAL CONTROL SIMULATIONS

### Objective

The objective of this research is to develop personal computer-based simulation models for available chemical control techniques. These models will also be composed of separate modules for predicting the fate of herbicides in the environment following application and for predicting the effect of the subsequent herbicide dose(s) on the target plant population. By coupling this module with the appropriate plant growth model, the overall chemical control simulation model will provide a simulation of the interaction of a herbicide application on the target plant population and the response or regrowth of the plant population following treatment.

### FY 1987 accomplishments

*Development of FATE.* A first-generation fate and target species effects model (FATE) was developed. FATE (Version 1.0) evaluates the effectiveness of 2,4-D (DMA) applications for waterhyacinth control. A detailed description of this model is given by Rodgers, Clifford and Stewart in this proceedings (pp 171-178).

### FY 1988 scheduled work

*Validation of FATE.* Validation tests of FATE (Version 1.0) will be conducted using laboratory and field data. These data will evaluate algorithms for 2,4-D uptake and translocation, percent mortality versus dose, and plant regrowth following treatment effect.

*Inclusion of additional herbicides in FATE.* FATE will be modified to include additional herbicide/plant species combinations. Herbicides that will be included are diquat, endothall, glyphosate, and fluridone. Effective algorithms for these herbicides will be developed to include Eurasian watermilfoil and hydrilla.

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# Field Validation and Improvements to INSECT, a Personal Computer Simulation Model

by

Fred F. Howell\*, Kunter S. Akbay\*\*, and R. M. Stewart†

## INTRODUCTION

In the development of any simulation model, it is necessary to eventually “test” the model’s performance against “real world” conditions to establish the credibility of the model’s assumptions and to test the accuracy of its algorithms. The eventual usefulness of a model will depend upon how well the completed model and its components represent reality (Bell 1981).††

INSECT is being developed by the Aquatic Plant Control Research Program (APCRP), Waterways Experiment Station, to predict waterhyacinth growth and the impacts of biocontrol agents on the growth and productivity of the host plants. The first generation model of INSECT (Version 1.0) is described in Akbay, Wooten, and Howell (1988).‡ Potential uses of the model are described in Howell, Wooten, and Akbay (1987).‡‡

APCRP initiated a 2-year field study in early 1986 to specifically collect data for validation of INSECT. The 1986 portion of those data was made available to us early in 1987. In this paper we describe our progress in making improvements to Version 1.1 of the model and illustrate the model’s performance against field data collected from two geographic regions in Florida in 1986. Our primary interests at midpoint in this validation study are: (a) that the timing of development for insects be an accurate reflection of field situations, (b) that magnitude of abundances of insect life stages be reasonable, and (c) that the predicted plant biomass fall within or near field estimates. Since field sampling at these sites has continued uninterrupted, the completed validation study using 2-year comparisons for each site will be made during the coming contract year.

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††Bell, C. J. 1981. “The Testing and Validation of Models,” *Mathematics and Plant Physiology*, D. A. Rose and D. A. Charles-Edwards, eds., Academic Press, New York, pp 299-309.

‡Akbay, K. S., Wooten, J. W., and Howell, F. G. 1988. Computer Simulation Modeling of Waterhyacinth and Their Biocontrol Agents,” Technical Report A-88- , US Army Engineer Waterways Experiment Station, Vicksburg, Miss.

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## FIELD VALIDATION DATA

Field data used in this study were collected from three sites in north Florida by Kim Haag, US Department of Agriculture (USDA)—University of Florida, and from four sites in south Florida by Ted Center, USDA—Fort Lauderdale. The data base provided us by Center and Haag included monthly samples (on per square-metre basis) for plant biomass (kg), plant density (number), detritus (kg), numbers of adults, pupae, and larvae for *N. eichhorniae* (at all sites) and *N. bruchi* (one site in north Florida and at all sites in south Florida.) Additional insect data for all sites included male to female ratio, number of females with eggs, and number of males and females with wing muscles. Regional weather data sets accompanied the biological data. These data sets included daily accumulated solar radiation (in watts), and minimum and maximum temperature (°C) for each 24-hr period.

## SIMULATIONS VERSUS FIELD DATA

For the sake of this presentation, validation results for only three data sets are shown. Because several algorithms used by the model were altered, it was necessary to reevaluate the model's performance against the original validation data used in the First Generation Model (Version 1.0), i.e. 1976 Lake Alice data.\* Incorporation of these changes has led to the creation of Version 1.1 of INSECT. The other two validation results represent one site from north Florida (site PP) and one site (CA) from south Florida. Validation results for all six 1986 Florida sites are described in Wooten, Akbay, and Howell (1987).\*\*

Approximate starting numbers for insects were estimated by using the first three sampling periods from a site specific data set and back-calculating to determine numbers of individuals. "Ages" were assigned by back-calculating with day-degree data to determine "best-fit." Often multiple runs had to be made to arrive at reasonable starting numbers. Plant biomass for a simulation was estimated based upon the first biomass value reported in 1986 for a given site.

### Lake Alice 1976 data.

Starting conditions for the 1976 Lake Alice simulations were as follows: plant biomass - 0.705 kg/sq m; adults - 5 on Julian Day (JD) 42; 5 on JD 68; 10 on JD 95; and pupae - 8 on JD 42. Simulation results of the model's performance plotted against the 1976 Lake Alice data are presented in Figures 1A through 1C. The major improvement in estimates of plant biomass were in the mid-growing season. Version 1.1 presented a more accurate representation of the 1976 field data; whereas Version 1.0 tended to over estimate midseason waterhyacinth biomass. For adult *N. eichhorniae*, Version 1.1 failed to meet the 95 percent confidence intervals of field data in only 3 of 44 cases beyond JD 60;

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\*Akbay, Wooten, and Howell, op. cit.

\*\*Wooten, J.W., Akbay, K.S., and Howell, F.G. 1987. "Computer Simulation Modeling of Aquatic Plants and Their Biocontrol Agents," Final Report: Phase II, prepared for the Aquatic Plant Control Research Program, US Army Engineer Waterways Experiment Station, Vicksburg, Miss.

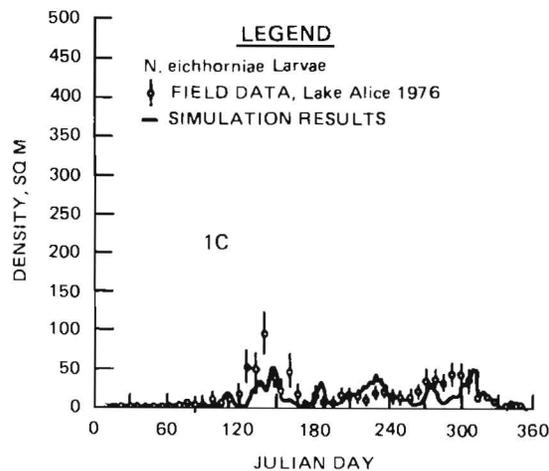
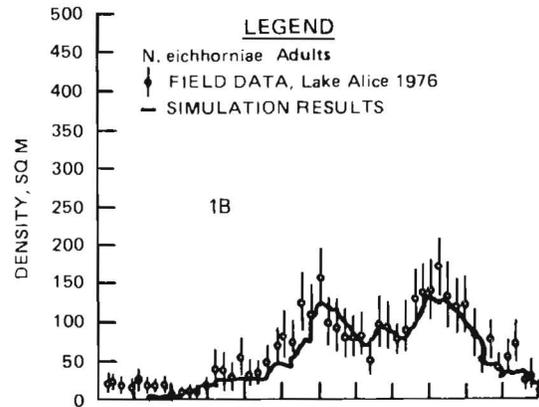
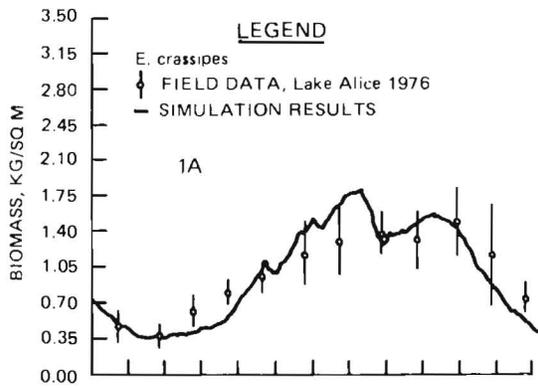


Figure 1. Version 1.1 simulation results plotted against 1976 Lake Alice field data (see text for starting conditions). A = plant biomass data; B = adult *N. eichhorniae* data; c = larval *N. eichhorniae* data

Version 1.0 failed to meet these intervals in 12 of the same 44 cases. Whereas estimates of larval density appeared “out-of-phase and overestimated” in Version 1.0, simulation results generated by Version 1.1 indicated a congruent trend in both timing and numbers of individuals. Adjustments made in the model appear to produce better estimates of both waterhyacinth and *Neochetina* than did the first generation model.

### North Florida 1986 Site “PP”

Starting numbers for model simulation of waterhyacinth and *Neochetina* dynamics sampled at site “PP” in north Florida were determined to be as follows: plant biomass - 1.11 kg/sq m on JD 1; *Neochetina* spp. - pupae - 25 on JD with one-third development complete; larvae - 37 on JD 1 with one-third development complete. PP is the only site among the three north Florida sites sampled which contained *N. bruchi* as well as *N. eichhorniae*. *Neochetina eichhorniae* exceeded *N. bruchi* by about 10 to 1.

Plant biomass values generated by the model closely tracked the monthly means for field biomass data for this site (Figure 2A). A major departure between model predictions and field data occurred during the last two months of the year. In this case, the model simulation showed a continual decrease in plant biomass after approximately

JD 250, while field estimates showed a gradual increase during this same time period. This discrepancy between model simulations and field estimates was common for all three north Florida sites. Assuming the field data are accurate, the tendency of the model to underestimate plant biomass during the end of the year at certain sites indicates a need to improve and refine model relationships that describe seasonal plant growth characteristics (e.g. root:shoot ratios).

Model predictions for adult *N. eichhorniae* tracked field data for all the north Florida sites. Figure 2B illustrates the comparison for site PP, although total numbers of insects rarely exceeded the field data. In all cases, population peaks between the field and simulation data sets were accurate. Simulations for *N. bruchi* adults (Figure 2C) at site PP met all the 95 percent confidence intervals of associated field data. Trends of population peaks were in agreement.

Predictions of abundance of approximate third instar larvae are probably the most critical in terms of model accuracy since this insect stage has the major impact to host plants. Too, timing and magnitude of occurrences of third instar larvae probably serve as the most important indicators of accurate starting numbers, simply because individuals do not persist as larvae as long as they do as adults. Therefore, accurate reflections of larval abundance and timing will ultimately determine the accuracy and efficacy of the model.

Model predictions for the third instar larval populations at the north Florida sites for the most part delivered good representations of those collected from the field. Some of the difficulties here are that, particularly during early to mid-growing season, third instars can develop faster than the intervals sampled. This practice can, and probably does, lead to misconceptions concerning larval abundances. The early-year “misses” as illustrated in Figures 2D are due to the inability of the operating version of the model to record “age adjusted” entry numbers. (This problem was detected too late for correction for this paper but will be corrected during the coming contract year.) Otherwise, model predictions followed the same trend as field data, i.e. larger numbers during the early part of the year, followed, in turn, by progressively smaller numbers toward the end of the year.

### **South Florida 1986 site “CA”**

Starting numbers for model simulations of waterhyacinth and *Neochetina* spp. dynamics sampled at site “CA” in south Florida were as follows: plant biomass - 1.2 kg/sq m on JD 1; 17 larvae on JD 1 with one-third development completed; 14 pupae on JD 1 with one-third development completed; and 7 adults on JD 23. Percent *N. eichhorniae* was 62; percent *N. bruchi* was 38.

Plant biomass values generated by the model for south Florida site CA are shown in Figure 3A. At this site, the trend between field data and model data was almost identical. Unlike ending numbers for the north Florida sites, estimates of plant biomass at the end of the year showed a trend of decreasing magnitudes. Model estimates of plant biomass reflected this same trend and matched the estimated field values.

Model predictions for adult *N. eichhorniae* (Figure 3B) showed good agreement with field data collected from these sites. In this case (as in simulations for the other two south

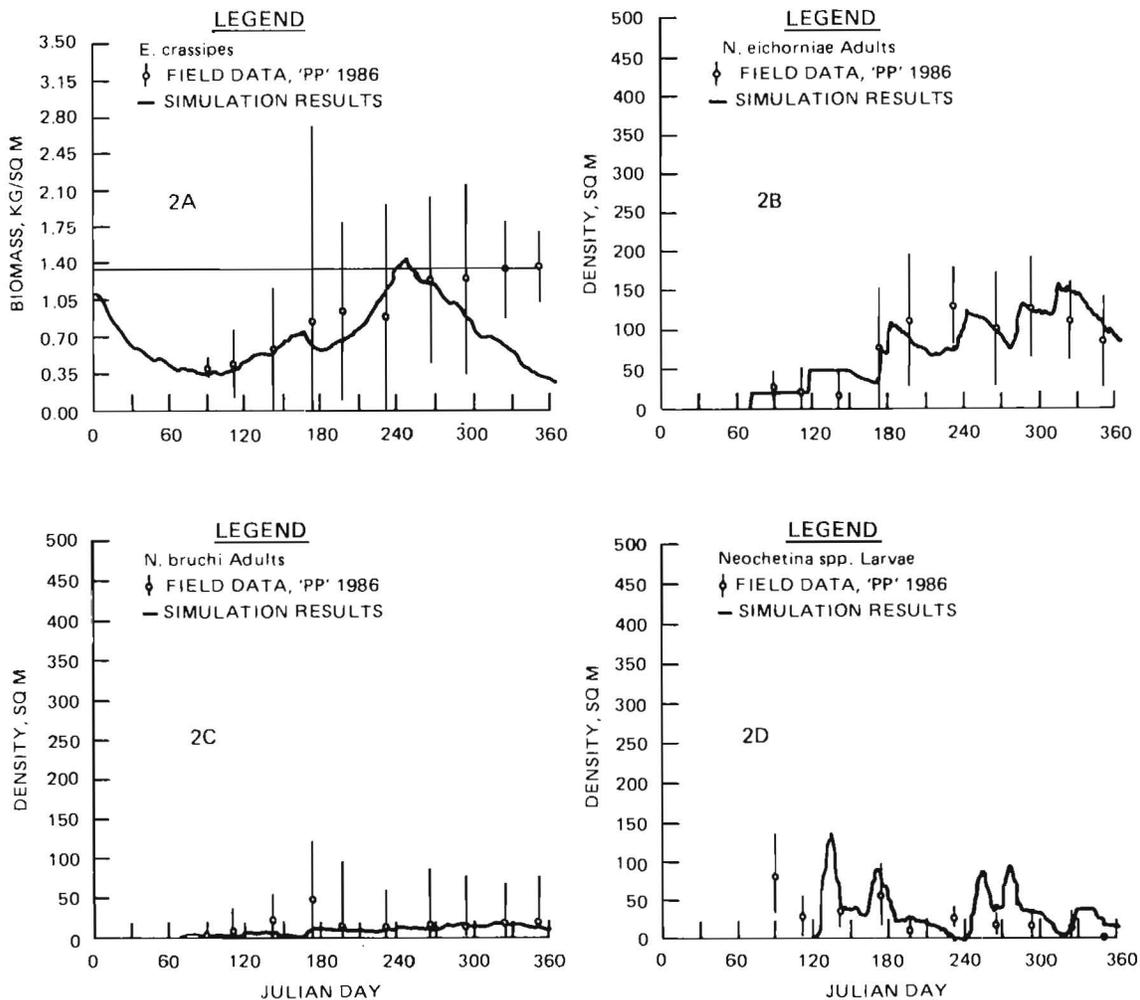


Figure 2. Version 1.1 simulation results plotted against 1986 north Florida site "PP" field data (see text for starting conditions). A = plant biomass data; B = adult *N. eichorniae* data; C = adult *N. bruchi* data; D = larval *Neochetina* spp. data

Florida sites), peaks of abundances, or timing, were accurate, and magnitudes were within or close to the 95 percent confidence intervals. Simulations for adult *N. bruchi* (Figure 3C) met the 95 percent confidence intervals in all but one case.

Basically, the same trends in large larvae abundances as observed at the north Florida sites also were reflected in the field data from the south Florida sites. The same comments made above for larvae also apply here.

## IMPROVEMENTS TO INSECT

The 1986 field data allowed us to examine several of the model's algorithms built on original but often unrelated literature.\* Because the 1986 data represented information collected within the same time frame from replicated sites from two geographic areas, it was possible to assess and compare site specific phenomena, particularly for the insect

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\*Akabay, Wooten, and Howell, op. cit.

populations. A more detailed account of these interpretations can be found in our annual report to APCRP.\* Based upon these inspections and interpretations, the following adjustments were made to INSECT (Version 1.1).

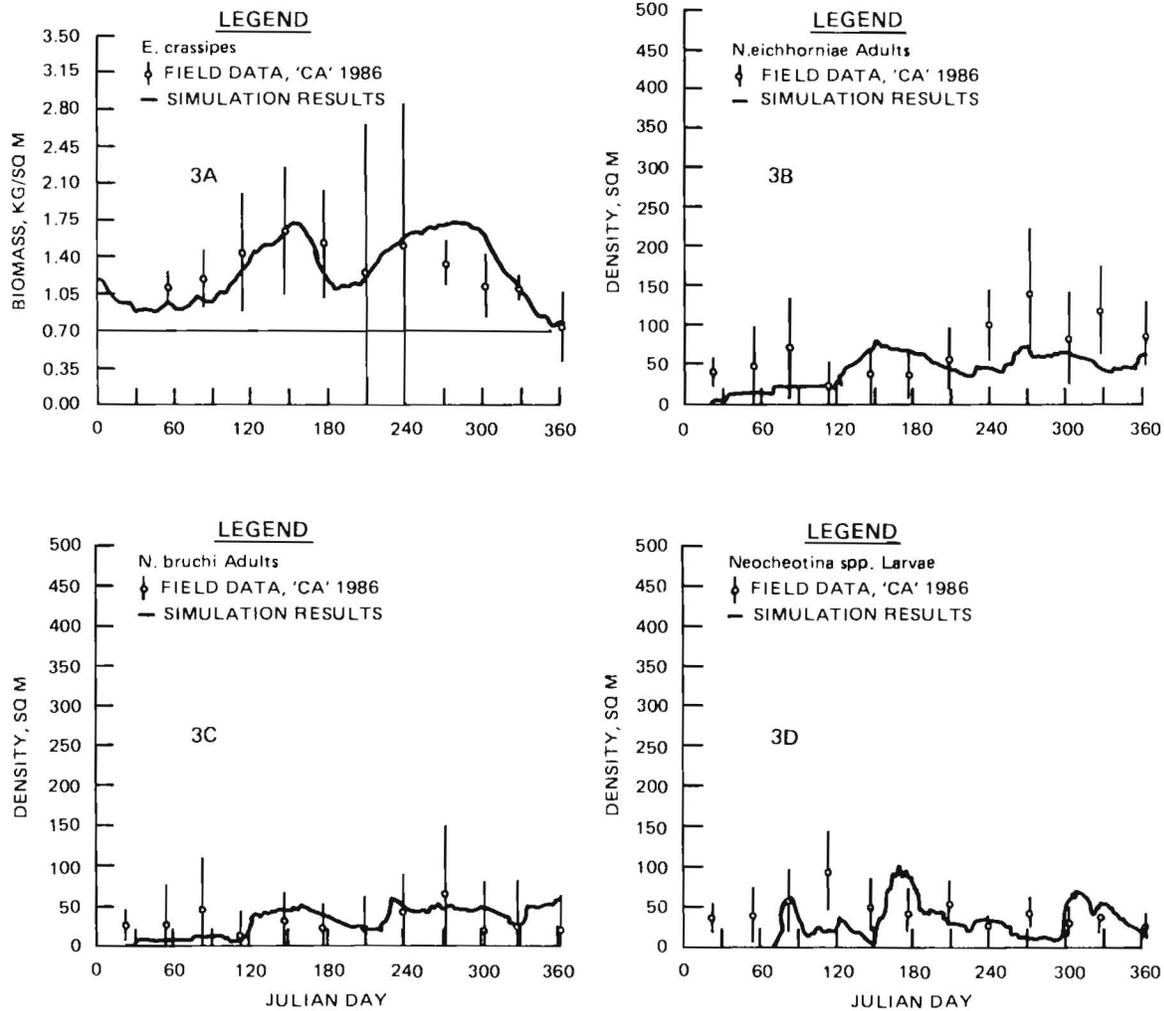


Figure 3. Version 1.1 simulation results plotted against 1986 south Florida site "CA" field data (see text for starting conditions). A = plant biomass data; B = adult *N. eichhorniae* data; C = adult *N. Bruchi* data; D = larval *Neochetina* spp. data

### Upper temperature threshold

One of the problems in developing the First Generation Model (Version 1.0) was a lack of information from other geographic regions. While we believed that *Neochetina* spp. were probably responding to upper temperature extremes, we could not make assumptions regarding its impacts. Therefore, no upper temperature limit was imposed in the model. The 1986 field data from two geographic regions in Florida, along with our

\*Wooten, Akbany, and Howell, op. cit.

knowledge of how development is calculated and “timed” in the model, permitted us to make preliminary assumptions regarding the delaying effects of high ambient temperatures upon the development of *Neochetina*. It should be understood that we have no special data which verify these assumptions, and it is entirely appropriate that some laboratory/field work be done to establish these relationships.

However, insect development as calculated in the model (Version 1.1) now reflects the effects of high ambient temperatures. This relationship is accomplished by allowing development, or acquisition of cumulative degree-days, to “free-run” between daily average temperatures from 11° to 27.0° C. At 27.0°, 16 degree-day units are added to a cohort’s cumulative total. Between 27.0° and 29.0°, only 8 degree-day units are allowed and none is accumulated above 29.0°. This algorithm, therefore, delays development of a cohort for each day in which average air temperature exceeds 27.0°.

### **Fecundity/temperature relationships**

All of the weather data sets which are currently used in INSECT, i.e. north Florida 1975 through 1980 and south Florida 1986, show that spring and early summer average air temperatures persist for extended periods in the optimum oviposition temperature ranges for *Neochetina* spp. Inspections of field data also reveal that except in cases where plant biomass becomes limiting, second generation *Neochetina* adults are more numerous than first generation adults.

This apparent relationship between oviposition and weather has been installed in Version 1.1. The fecundity/temperature relationship was adjusted so that the optimum oviposition temperature (average air temperature) is 20° C. Though slight in magnitude and given nonlimiting conditions, these changes allow the model to create a larger second generation whenever the first generation of adults is exposed to extended periods in which average air temperatures persist at or near the optimum air temperature (20° C).

### **Assignment of “age” to beginning cohorts**

Often individuals entering the population on JD 1 can not accumulate sufficient development time to appear as the next life stage “on cue” later in the year. The result is that life stages appear out of phase when compared with field data. Version 1.0 assigned individuals being used to initialize the model with zero ages, thereby ignoring whatever physiological age already accumulated by the individuals represented. The model was modified, therefore, to permit the assignment of “ages” to cohorts being used to initialize the model. A more detailed account of this mechanism is presented in our report to APCR.\*

### **Effects of sub-freezing temperatures**

In construction of these algorithms during development of the First Generation version, our interpretations of the 1976 Lake Alice data suggested that early year mortalities were mostly due to effects of subfreezing temperatures. Some of this may yet be happening, but inspection of the 1986 data sets makes us believe that early year mortalities, particularly for adults, are a result of age. Other life stages, i.e. larvae and

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\*Wooten, Akbay, and Howell, op. cit.

pupae, do not appear to decrease significantly with minor freezing temperatures. Therefore, adjustments were made in Version 1.1 to minimize the impact of subfreezing temperatures during winter and spring conditions; subfreezing temperatures are permitted to impact the end-of-year populations as before. The assumption is that early winter populations (end of year) are more susceptible to freezing conditions, whereas late winter populations (early year) have either already been selected for freeze tolerance or individuals have adjusted their positions within the habitat and are in less vulnerable places. Eggs, however, remain vulnerable regardless of time of year.

### **Insect losses due to extremely low (plant) biomass**

An algorithm has been installed in both NEOCE and NEOCB subroutines to respect the biological reality that insect density can not remain high without sufficient plant biomass being present. If plant biomass decreases to 100 g/sq m, then individuals from adults, larvae, and eggs are removed at the rate of 5.63 percent per day. Pupae, confined to the root zone of host plants, are removed at rate of 1 percent per day. It is possible that this biomass threshold should be higher (greater than 100 g), but (a) we have no specific data for the algorithm, and (b) the plant module often generates very low (lower than field data indicate) biomass during the early part of the growing season which will satisfy any reasonable threshold value. When this condition occurs, our ability to follow the various cohorts becomes very difficult. This point needs attention.

## **DISCUSSION**

The 1986 field data collected by Center and Haag from the Florida sites are extremely valuable for the development of INSECT from several viewpoints. One is that we now possess replicate samples of the population dynamics of *Neochetina* spp. along with a variety of complimentary sets of information which reflect bioecological characteristics of these species under defined conditions. Analysis of this information provides insight to the dynamics in operation at these sites. We are especially encouraged that similar information for the second year will be forthcoming. One of the areas, however, which requires additional work is that of control data for waterhyacinths grown in the absence of biocontrol agents. Further development and refinement of the plant module is limited until such data are made available.

Inspections of the 1986 field data allowed us to make or modify several important assumptions regarding the interactions of the target populations. For example, Version 1.1 of INSECT now includes (in a limited way) a mechanism for consideration of the physiological ages of entering cohorts. Physiological "age" can be assigned to some individuals entering the population on JD 1. Inclusion of this mechanism allowed us to test the importance of being able to assign physiological ages to entering cohorts.

One of the most important features of a simulation model is its ability to regulate development. Without proper timing the model is useless. The mechanism used in the first generation version of INSECT used a totally linear temperature/development relationship. No upper temperature limit was imposed. Insect development proceeded at whatever rate would be allowed by mean temperatures, even if daily mean temperatures reached very high levels. This procedure is not biologically acceptable. In Version 1.1, we

have essentially made the temperature/development relationship nonlinear at upper daily mean temperatures. This relationship has been accomplished by limiting development to only 8 day-degrees at temperatures above 27.0° C and zero day-degrees above 28.5° C. This mechanism appears to be working well with simulations made for both north and south Florida. However, since these simulations have been made for only one year, multiple-year simulations may require additional refinements of this relationship. Presently no allowances other than a linear relationship is made for lower temperatures. *It needs to be stressed that we are working with assumptions regarding temperature and insect development. We have no data which specifically validates our assumptions, only inferences made from field data and unrelated literature on insect development in general.* Presently, results of our validation simulations agree with these assumptions as evidenced by the timing of population peaks between model and field data.

## CONCLUSIONS

Based upon the validation tests shown here and in available literature, the timing and magnitude features of both insect and waterhyacinth populations as estimated by Version 1.1 have been shown to be reasonably accurate for a given simulation. Once proper starting values are in place, the model can be expected to track field data. This step is important because the model can now be operated, with reasonable confidence, according to *site specific* conditions; no special condition has to be set in the model to allow for geographic region. The major restriction is that the operator understands how to derive proper starting values.

The difficulties in finding appropriate starting numbers of individuals to initialize INSECT has to be reconciled and eventually expressed in a computer oriented format. While programming is not a problem, estimating physiological ages of immatures and judging the reproductive status of adults during winter months are. We expect that examination of the 1987 data from these sites will help clarify these problems. In addition to completing this validation portion of the study, we shall write an initialization routine for INSECT during the coming contract year.

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\*Wooten, Akbay, and Howell, op. cit.

# **HYDAMUR: Hydrilla and Triploid White Amur Growth Model**

by  
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## **INTRODUCTION**

The purpose of this paper is to present a brief overview of a first generation computer simulation model for hydrilla and triploid white amur. This research is a part of the continuing efforts within the Aquatic Plant Control Research Program to develop computer-oriented operational tools for the biological control of aquatic plants.

## **OVERVIEW OF THE MODEL**

The Hydrilla-Triploid White Amur model (HYDAMUR) has two major model components: the hydrilla model component (or plant module) and the triploid white amur component (or white amur module).

Conceptually, the hydrilla model is concerned with the biomass growth in 1-m  $\times$  1-m  $\times$  4-m water columns. Each three-dimensional column is divided into 1-m  $\times$  1-m  $\times$  0.10-m three-dimensional layers. The model assumes that the plants are located only on the top surface of the layer and the amount of light and water temperature differs from layer to layer. In the model, the plant biomass is affected by gross photosynthesis, amount of light available, water temperature, pH level of the water, fruit production, tuber production, dark respiration, photorespiration, natural plant mortality, and the grazing by white amur. Once the daily change in plant biomass is computed for each layer, the biomass in each layer is summed to obtain total hydrilla biomass available for white amur consumption.

The conceptual model for triploid white amur assumes that there are no predators affecting the population. The constant movement of the fish in the water column makes it very difficult to treat each fish separately. Therefore, the model simulates the total live weight of fish in a 1-sq-m area. The change in white amur weight is affected by the consumption rate, egestion rate, excretion rate, respiration rate, natural mortality, water temperature, and the amount of hydrilla biomass available.

Once the amount of reduction in hydrilla biomass due to white amur consumption is determined, the remaining total plant biomass is redistributed among the layers of the water column to initiate the next growth cycle.

In the following sections, the conceptual model for each module will be described.

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## HYDRILLA MODULE

The daily change in hydrilla biomass for each layer of the water column is a function of gross photosynthesis rate, dark respiration rate, photorespiration rate, and mortality rate for a given layer. This relationship can be summarized with the following mass balance equation:

$$\frac{d\text{HBIOM}}{dt} = \text{HGP} - (\text{HDR} + \text{HPR} + \text{HMORT})$$

where

$\frac{d\text{HBIOM}}{dt}$  = change in hydrilla biomass (HBIOM) in grams per square metre per day for a given layer

HGP = gross photosynthesis rate per square metre

HDR = dark respiration rate per square metre per day

HPR = photorespiration rate per square metre per day

HMORT = mortality rate per square metre per day

### Gross photosynthesis rate

The daily rate of photosynthesis is assumed to be affected by the maximum growth rate, the amount of light available, the water temperature, the pH level of the water, and the amount of fruit and tuber production. This relationship can be represented by the following expression:

$$\text{HGP} = \text{HGPMAX} * \text{F(L)} * \text{F(T)} * \text{F(pH)} * \text{HBIOM} - (\text{FP} + \text{TP})$$

where

HGPMAX = maximum growth rate

F(L) = limiting factor due to available light

F(T) = limiting factor due to water temperature

F(pH) = limiting factor due to pH level of the water

FP = fruit production rate

TP = tuber production rate

**Light limiting factor.** The light intensity is reduced considerably as it penetrates from the top to the bottom of the water column. The reduction in the light intensity is affected by the extinction coefficient of the water, the self shading due to plant biomass in the above layers, and the depth of the water for a given layer.

**Temperature limiting factor.** The rate of photosynthesis increases with increased water temperature to a maximum level and decreases rapidly at lethal temperatures.

**pH limiting factor.** In the initialization phase, the user of the HYDAMUR model is asked to enter the pH level for the body of the water simulated. Based on this input, the model determines the value of the pH limiting factor using a table look-up function. In

this version of the model, the pH limiting factor remains constant throughout the simulation run.

***Fruit production rate.*** The effect of fruit production on gross photosynthesis is still under development; therefore, it is not currently included in the model.

***Tuber production rate.*** The reduction in photosynthesis due to tuber production takes place only if certain temperature and photoperiod requirements are met. Even under environmental conditions conducive to tuber production, laboratory and field data reflect large variation between sites and individual plants within a given site. Recognizing this inherent variation, algorithms were developed which allow the user to choose between three levels (high, medium, and low) of tuber production during the initialization process. Based on this input, actual tuber production during the simulation is derived from a table look-up function containing monthly rates of tuber production for each of the three levels. The weight of individual tubers is then determined by regression equations that account for the effects of temperature and photoperiod on tuber dry weight.

***Dark respiration rate.*** The maximum daily dark respiration rate for a given layer is reduced because of water temperature to determine the actual dark respiration rate. This relationship can be expressed by the following equation:

$$\text{HDR} = \text{HDRMAX} * \text{FTDR} * \text{HBIOM}$$

where

HDRMAX = maximum daily dark respiration rate

FTDR = effect of water temperature on dark respiration

The dark respiration rate increases with increased water temperature to a maximum level and remains constant at higher temperatures.

### **Photorespiration**

The photorespiration rate increases if the amount of light intensity at a given layer is high. This relationship is described by the following expression:

$$\text{HPR} = \text{HEXMAX} * (1 - \text{F(L)}) * \text{HBIOM}$$

where

HEXMAX = maximum daily photorespiration rate

F(L) = light limiting factor (between zero and one)

Based on this expression, the reduction in the maximum photorespiration rate is less if the light intensity is low.

### **Mortality rate**

The mortality rate of the hydrilla plant is affected by the water temperature and sloughing. Long periods of low water temperatures will cause intrinsic death of whole plants. During certain time intervals, sloughing results in the death of leaves and stems.

## WHITE AMUR MODULE

The daily change in white amur weight is a function of daily feeding, egestion, excretion, respiration, and mortality rates. This relationship can be summarized by the following mass balance equation:

$$\frac{d\text{FBIOM}}{dt} = \text{FFEED} - (\text{FEGES} + \text{FEXCR} + \text{FRESP} + \text{FMORT})$$

where

$$\frac{d\text{FBIOM}}{dt} = \text{change in total fish weight (FBIOM) per square metre per day}$$

FFEED = feeding or consumption rate per square metre per day

FEGES = egestion rate per square metre per day

FEXCR = excretion rate per square metre per day

FRESP = respiration rate per square metre per day

FMORT = mortality rate per square metre per day

In the following sections, the components of this equation will be described briefly.

### Feeding rate

The daily feeding rate or the food consumption rate of the white amur is given by the following equation:

$$\text{FFEED} = \text{FEED (SIZE)} * \text{FEED (RATION)} * \text{FEED (TEMP)}$$

where

FEED (SIZE) = effect of fish size in feeding

FEED (RATION) = effect of total plant biomass and fish weight on feeding

FEED (TEMP) = effect of water temperature on feeding

***Effect of fish size.*** The feeding rate is higher when the fish are smaller. As fish get larger, the feeding rate decreases proportionally.

***Effect of plant biomass and fish weight.*** Hydrilla is the only type of food available for white amur, or it is the only preferred source of food for growth. This assumption can easily be modified to include relative preference of different food sources for white amur.

As hydrilla biomass becomes very large, the white amur feeding rate becomes dependent only on the total weight of fish available to consume the unlimited food. As white amur weight becomes very large, feeding is proportional only to the quantity of food available. At intermediate levels, the feeding rate is affected by both hydrilla biomass and white amur weight.

***Effect of water temperature.*** The feeding rate increases with increased water temperature to a maximum level and decreases rapidly at lethal temperatures. In

determining the effect of water temperature on feeding rate, an average water temperature in the top 2-m portion of the water column is used as the temperature input.

### **Egestion rate**

The daily egestion rate is defined as the rate of fecal production. This rate is related to feeding by a proportionality constant. This constant represents the fraction of consumed plant material which is not assimilated. This relationship is shown below:

$$FEGES = FEGESR * FFEED$$

where

FEGESR = fraction of consumed hydrilla biomass which is not assimilated

### **Excretion rate**

The daily excretion rate is considered to be the urine loss and is also related to feeding by a proportionality constant as shown below:

$$FEXCR = FEXCRR * FFEED$$

where

FEXCRR = fraction of consumed hydrilla biomass which is lost due to urine production

### **Respiration rate**

The daily respiration rate of the triploid white amur is given by the following relationship:

$$FRESP = RESP(SPDYN) + RESP(SIZE) * RESP(DEN) * RESP(TEMP) * FBIOM$$

where

RESP (SPDYN) = specific dynamic action

RESP (SIZE) = effect of fish size on respiration

RESP (DEN) = effect of density on respiration

RESP (TEMP) = effect of water temperature on respiration

***Specific dynamic action.*** Specific dynamic action can be defined as the metabolic cost of digestion, absorption, and assimilation. A simple proportionality is used with feeding or consumption rate. Therefore, respiration is increased linearly as the feeding rate increases.

***Effect of fish size.*** It is assumed that the respiration rate is higher when the fish are smaller. As fish get larger, the respiration rate decreases proportionally.

***Effect of fish density.*** It is assumed that the metabolic cost (respiration) increases in proportion to fish density (kilograms per square metre) and maximizes when fish density reaches the carrying capacity.

***Effect of water temperature.*** The rate of respiration for white amur increases with increased water temperature to a maximum level and decreases rapidly at lethal temperatures.

## Mortality rate

The mortality rate of the white amur is given by the following expression:

$$FMORT = MORT(INST) * MORT(SIZE) * MORT(TEMP) * MORT(DEN) * FBIOM$$

where

MORT (INST) = instantaneous rate of natural mortality (constant)

MORT (SIZE) = effect of fish size on mortality

MORT (TEMP) = effect of temperature on mortality

MORT (DEN) = effect of density on mortality

This relationship only allows for natural mortality; therefore, predator mortality and exploitation by humans are not considered.

**Effect of fish size.** It is assumed that for smaller fish the natural mortality rate is higher, and as fish get larger the size related mortality is reduced proportionally.

**Effect of temperature.** The mortality rate of white amur increases as a power function if the maximum temperature on a given day exceeds the critical temperature. Otherwise, there is no increase in the mortality rate due to high temperatures.

**Effect of fish density.** The mortality is assumed to increase in proportion to fish density (kilograms per square metre) levels and maximize when fish density reaches the carrying capacity.

## USING THE MODEL

### Computer code

The model is written in FORTRAN for IBM-AT microcomputers. The software used (IBM Professional FORTRAN by Ryan-McFarland Corporation) requires that a 80287 math coprocessor chip be installed in the computer. This FORTRAN language is designed according to the specifications of the American National Standard Programming Language FORTRAN 77. The model takes approximately 3 min to simulate the interactions between the hydrilla plant and the white amur fish for a year.

### Input requirements

At the beginning of the simulation, the user is asked to establish the initial conditions for the simulation. The model requires the following information to be entered by the user:

- The weather data to be used (code)
- First day of simulation (Julian date)
- Last day of simulation (Julian date)
- Average depth of the lake (metres)
- Initial dry weight of the plant biomass (kilograms per square metre)
- The pH level of the water (code)
- Level of tuber production (code)
- Stocking rate (number of white amur per acre)
- Weight of each white amur stocked (kilograms)

Each weather data set to be used with the model must contain the following information:

- Julian date
- Solar radiation (Langleys per square metre)
- Maximum daily temperature (Fahrenheit)
- Minimum daily temperature (Fahrenheit)

### **Input information**

The model is capable of generating a wide range of data concerning different parameters. However, a standard output includes the following daily information:

- Total hydrilla biomass (kilograms per square metre)
- Change in hydrilla biomass
- Total live weight of white amur (kilograms per square metre)
- Change in total weight of white amur
- Total hydrilla biomass consumed by white amur (kilograms)

## **SUMMARY AND CONCLUSIONS**

In this paper, a brief overview of a first generation computer simulation model for hydrilla and triploid white amur is presented. The contract report submitted by the authors in October 1987 can be referenced for detailed explanation of the algorithms. The model is still in its developmental stage, and the authors are continuing to improve the model. The next step is to compare the model results with reliable field data for verification and validation purposes.

There are numerous expected benefits from a model such as this. The model can be used to study hydrilla and white amur population dynamics as well as the interaction between them by varying certain parameters and keeping others constant. This model can also help other researchers model similar submersed aquatic plants and their similar biological control agents.

The ultimate benefit that can be achieved from this research is to make this model available to aquatic plant control managers as an effective decision tool. The managers can integrate this model in their decision — making process by asking many different “what if” questions and running the model to provide answers to these questions. However, this model should not replace the manager, but instead it should assist the manager in his/her decision making.

# Development of A Coupled Herbicide Fate and Target Plant Species Effects Model (FATE)

by

John H. Rodgers, Jr.,\* Philip A. Clifford,\*  
and R. Michael Stewart\*\*

## INTRODUCTION

Computer-aided methodologies are needed that will allow operational aquatic plant management personnel to optimally utilize available control techniques. A variety of site-specific environmental factors may strongly influence successful application of an aquatic plant control technique. Computer-based simulation models have proven useful in similar complex situations as decision support systems (Reinert and Rodgers 1986; Reinert, Rocchio, and Rodgers 1987).

Chemical control techniques, including herbicides such as 2,4-D, are used in a variety of aquatic environments. After an herbicide is applied to an aquatic environment, the rate of decrease of the herbicide in the environment is a function of in situ biological, chemical, and physical processes. These processes are relatively well known for many aquatic herbicides (Reinert and Rodgers 1987) and can be modeled by mass balance and kinetic methods (Donigian 1982, Lassiter 1982).

The response of target aquatic plant species to time-varying concentrations of herbicides is perhaps less well understood. Information from both field and laboratory studies are being used to model herbicide exposure (concentration \* time) versus effects for an aquatic plant species. This research effort is directed toward developing concepts and algorithms for a coupled herbicide fate and target species effects model (FATE - Fate and Target Effects). This simulation requires input data on site-specific characteristics. The simulation model provides estimates for the required initial herbicide concentration to be used for a desired level of plant control. Using this simulation, different herbicide dose rates can be simulated to determine a particular level of plant control. We can also begin to examine such strategies as timing of treatment and the effects of repeated treatments. In developments of the first-generation FATE model, waterhyacinth (*Eichhornia crassipes* (Mart.) Solms) and 2,4-D were used since considerable data were available. This paper gives a brief overview of the FATE model and describes its present state of development.

## OVERVIEW OF FATE

### Organization of the model

The first year's research efforts were devoted to developing a conceptual plan and first-generation FATE model that contains algorithms for herbicide fate and target

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species effects. FATE is a user-oriented software package written in BASIC (Microsoft Corp.) for use with IBM compatible personal computers. A flowchart of the first generation FATE model is shown in Figure 1. The FATE model, composed of three coupled modules, simulates herbicide fate and compartmentalization in the environment (Module I), the effects of the determined herbicide dose on the target plant population (Module II), and the regrowth of the target plant population following herbicide treatment (Module III). The FATE model is driven by the assumption that the target plant must receive a sufficient herbicide exposure (concentration \* time) in order to obtain the desired effect or degree of plant control.

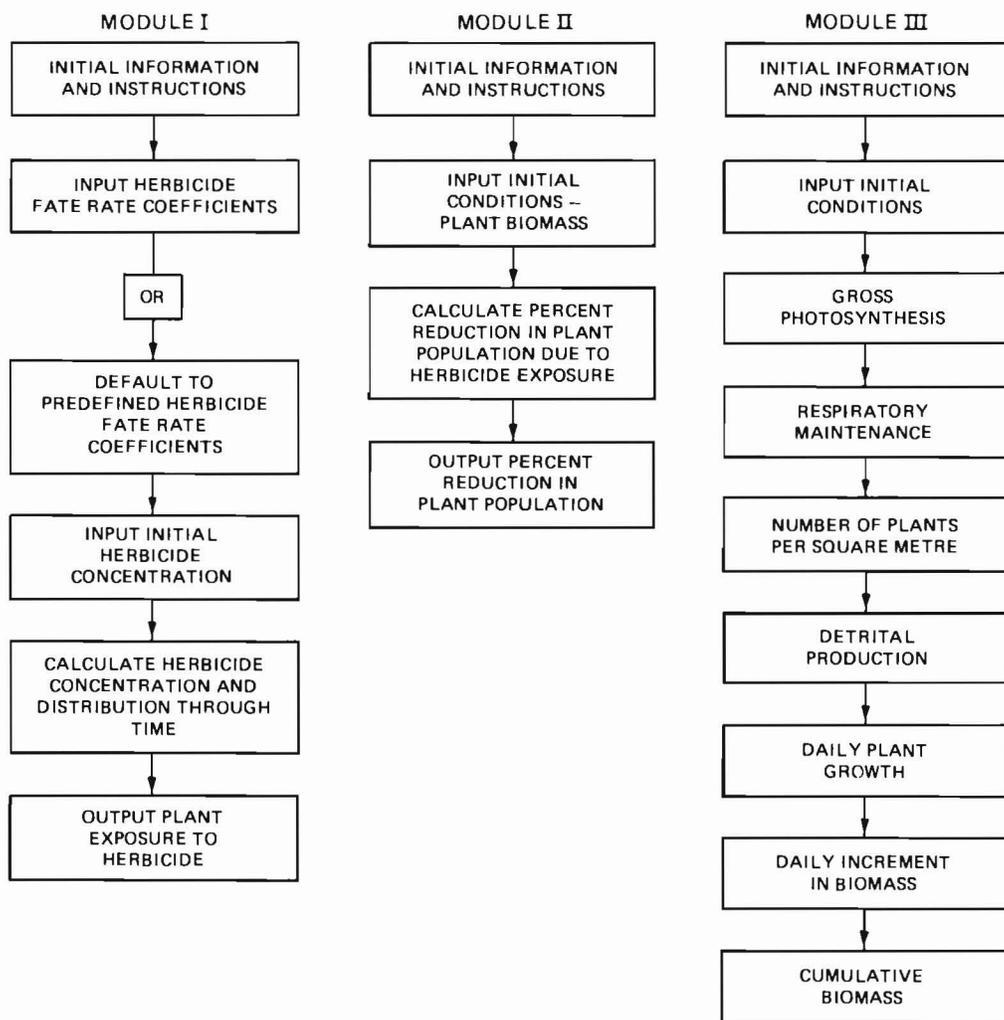


Figure 1. Flowchart of the FATE model

In Module III, the regrowth of the treated plant population is simulated for a user-defined period of time. Plant regrowth is simulated by incorporation of an extant plant growth algorithm for a particular plant species. Currently, the algorithm is limited to only one plant species – waterhyacinth. The assumptions used in the waterhyacinth plant growth algorithm can be found in Akbay, Wooten, and Howell (1988).

### Utility

The FATE model has been developed to assist aquatic plant control managers in the selection of herbicides and application strategies for control of problem aquatic plants. The first-generation model provides estimates for the compartmentalization and persistence of 2,4-D (DMA) and the effects of this herbicide on waterhyacinth. The waterhyacinth plant growth algorithm allows the user to simulate the regrowth of this plant species under site specific conditions of temperature and solar radiation following 2,4-D treatment. (Algorithms for other herbicides and aquatic plant species are currently under development). For a given set of site-specific environmental conditions, the first-generation FATE model can be used to:

- Predict the 2,4-D exposure received by a waterhyacinth population.
- Predict the effects of the determined 2,4-D exposure on the waterhyacinth population.
- Predict the regrowth of the waterhyacinth population following chemical treatment.

The model calculations provide a means of comparing the overall effectiveness of various 2,4-D application strategies for controlling waterhyacinths. Factors related to application technique that can be easily evaluated are the application rate and the timing of application. The model calculations will also aid the user in scheduling necessary repeat applications at a given site in order to maintain a desired level of control.

### Model input requirements

To use the FATE model requires initialization of all three modules. The initialization process is accomplished by a combination of user-supplied data (i.e. input from the keyboard), model-supplied data (using internal default data sets), and model-generated data. Specific data that must be input by the user are described below.

- Module I.* Module I has been designed to facilitate fate calculations for commonly used aquatic herbicides. The initial input requirement is the herbicide formulation. These user-supplied data are used to select the appropriate default data set for the herbicide. Table 1 lists the type of information included in this default data set. The user has the option of using the information supplied from the default data set or changing through keyboard entry any value of the variables to better reflect site-specific conditions. The default data set contains reasonable values for the input variables and will result in a “generic” simulation of herbicide fate; however, greater accuracy will be achieved by inputting site specific information.
- Module II.* The input requirement of Module II is the plant species. This input is used to select an internally stored file that contains values for the slope and y-intercept parameters of the logistic equation that expresses mortality for the particular herbicide and plant species combination. Mortality is then calculated by solving the logistic equation for the predicted exposure output from Module I.

Table 1  
Input Requirements of Module I that Determine the Effects of Transfer/Transformation Processes on Herbicide Fate

<i>Transfer Processes</i>	<i>Input Requirements (units)</i>
Drift	<ul style="list-style-type: none"> <li>● Loss of herbicide due to drift (percent)</li> </ul>
Dilution	<ul style="list-style-type: none"> <li>● Application rate of formulation (per acre)</li> <li>● Active ingredient fraction of formulation (percent)</li> <li>● Herbicide formulation release half-life (days)</li> <li>● Average depth of treated area (feet)</li> <li>● Flow rate from treated area (feet per minute)</li> </ul>
Sorption	<ul style="list-style-type: none"> <li>● Herbicide sediment layer partition coefficient</li> <li>● Total suspended solids in water</li> <li>● Sedimentation rate (inches per year)</li> <li>● Sediment resuspension rate (inches per year)</li> <li>● Depth of active sediment layer (inches)</li> <li>● Sediment water content (percent)</li> <li>● Sediment diffusion exchange rate (inches per day)</li> </ul>
Volatilization	<ul style="list-style-type: none"> <li>● Herbicide volatilization half-life in water (days)</li> </ul>
Bioaccumulation	<ul style="list-style-type: none"> <li>● Bioaccumulation factor of herbicide</li> </ul>
<i>Transformation Processes</i>	
Oxidation	<ul style="list-style-type: none"> <li>● Herbicide oxidation half-life in water (days)</li> <li>● Herbicide oxidation half-life in sediments (days)</li> </ul>
Hydrolysis	<ul style="list-style-type: none"> <li>● Herbicide hydrolysis half-life in water (days)</li> <li>● Herbicide hydrolysis half-life in sediments (days)</li> </ul>
Photolysis	<ul style="list-style-type: none"> <li>● Herbicide photolysis half-life in water (days)</li> <li>● Herbicide photolysis half-life in sediments (days)</li> </ul>
Biodegradation	<ul style="list-style-type: none"> <li>● Herbicide biodegradation half-life in water (days)</li> <li>● Herbicide biodegradation half-life in sediments (days)</li> </ul>

c. *Module III.* The inputs for Module III include the initial plant biomass and the starting and ending dates for the plant regrowth period. The user also selects a weather data file that is used in conjunction with the plant growth algorithm. The weather data include daily values for solar radiation (langleys/sq m) and air temperature (degrees C). Currently, the user is able to choose from a list of historical weather files from sites in Florida, Louisiana, and Texas.

### Model output

The FATE model generates tabular data and graphical plots on herbicide fate, a graphical plot of herbicide effects, and a graphical plot of the simulated regrowth of the treated plant population. Currently, the user may choose to display these outputs on the monitor or to obtain a hardcopy product.

## SIMULATION USING THE FATE MODEL

To demonstrate this utility, an example simulation run is presented. Figure 2 contains the input values used for this simulation. For this simulation, the values of some of these variables were entered by the keyboard while others were extracted from the default data set for 2,4-D (DMA). The graphical output from the simulation using the indicated values in Figure 2 is presented in Figures 3 through 5. Figure 3 shows the simulated partitioning of 2,4-D at the site following application. A review of Table 1 and Figure 2 indicates that values for processes affecting the transfer of 2,4-D to the sediments were all set to zero by keyboard entry. As a result, there was no partitioning of 2,4-D in the

sediments (Figure 3). From the curve representing 2,4-D concentration in the plant tissues (Figure 3), the predicted 2,4-D exposure to the plant population is 7.102 ppm-days. Figure 4 shows the simulated effect of this exposure to the waterhyacinth population. As indicated in Figure 4, an exposure of less than one-half (2.3 ppm-days) of the simulated exposure would have resulted in the same level of control (99 percent) under the specified conditions. Figure 5 includes plots of two waterhyacinth growth simulations. Simulation 1, the Module III output for the example FATE run, estimates regrowth following a 99 percent reduction in the waterhyacinth population. Simulation 2 estimates growth of an “untreated” waterhyacinth population over the same time period. For both simulations, initial plant biomass was 100 tons per acre. In general, growth in the “treated” population was considerably reduced as compared to the “untreated” population. Regrowth in the “treated” population was not obvious until late July, and maximum density levels (approximately 85 tons per acre) did not occur until October. In the “untreated” population, density values increased to near 135 tons per acre by late July and remained at this level for approximately two months. Both the “treated” and “untreated” populations showed the same biomass levels for the winter months.

### Input Values

Compound Selected is: 2,4-D (DMA) [LIQUID]  
 Plant Species is: Water Hyacinth (*Eichhornia crassipes*)  
 Average Depth of Area to be Treated (feet) 1.000\*  
 Water Flow Rate From Treated Area (feet/minute) 0.000  
 Total Suspended Solids in Water (parts per million) 10.000\*  
 Depth of Active Sediment Layer (inches) 0.000\*  
 Sediment Water Content (percent) 0.000\*  
 Sediment Diffusion Exchange Rate (inches/day) 0.000\*  
 Sedimentation Rate (inches/year) 0.000\*  
 Sediment Resuspension Rate (inches/year) 0.000\*  
 Herbicide Formulation Release Half-life (days) 0.000  
 Active Ingredient Fraction of Herbicide (percent) 46.800\*  
 Application Rate of Formulation (gallons/acre) 1.000  
 Loss of Herbicide Due to Drift (percent) 0.000\*  
 Herbicide Sediment Layer Partition Coefficient [KP] 0.250  
 Herbicide Hydrolysis Half-life in Water (days) 1000.000  
 Herbicide Hydrolysis Half-life in Sediment (days) 1000.000  
 Herbicide Oxidation Half-life in Water (days) 1000.000  
 Herbicide Oxidation Half-life in Sediment (days) 1000.000  
 Herbicide Biotransformation Half-life in Water (days) 3.900  
 Herbicide Biotransformation Half-life in Sediment (days) 3.900  
 Herbicide Photolysis Half-life in Water (days) 1000.000  
 Herbicide Volatilization Half-life in Water (days) 1000.000  
 Bioconcentration Factor of Herbicide for this Plant Species 7.000  
 Plant Biomass to be Treated (tons/acre) 100.000\*  
 Percent Dry Weight of Plant Tissue (percent) 10.000

**Figure 2. Screen display of input values used in the example simulation run. Values marked by an asterisk were entered from the keyboard during initialization of Module I, and were chosen to simulate a one-time treatment with 2,4-D (DMA) in a site where herbicide transfer to sediments is negligible**

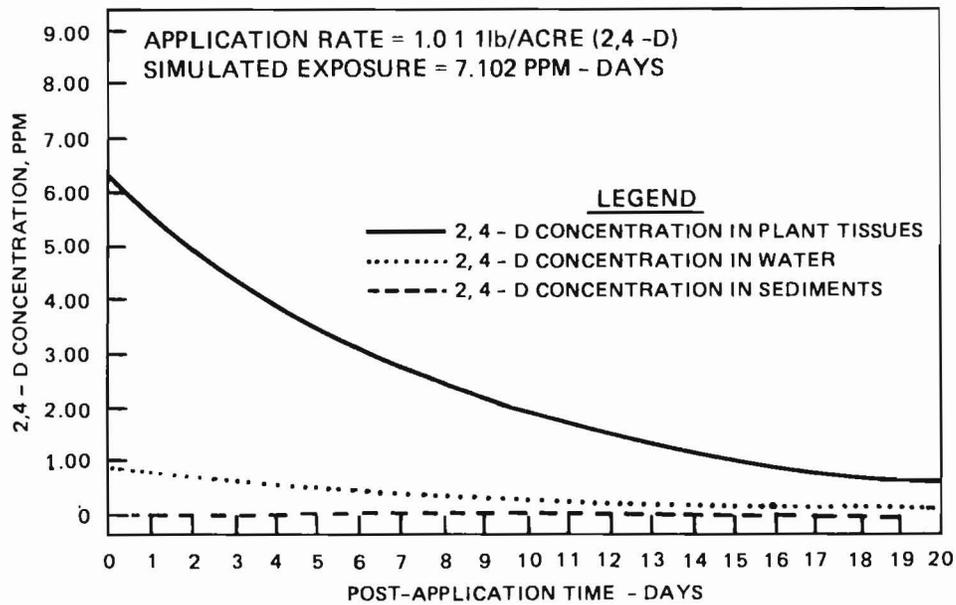


Figure 3. Herbicide ((2,4-D (DMA)) compartmentalization and persistence predicted in the example simulation. Waterhyacinth exposure to 2,4-D (DMA) (7.102 ppm-days) is estimated as the area beneath the curve for herbicide concentration in plant tissues (solid line)

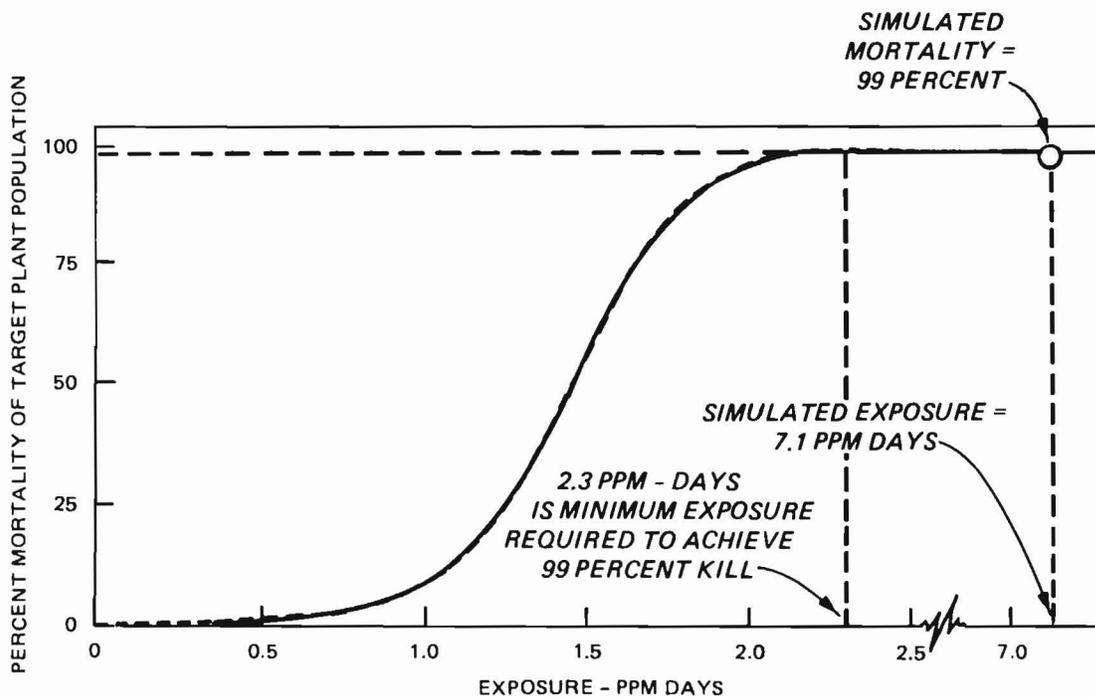


Figure 4. Exposure-response (effects) relationship used for 2,4-D (DMA) and waterhyacinth in Module II. The circle at the upper right hand corner of the curve indicates percent kill calculated in the example simulation. For this example, 99 percent was the maximum allowable kill. An exposure of 2.3 ppm-days can be estimated from the curve as the minimum exposure that would result in 99 percent kill

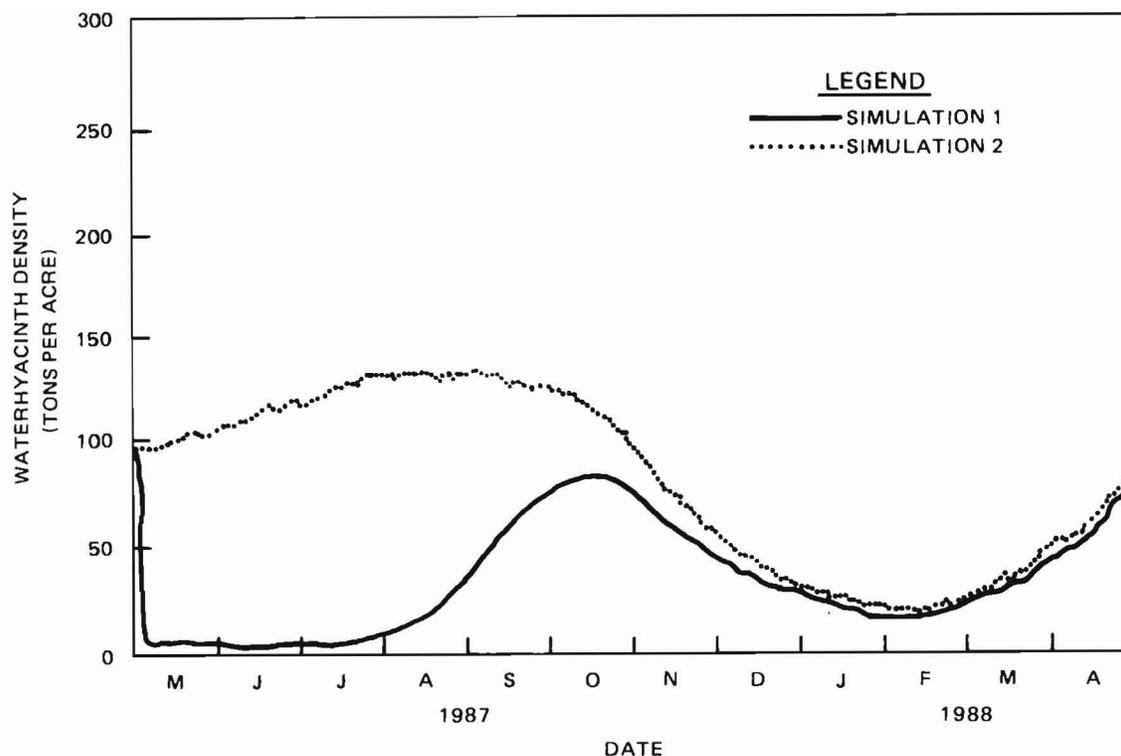


Figure 5. Regrowth of the treated waterhyacinth population (Simulation 1) compared to growth of an untreated waterhyacinth population (Simulation 2). The simulation period of both was May 1, 1987, to April 30, 1988. Initial biomass for both simulations was 100 tons per acre (fresh weight). Weather data for both simulations were collected during 1976 from a site near Gainesville, Florida

Depending on the control requirements of this particular site, the aquatic plant manager could use the results of this “regrowth” simulation to schedule additional 2,4-D applications. For example, if 50 tons per acre was the tolerance level for waterhyacinth at the site, Figure 5 indicates that a second application should be made in late August. Further, Figure 5 shows that the “treated” population would recover from winter die-back at the same level as the “untreated” population. Thus, a similar application schedule would probably be necessary during the 1988 growing season.

## STATUS OF THE MODEL AND RECOMMENDATIONS

The current version of the FATE model is operational on IBM compatible personal computers. Minimum system requirements are 240 K RAM, two 5-1/4-in. floppy disk drives, a math coprocessor, a graphics adaptor, a color monitor, and a dot matrix printer.

During FY 88, the FATE model will be expanded to include additional herbicides and other aquatic plant species. The FATE model has been designed to facilitate these additions and updates. Herbicides to be included during FY 88 are diquat and endothall. Plant growth models for *Hydrilla verticillata* and *Myriophyllum spicatum* are under development and will be tested for inclusion in FATE.

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# **Chemical Control Technology Development**

# Chemical Control Technology an Overview

by  
Howard E. Westerdahl

## DIRECT-ALLOTTED RESEARCH FOR FY 87

The Chemical Control Technology area in FY 87 was comprised of five work units which included:

- a. ***Herbicide concentration/exposure time relationships.*** This work unit determines the relationship between chemical concentration and exposure time for a variety of aquatic plants under controlled-environment conditions. This information will assist developers and commercial applicators in evaluating alternative formulations for specific flowing water environments. Tests were completed on the herbicide 2,4-D during FY 87. Herbicides to be tested include endothall, diquat, dichlobenil, and bensulfuron methyl.
- b. ***Herbicide/adjuvant evaluation.*** Testing of available adjuvant types has been completed under laboratory conditions at low flow velocities, i.e. 0.03 m/sec. Additional testing at higher flow velocities is not possible using existing facilities. Results to date suggest that herbicides applied with polymers and inverters do not provide a significant advantage over the use of herbicide formulations alone.
- c. ***Herbicide application technique evaluation and development.*** Based on the information being developed on herbicide concentration/exposure time relationships in a previous work unit (a), application techniques are being evaluated and developed to provide the appropriate contact time necessary to achieve control of the target plants. During FY 87, effectiveness of a conventional liquid formulation of dipotassium endothall was compared with an endothall/polymer mixture. Moreover, efforts are being made to quantify water movement in submersed plant stands and develop guidance on the appropriate use of herbicides in environments where water movement may impact efficacy of a herbicide.
- d. ***Field evaluation of selected herbicides.*** Field testing of new application techniques and herbicide formulations must be conducted under operational, field conditions. Tests of this magnitude generally require an Experimental Use Permit (EUP) from the US Environmental Protection Agency if they involve a new formulation, change in site use, or amendment of residue tolerances. This research involves cooperative efforts between chemical companies, other Federal agencies, and the Waterways Experiment Station (WES). Efforts were made in FY 87 to obtain an EUP for the herbicide dichlobenil (Casoron 10G), but approval was delayed until industry provided additional toxicological data. Field test plans for the new herbicide bensulfuron methyl (Mariner) were developed cooperatively with DuPont Corporation, US Department of Agriculture-Agriculture Research Service, in Davis, California, and the Bureau of Reclamation in Denver, Colorado.

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

- e. *Plant growth regulators for aquatic use.* Since the dichlobenil EUP field test was delayed, a contract was approved for Purdue University to develop a bioassay system for plant growth regulating chemicals (PGRs). This bioassay system will determine effects of PGRs on aquatic plants to assess their potential for aquatic use.

### RELATED PROJECTS DURING FY 87

The District project initiated during FY 87 was determination of water exchange patterns in hydrilla. Through support from the Jacksonville District, tracer dye studies were initiated in the Three Sisters Canals, Crystal River, Florida. During late summer and fall, nine dye or dye/herbicide applications were evaluated in the canal system. Based on the information obtained from these studies, an application technique was recommended and, when implemented by Citrus County operations personnel, resulted in excellent control of hydrilla. This study will be continued for FY 88 by the District.

### DIRECT ALLOTTED RESEARCH FOR FY 88

The FY 88 chemical control research will involve four work units:

- a. *Plant growth regulators for aquatic plant management.* Research will continue under contract to develop bioassay procedures to determine the effects of PGRs on selected aquatic plants. The most promising formulations will be tested to identify the minimum contact time required to inhibit the development of buds, branches, roots, flowers, and reproductive propagules.
- b. *Herbicide concentration/exposure time studies.* The herbicide endothall will be tested this year, and the target plant species will be Eurasian watermilfoil and hydrilla. Results from this research will also assist WES personnel in providing the Jacksonville District with appropriate techniques for using endothall in the previously mentioned Three Sisters canal system for controlling hydrilla.
- c. *Herbicide application technique evaluation and development.* Water movement through plant stands in the Pend Oreille River, Washington, and north Florida will be characterized using tracer dye and flow meters. Once defined, application techniques and chemical formulations will be evaluated and the best combinations recommended for testing in FY 89. Results of the previous work unit (b) will complement this effort.
- d. *Field evaluation of selected herbicides for new aquatic uses.* Testing of the herbicide Mariner under an EUP will be conducted in Lake Seminole, Georgia, cooperatively with DuPont Corporation. Similar studies will be conducted by the Bureau of Reclamation at Banks Lake or equivalent location. The EUP for Casoron 10G should be approved this year and scheduled for testing during FY 89.

### RELATED PROJECTS FOR FY 88

The Jacksonville District will fund an effort to develop techniques for controlling hydrilla in tidal canals of the Crystal River, Florida. Moreover, Seattle District and the Washington State Department of Ecology will assist the WES with manpower and logistical support for the tracer dye studies on the Pend Oreille River.

# Development of Herbicide Application Techniques for Flowing Water

by  
Kurt D. Getsinger\*

## INTRODUCTION

Although submersed plants are effectively controlled with herbicides in static water, satisfactory control in environments with significant water movement, or exchange, (e.g., rivers, streams, canals, and tidal areas) has proven to be very inconsistent. Control of submersed plants in flowing water is directly related to herbicide concentration and exposure (contact) time. An ongoing work unit within the Chemical Control Technology area is determining herbicide concentration/exposure time relationships. Achieving the herbicide concentration/exposure time required for successful control is dependent upon site-specific factors, e.g., water exchange within plant stands and plant density. Therefore, understanding water exchange patterns within submersed plant stands is necessary prior to selecting a herbicide formulation and application technique. Once water exchange is characterized for a flowing-water environment, this information can be used to evaluate suitable herbicide application techniques.

The objectives of this work unit are to: (a) characterize flow velocities and water exchange in submersed plant stands under field conditions, and (b) evaluate application techniques which maximize herbicide contact time in flowing-water environments. Flow velocity meters and tracer dyes are used to measure water movement/exchange in submersed plant stands growing in streams, rivers, irrigation/drainage canals, and estuarine tidal areas.

This article presents results of a 2,4-D application technique pilot study. The objectives of this study were to: (a) compare 2,4-D release rates from granular and liquid formulations when applied to Eurasian watermilfoil stands in flowing water, and (b) evaluate three different 2,4-D application techniques for use in flowing water. In addition, this article highlights recent findings from ongoing field flow velocity studies. An update on the use of fluorescent dyes for measuring water exchange patterns in hydrilla-infested, tidal canals follows this article.

## MATERIALS AND METHODS

### 2,4-D application technique pilot study

Duplicate stands of Eurasian watermilfoil (*Myriophyllum spicatum*) were established

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\*Getsinger, K.D., and Westerdahl, H.E., 1986. "Evaluation of 2,4-D/Adjuvant Mixtures in Flowing Water," Miscellaneous Paper A-86-3, US Army Engineer Waterways Experiment Station, Vicksburg, Miss.

in the Waterways Experimental Station (WES) hydraulic flume, as described by Getsinger and Westerdahl (1986),\* for each experiment. The herbicide formulations tested were granular 2,4-dichlorophenoxyacetic acid (2,4-D) butoxyethanol ester (BEE), and liquid 2,4-D dimethylamine (DMA). All formulations were applied at 45-kg active ingredient (ai)/ha (40 lb ai/acre). Incoming flow velocities were maintained at 3 cm/sec (0.1 ft/sec) during the experiments.

In the first experiment, liquid 2,4-D (DMA) was injected below the surface throughout each plant stand using a pressurized sprayer. In the second experiment, granular 2,4-D (BEE) was broadcast over each plant stand using a shaker-box. In the third experiment, 2,4-D (BEE) was loaded into a porous pipe and suspended, at middepth, 1 m upstream from each plant stand. The porous pipe consisted of electrical conduit (60 cm long x 2-cm ID) perforated with 2-mm-diam holes at 1-cm intervals along its length. Identical series of holes were drilled at three equidistant locations around the circumference of the conduit, creating a pipe with 240 openings. The open ends of the pipe were sealed with rubber stoppers to prevent loss of granules during the experiment.

Water samples, for herbicide residue analysis, were collected in the center of each flume channel, 175 cm downstream from the plant stands, using an ISCO Model 2100 automatic water sampler adapted to sample a water column depth of 10 to 60 cm. Samples were collected at 2-min intervals posttreatment for 180 min and composited to represent 12-min periods per sample.

Residues of 2,4-D in water were determined using high-pressure liquid chromatography procedures (American Public Health Association 1976)\*\* and were performed by the Laboratory Branch, Tennessee Valley Authority (TVA), Chattanooga, Tennessee.

### Flow velocity studies

Flow velocities were measured at 0.5-m intervals along transects across submersed plant stands (perpendicular to direction of flow) using a Montedoro-Whitney Model PVM-2A portable velocity monitor (accuracy  $\pm 2$  percent). The electromagnet velocity sensor was attached to a wading rod and held in place on, and perpendicular to, the bottom. Velocities were measured from the surface to the bottom, at 12-cm increments through depth.

Flows were measured in stands of Eurasian watermilfoil, sago pondweed (*Potamogeton pectinatus*), coontail (*Ceratophyllum demersum*), and elodea (*Elodea canadensis*) in irrigation and drainage canals of the Sacramento Valley, California. Plant stands ranged from 100 sq m to 0.5 ha in size and occurred in water 1 to 1.5 m deep.

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\*Getsinger, K.D., and Westerdahl, H.E., 1986. "Evaluation of 2,4-D/Adjuvant Mixtures in Flowing Water," Miscellaneous Paper A-86-3, US Army Engineer Waterways Experiment Station, Vicksburg, Miss.

\*\*American Public Health Association, 1976. *Standard Methods for the Examination of Water and Wastewater*, 14th ed., Washington, DC.

## RESULTS

### 2,4-D application technique pilot study

Herbicide release rates from the 2,4-D (DMA) and (BEE) formulations are compared in Figure 1. Application of the liquid, 2,4-D (DMA) formulation resulted in a quick release, or flush, of herbicide ( $>1.5$  mg/l) following treatment. Residue levels decreased substantially ( $<0.3$  mg/l) by 24-min posttreatment and were below detection by 36-min posttreatment.

Broadcast application of granular 2,4-D (BEE) extended the herbicide exposure time in the water column to 84-min posttreatment; however, 2,4-D concentrations were quite low ( $<0.2$  mg/l). Low levels of 2,4-D in the water column should be expected following this type of application, since granules may release 2,4-D after sinking to the bottom.

The porous-pipe application of 2,4-D (BEE) resulted in a relatively constant release of 2,4-D throughout the study. In addition, herbicide residues remained moderately high (0.45 to 0.75 mg/l) during this 180-min sampling period.

### Flow velocity studies

Cross-sectional flow velocities in California canals, with and without vegetation, are shown in Figures 2 through 4. Flow velocities were considerably less within sago pondweed stands compared with areas outside the stands, or in sections of the canal without vegetation (Figures 2 and 3). Stands of Eurasian watermilfoil caused similar velocity dampening effects in a different canal (Figure 4). Flow velocities within these submersed plant stands followed patterns measured in the WES and TVA flumes, and in the Holston River (Getsinger 1987).\*

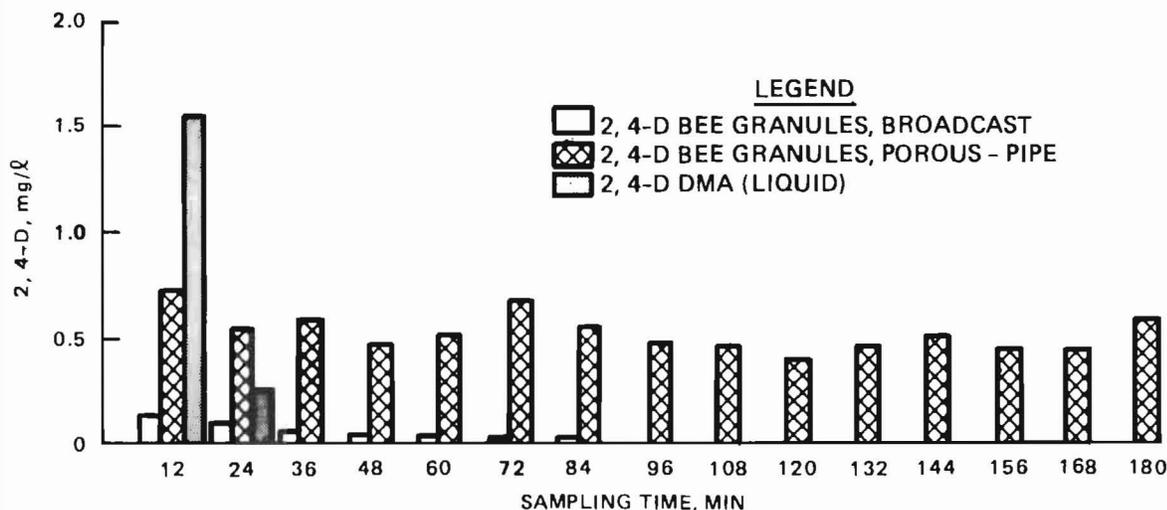


Figure 1. Effect of time on 2,4-D residues downstream of plant stands using different application techniques at a flow velocity of 3 cm/sec

\*Getsinger, K.D. 1987. "Herbicide Application Technique Development for Flowing Water," *Proceedings, 21st Annual Meeting, Aquatic Plant Control Research Program*, Miscellaneous Paper A-87-2, US Army Engineer Waterways Experiment Station, Vicksburg, Miss.

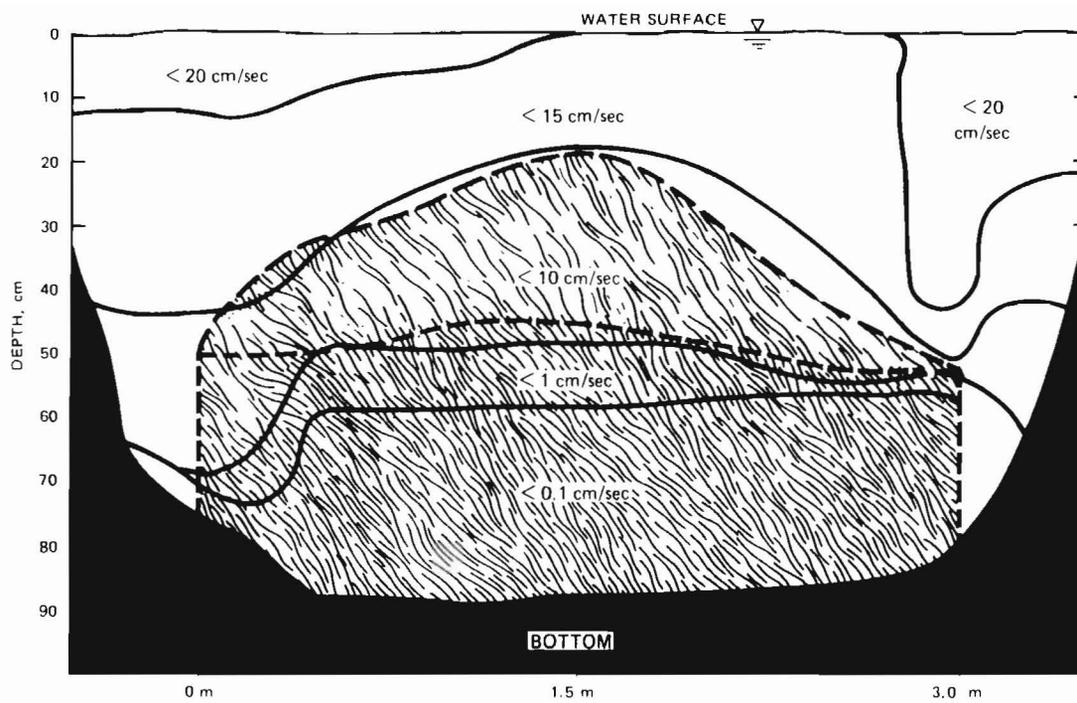


Figure 2. Cross section of flow velocity patterns in sago pondweed stand

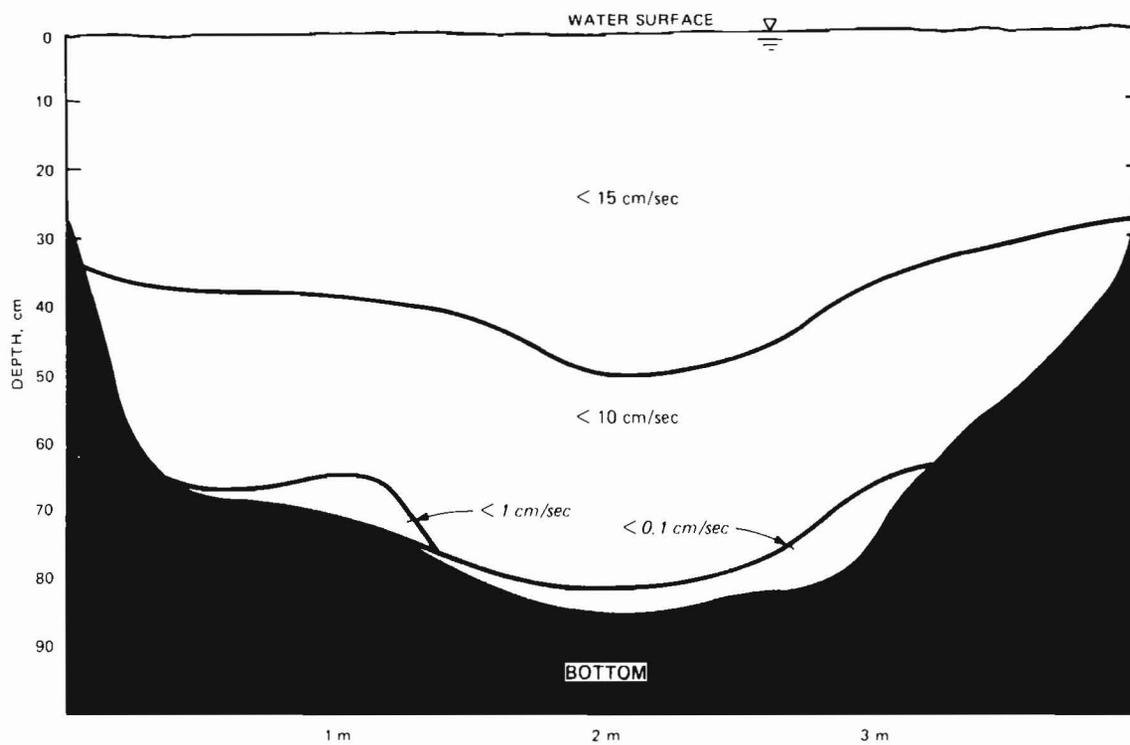


Figure 3. Cross section of flow velocity patterns in canal without plants

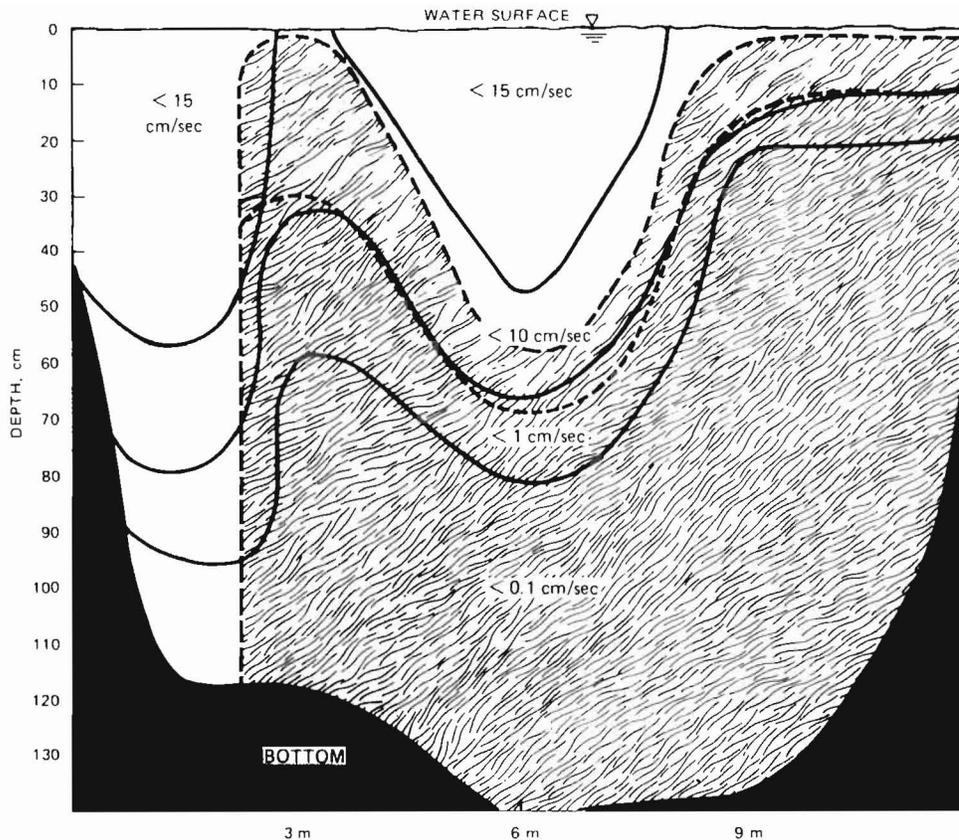


Figure 4. Cross section of flow velocity patterns in Eurasian watermilfoil stand

## CONCLUSIONS

Results of the 2,4-D pilot study suggest that a porous-pipe application technique of a granular formulation can extend the release profile of 2,4-D at a flow velocity of 3 cm/sec, compared to conventional liquid and granular application techniques. The porous-pipe technique should be evaluated at higher flow velocities and on larger plant stands.

Preliminary results of the flow velocity studies show that velocities are greatly reduced upon entering submersed plant stands. Therefore, herbicide placement and delivery techniques will be critical factors in the successful control of submersed plants in flowing-water environments. An understanding of water exchange within submersed plant stands is a necessary element in the identification and development of herbicide application techniques in flowing water.

## PRESENT RESEARCH

A two-year field study to determine water exchange patterns in submersed plant stands in the Crystal River, Florida, using a tracer dye (Rhodamine WT), was initiated in FY 87 in cooperation with the Jacksonville District. Information obtained from this study will be used, in conjunction with other studies, to develop guidance in herbicide

selection and use. A field study to evaluate herbicide application techniques in rivers with high water flow will be initiated in Washington State during FY 88. The first phase of the study will measure water exchange in submersed plant stands using Rhodamine WT.

### **ACKNOWLEDGEMENTS**

The author thanks Ed Wilkerson, Yvonne Vallette, and Kristy Smith of WES for their technical assistance in the 2,4-D pilot study. Chemicals used in this study were provided by Rhone-Poulenc. In addition, thanks are extended to Richard Young and Eric Morgan, The Advent Group, Inc., and to David Spencer and Greg Ksander, US Department of Agriculture Aquatic Weed Control Laboratory, Davis, California, for their assistance in measuring flow velocities in the Holston River and in canals of central California, respectively.

# **Preliminary Study of the Dilution of Dyes in Tidal Canals of the Crystal River, Florida**

by

Alison M. Fox,\* William T. Haller,\* and Kurt D. Getsinger\*\*

## **INTRODUCTION**

The Three Sisters Canal system is a series of five dead-end canals in the eastern headwaters of Crystal River, Florida. For many years these clear freshwater, residential canals have supported an extensive growth of hydrilla which has been controlled by both mechanical and chemical methods. Application of aquatic herbicides has often resulted in less than desired levels of control, and generally effects have been unpredictable.

Water movement in the canals is influenced by freshwater springs and tides, which ebb and flow along the 8-km river connecting the headwaters to the Gulf of Mexico. It is widely believed that this water movement dilutes herbicide concentrations, thereby reducing effectiveness of herbicides applied to these canals. A better knowledge of the water movement characteristics in these canals may result in more predictable hydrilla control, which would reduce the amount and cost of herbicides used in future treatments. In addition, the Three Sisters Canals may be suitable as an experimental system for studying aquatic plant management techniques needed in other freshwater, tidally-influenced water bodies.

Dyes are regularly used to determine the velocity and movement of water (Pilgrim and Summersby 1966, Johnson 1984). The fluorescent dye Rhodamine B has been used to simulate the movement of herbicide residues in canals (Demint 1970) and lakes (Langeland and DeMont 1986), but this dye has the disadvantage of being readily adsorbed onto sediments (Smart and Laidlaw 1977). Rhodamine WT, which is resistant to adsorption, was specifically developed for tracing work and has been approved by the US Environmental Protection Agency and the US Geological Survey for use in potable water at concentrations up to 10 ug/l.

## **OBJECTIVES**

The objectives of this study were to: (a) characterize water movement and dye dilution, using Rhodamine WT, in four canals under similar tidal and vegetated conditions; (b) determine whether these canals can be used as replicates in future studies; (c) compare the influence of spring and neap tidal cycles on the rate of dye dilution; and (d) compare the rate of dye dilution from total canal treatments in unvegetated and vegetated conditions.

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

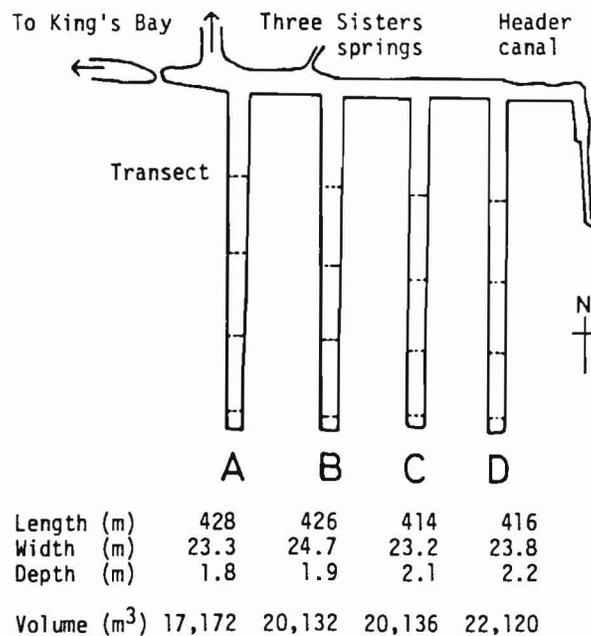
## CANAL DIMENSIONS AND TIDES

The four canals used in the study run north-south and are connected to King's Bay by a header canal. It is likely that there are small springs in each canal; however, a major influence on water exchange in these canals would be from the Three Sisters Spring, located in the header canal, between canals A and B (Figure 1). The dimensions of the canals were calculated using an aerial photograph (Department of Transportation 1985) and measured in the field. Nine transects were established in each canal and depth measurements taken at 1-m intervals across the canal to determine the cross-sectional area.

Depth and volume estimates were corrected to a datum level for comparison throughout the tidal cycles. Water levels were continuously recorded in canals A and D using Stevens' Type F recorders, and an arbitrary datum level was gauged in canal A.

The tides in King's Bay show a predictable synodic, semidiurnal pattern (Macmillan 1966). Two types of tides, spring (maximum) and neap (minimum) are recognizable in the 29-day lunar cycle. The range of water levels is greater between the high and low tides of a spring cycle than during a neap cycle (Figure 2). Therefore, during neap tides (when a smaller proportion of the total volume is being exchanged between high tides) it is expected there will be a slower dilution rate of dye in the canals.

A feature of neap tides in King's Bay is that the range of one of the daily tidal cycles is much more reduced than the other (Figure 2, September 16), so that there is effectively only one tide in 24 hr. This factor would also be expected to prolong the persistence of



**Figure 1. Scale plan of the Three Sisters Canals showing the mean dimensions and the transects at which the dye was monitored.**

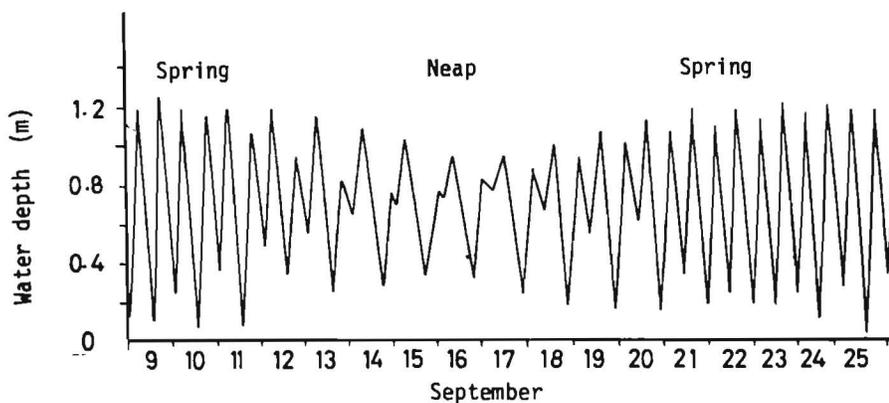


Figure 2. The predicted tidal cycles for King's Bay, in September 1987

dye in the canals. Water levels and exchange may also be influenced by less predictable factors, such as strong winds, atmospheric pressure, and heavy rainfall.

## METHODS

Direct measurements of the dye concentration in the canals were made from a boat using a Turner Designs Model 10-005 fluorometer fitted with the Model 10-020 high-volume, continuous-flow cuvette system. The limit of detection of the dye is 10 ng/l or 1,000 times less than the application rate approved for potable water. Water was pumped through the fluorometer by a bilge pump dropped on a hose to the desired depths. The temperature of each sample was recorded by a temperature probe attached near the pump. The fluorescence of Rhodamine WT varies with temperature, and a correction factor (Smart and Laidlaw 1977) was used to calculate actual concentrations. No significant loss of dye due to photochemical or microbial action was found in transparent plastic bags, filled with dye-treated water, which were suspended in the canals.

Rhodamine WT was applied to the canals at low tide from an airboat, with the intention of achieving a mean concentration of approximately 10 ug/l at the subsequent high tide. Dye was injected through the water column using trailing hoses and a perforated PVC pipe (approximately 3 m in length), which was pushed to the canal bottom and beneath boat docks to release dye in areas of the canal not reached by the hoses.

Dye concentration was monitored only at high tides at four transects along the length of each treated canal (Figure 1). Measurements were made at three points across each transect and at 0.5-m-depth intervals, starting at 0.5 m from the bottom, to the water surface.

The canals were relatively free of hydrilla at the beginning of the study due to repeated

applications of the herbicide endothall and mechanical harvesting by the Citrus County Aquatic Plant Control Program.

## RESULTS AND DISCUSSION

Dye treatment dates and comparisons between treatments are shown in Table 1, with each dye treatment represented by the canal name (A, B, C, D) and the number of the application in that canal.

Table 1  
Comparisons between dye treatments in tidal canals in Crystal River, Florida

<i>Date</i>	<i>Treatment</i>	<i>Tide</i>	<i>Vegetation</i>	<i>For Comparison</i>	<i>Objective</i>
25 Aug	A <sub>1</sub>	Spring	-	B <sub>1</sub> C <sub>1</sub> D <sub>1</sub>	1
27 Aug	C <sub>1</sub>	Spring	-	A <sub>1</sub> B <sub>1</sub> D <sub>1</sub>	1
31 Aug	A <sub>2</sub>	Neap	-	A <sub>1</sub>	2
8 Sep	D <sub>1</sub>	Spring	-	A <sub>1</sub> B <sub>1</sub> C <sub>1</sub>	1
10 Sep	B <sub>1</sub>	Spring	-	A <sub>1</sub> C <sub>1</sub> D <sub>1</sub>	1
5 Oct	A <sub>3</sub>	Neap	+	A <sub>2</sub>	3

Incomplete mixing of the dye immediately after application caused the data from the first sampling period to be highly variable, and these data were omitted from the analyses.

Dilution of the dye from the canals appeared to follow an exponential curve; and when the natural logarithms of the temperature-corrected concentrations were calculated, a linear relationship resulted, of the form:

$$\log_e C_t = \log_e C_o + at$$

where  $C_t$  = concentration at time  $t$ ,  $C_o$  = initial concentration, and  $a$  = exchange rate of water (Fox 1987). From this equation, the half-life of the dye is:

$$\log_e e = \frac{(0.5)}{a}$$

Since the initial concentration of dye was determined by the amount of dye applied and the initial canal water volume, only differences between the canals of the dilution rate  $a$ , and not  $C_o$ , were of interest. These differences were identified by considering the canal and time interaction in the analysis of variance of all, or pair-wise combinations, of the canal data. Half-lives of the dye and linear regressions of concentration against time were calculated for each canal treatment (Figure 3).

The only two canals which have significantly different rates of dye dilution are B and C. This difference is not a major constraint to future use of the canals as replicates because in order to minimize the possibility of cross-contamination of dye, alternate pairs of canals (e.g. A and C or B and D) would be used for comparison.

No significant differences in the rate of water dilution from canal A occurred when

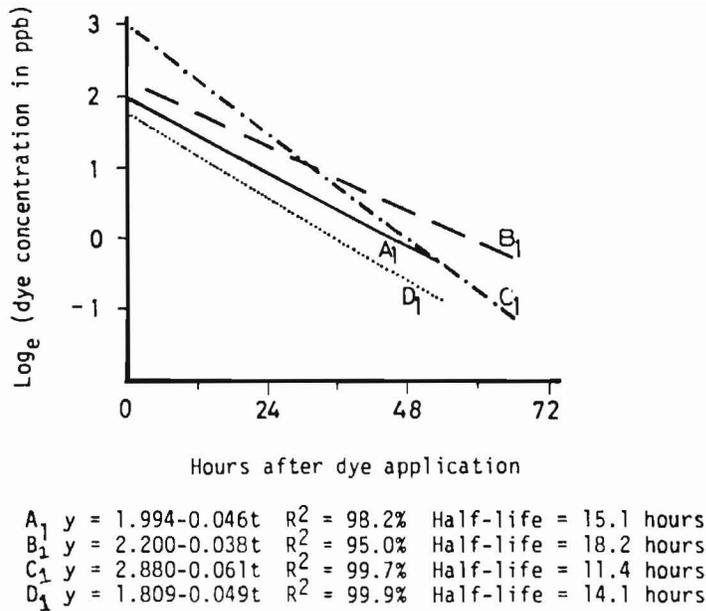


Figure 3. Regression lines showing the spring tide dissipation rates for Rhodamine W.T. in each of the canals

the spring (A<sub>1</sub>) and neap (A<sub>2</sub>) tide treatments were compared, although the dilution rate was more rapid than expected on the neap tide:

$$A_1 \quad y = 1.994 - 0.046t \quad R^2 = 98.2\% \quad \text{Half-life} = 15.1 \text{ hr}$$

$$A_2 \quad y = 1.499 - 0.058t \quad R^2 = 92.2\% \quad \text{Half-life} = 11.9 \text{ hr}$$

Examination of the changes in water level during the monitoring period (Figure 4) may indicate why the dilution rate for the neap tide was greater than expected. Further comparisons of the two cycles in other canals will be necessary to verify these preliminary findings.

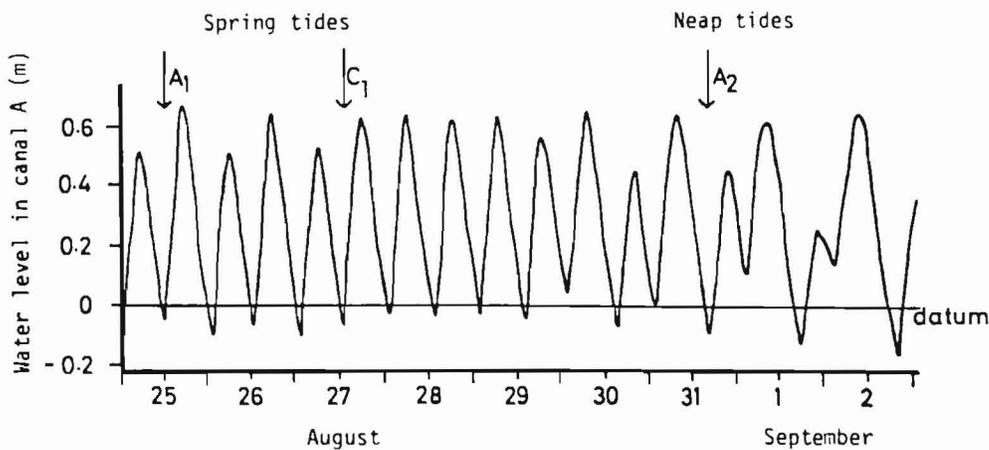


Figure 4. Water level recordings from canal A showing the times of dye treatments A<sub>1</sub>, C<sub>1</sub>, A<sub>2</sub>

Comparison of treatments A<sub>2</sub> and A<sub>3</sub> showed very significant differences in the dilution rates, with the half-life being extended from 12 hr to just over 5 days:

$$A_2 y = 1.499 - 0.058t \quad R^2 = 92.2\% \quad \text{Half-life} = 11.9 \text{ hr}$$

$$A_3 y = 1.696 - 0.0055t \quad R_2 = 73.0\% \quad \text{Half-life} = 126.0 \text{ hr}$$

Both treatments were carried out on neap tides with ostensibly only a difference in the density of vegetation. Fathometer tracings made along the center line of the canals at the times of treatment (Table 1) showed 43.6 and 73.5 percent vegetation in the longitudinal sections for treatments A<sub>2</sub> and A<sub>3</sub>, respectively.

In treatment A<sub>3</sub>, the concentration of dye was significantly greater in the bottom half of the water column (5.21 ug/l) than in the top half (2.96 ug/l), the opposite to that found in all of the other treatments (e.g. treatment A<sub>2</sub> -bottom 0.13 ug/l, -top 0.68 ug/l).

This differentiation might have been the result of the prolonged retention of dye-treated water within the dense plant beds, where unidirectional water velocity will be reduced (Marshall 1978; Getsinger 1987). However, it was also observed that the mean water temperatures for the two treatments were significantly different, which could also affect dilution and will be the subject of further investigation.

To take advantage of the unexpectedly long half-life of the dye it was recommended that an application of Aquathol K (endothall), at recommended label rates, be made by the Citrus County Aquatic Plant Control Program to canal A on the neap tide of 14 October 1987. This treatment was successful. Fathometer tracings showed a reduction in the amount of vegetation in the longitudinal section from 73.5 percent, pretreatment to 26.2 percent, 13 days after treatment.

In view of the efficacy of this herbicide treatment, it has been recommended that further dye studies should be carried out to determine the relative importance of temperature regimes and vegetation density on water dilution, and hence aid in the prediction of the optimum times for herbicide treatments in the Three Sisters Canals.

Dye applications could provide data on the minimum rate of herbicide loss that could be expected from water exchange (i.e. the maximum possible persistence). The concentration of herbicide will be further reduced by the uptake of plants, microbial degradation, and adsorption onto sediments. Half-lives of the dye in the canals of 11-18 hr may be relatively short in relation to the desired exposure of plants to herbicides, particularly since limited information exists on herbicide concentration/exposure time relationships. Studies to determine the concentration/exposure time requirements of endothall on hydrilla are being conducted at the Waterways Experiment Station. When all these data are combined with information on rates of endothall uptake and adsorption (e.g. Reinert et al. 1985, Langeland and DeMont 1986), the efficacy of endothall applications to the canals may be predictable.

## ACKNOWLEDGEMENTS

This study was conducted by the University of Florida (IFAS) Center for Aquatic Plants and the WES in cooperation with the Jacksonville District. Herbicide applications were made by the Citrus County Aquatic Plant Control Program, whose personnel

were most helpful throughout the study. Much appreciated technical assistance was provided, despite some difficult working hours, by W. Reed Green of the WES and Margaret Glenn of the University of Florida.

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# Herbicide/Adjuvant Evaluation in Flowing Water

by  
Kurt D. Getsinger\*

## INTRODUCTION

One method of managing submersed vegetation in flowing water is to use herbicide/adjuvant mixtures. Adjuvants, such as inverting oils and polymers, are designed to increase the effectiveness of liquid herbicide formulations by enhancing the placement of herbicides on target vegetation. A primary concern is the length of time that adjuvants can hold herbicides in the vicinity of target plants when exposed to high water exchange via stream flow, tides, or wind-generated currents.

In FY 87, a study was initiated to evaluate the use of herbicide/polymer mixture (endothall/Nalquatic) for controlling hydrilla (*Hydrilla verticillata*) in tidal canals of the Crystal River, Florida. The objectives of this study were to: (a) compare herbicide release profiles of a conventional endothall formulation, with an endothall/polymer mixture, following an operational application; (b) compare efficacy of the two formulations; and (c) if possible, determine the relationship between dye and herbicide dissipation under field conditions. This article describes the methods used and preliminary results of the endothall/polymer field study.

## MATERIALS AND METHODS

The study was conducted in the Three Sisters Canals in the Crystal River, Florida (Figure 1). Two herbicide formulations were evaluated: liquid endothall (Aquathol K), and an endothall/polymer (Aquathol/Nalquatic) mixture. The fluorescent dye, Rhodamine WT, was tank mixed with both herbicide formulations. The polymer, Nalquatic, was blended at 2 percent with one herbicide/dye formulation to produce a mucous-like mixture. Approximately 4 ml of the anti-foaming agent, Foam Buster, was added to each tank mix to prevent excessive foaming of the mixtures. Treatments were as follows: Canal B was treated with Aquathol K and Rhodamine WT; Canal D was treated with Aquathol K/Nalquatic and Rhodamine WT; and Canal C was an untreated reference. Herbicide/dye formulations were prepared to provide an initial endothall concentration of 3 mg/ℓ and Rhodamine WT concentration of 10 ug/ℓ in the canals.

The formulations were applied evenly to the canals, during low tide, using an airboat equipped with a 10-gallon per minute (gpm) piston pump connected to a rear-mounted manifold. A series of drop-hoses, attached to the manifold, injected the formulations approximately 15 cm below the water surface.

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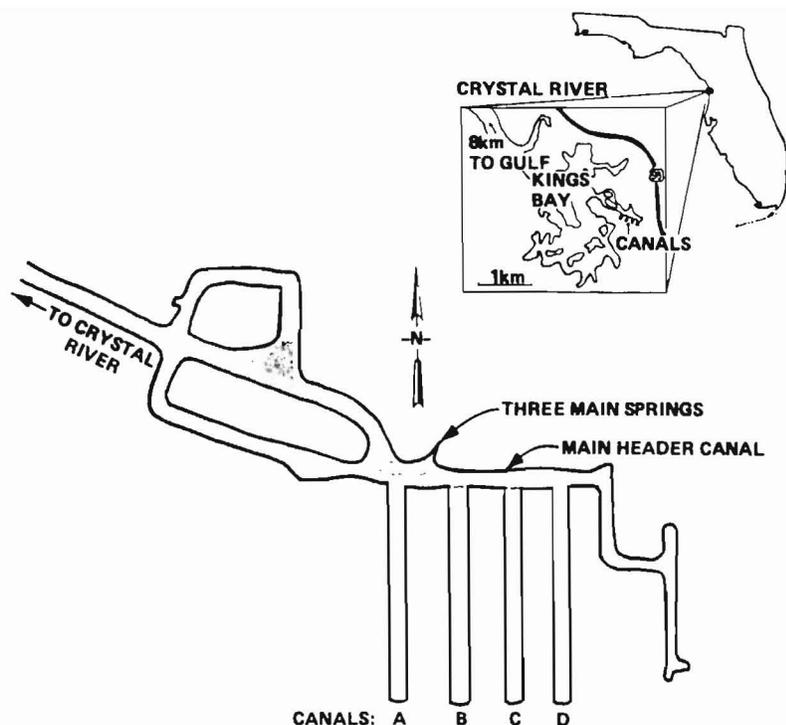


Figure 1. Three Sisters Canals study site, Crystal River, Florida

The dye was monitored at high tides for a 2-week posttreatment using a Turner Designs Model 10-005 field fluorometer fitted with a 10-020 high-volume, continuous-flow cuvette system and appropriate filters and light source for detecting Rhodamine WT. Water was pushed through the fluorometer by a bilge pump attached to the end of a hose. A temperature probe was also attached to the hose, enabling the simultaneous measurement of water temperature and dye concentration. Rhodamine WT concentrations were measured at three points across five transects established along each canal (Figure 2). Measurements were recorded at 0.5-m-depth intervals at each sampling station, starting at 0.5 m from the canal bottom and ending just below the water surface.

Water samples for endothall analysis were collected from 1 m below the water surface and 0.5 m above the canal bottom at the same time, and stations as used in the dye measurements. Water was collected from the outflow of the fluorometer so that dye and herbicide concentrations for each sample of water could be correlated. Samples were immediately stored on ice and frozen within 12 to 36 hr. Endothall residue analysis was performed by A & L Midwest Agricultural Laboratory, Omaha, Nebraska.

Water quality parameters (temperature, dissolved oxygen, conductivity, and pH) were measured using self-contained, submersible Hydrolab water quality samplers (Model 2030-DS). Water quality stations were established at one point in each canal (Figure 2). Parameters were measured every hour at 1 m below the water surface and 0.5 m above the canal bottom from pretreatment day 1 through posttreatment day 27.

Efficacy of herbicide treatments was assessed using fathometer tracings from the centers of the canals at pretreatment through posttreatment days 12, 27, and 43.

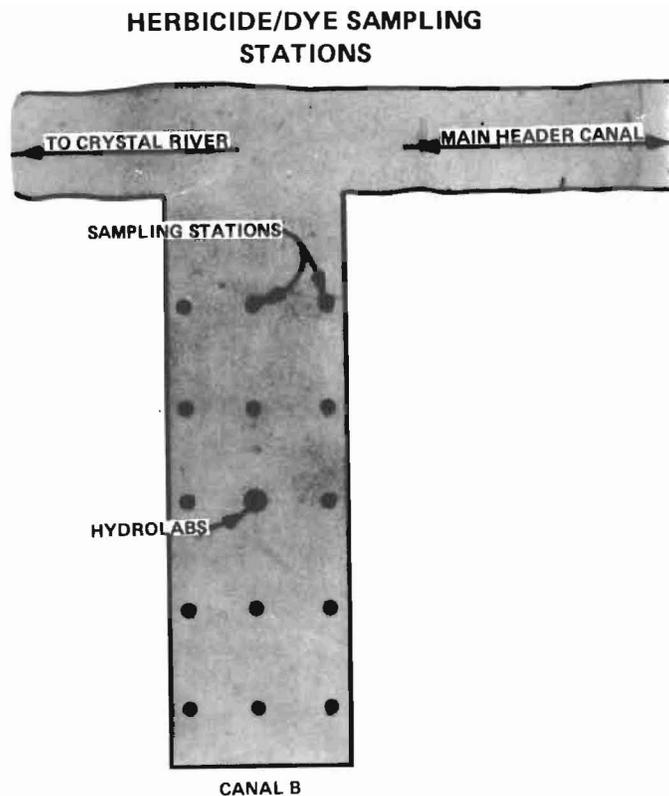


Figure 2. Rhodamine WT, endothall, and water quality sampling stations

## RESULTS

Only preliminary results are available at this time. The half-life of Rhodamine WT was 12 days in Canal B and 7 days in Canal D. Endothall residues are in the process of being analyzed. Fathometer tracings at posttreatment day 27 indicated good control of hydrilla in Canal B and fair control in Canal D, while hydrilla continued to flourish in the reference canal.

## ACKNOWLEDGMENTS

The author thanks Alison Fox, Bill Haller, and Margaret Glenn of the Center for Aquatic Plants, University of Florida; W. Reed Green of the Waterways Experiment Station; and the Jacksonville District for their assistance in this study. A special thanks is also extended to the Florida Citrus County Aquatic Plant Management Program for chemical application and posttreatment monitoring assistance.

# Herbicide Concentration/Exposure Time Studies on Eurasian Watermilfoil

by  
W. Reed Green\*

## INTRODUCTION

Standard herbicide application practices used in static aquatic systems generally produce unsuccessful results when applied in flowing-water environments. In flowing-water systems (e.g. rivers and reservoirs), water exchange enhances herbicide dissipation, dilutes herbicide concentrations, and reduces herbicide exposure time. Low residue concentrations and reduced exposure times reduce the effect of the herbicide on target plants. Basic relationships between herbicide concentration and exposure time for the control of specific target plants must be defined before successful strategies can be developed and implemented for use in flowing-water systems.

Herbicide concentration and exposure time correlations with plant control can be best defined in the laboratory where both herbicide concentration and exposure time can be controlled. The optimum concentrations exposed for the proper amount of time can then be applied and verified under field conditions. Concentration and exposure time relationships can then be adapted in field application procedures by adjusting the concentration of herbicides applied (up to the maximum label rate) to match the exposure time allowed within the system treated. These relationships will also be of value in the development of controlled release formulations and carriers to increase the amount of exposure time for known concentrations of herbicides.

## OBJECTIVES

In response to the necessity for the development of better technologies in the chemical control of aquatic plants, herbicide concentration/exposure time relationship studies were designed and developed by the Chemical Control Technology Development Project of the Aquatic Plant Control Research Program, US Army Corps of Engineers. The objective of the present research is to develop 2,4-D concentration and exposure time relationships for the control of watermilfoil (*Myriophyllum spicatum* L.)

## RELATED RESEARCH

The herbicide 2,4-D has been used for both terrestrial and aquatic purposes for over 40 years. Watermilfoil is controlled by 2,4-D, but little is known about the efficacious

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

relationships between concentration and exposure time. Under laboratory conditions, watermilfoil exposed to 0.10-mg 2,4-D acid equivalent (ae)/litre continuously for 70 days resulted in 100 percent control (total kill) (Hall et al. 1982). Further investigations (Westerdahl et al. 1983) found that watermilfoil was 100 percent controlled when exposed continuously to 2,4-D for 42 days at a concentration of 0.10 mg/l and for 21 days at a concentration of 0.20 mg/l.

Exposure times and residue dissipation rates in the field are dependent upon the hydrodynamics of the system treated. Exposure times and dissipation rates of 2,4-D are relatively similar when applied simultaneously to different sections within the same area of water. Applications producing the highest aqueous 2,4-D concentrations generally produce the greatest plant control. Many applications, however, do not provide a high enough concentration of 2,4-D for the exposure time allowed to have any effect on the target plant.

To evaluate 2,4-D residue persistence in the field, Getsinger and Westerdahl (1984) conducted an experiment in Lake Seminole, Georgia, using two application rates of a controlled release formulation (butoxyethanol ester, BEE) targeted for the control of watermilfoil. The initial 2,4-D exposure time (persistence) in the two BEE treatments (90-kg ae/ha and 22-kg ae/ha) was between 7 and 14 days. The half-life of residue persistence was approximately 5 to 7 days. Residues were again detected at posttreatment days 28 and 56 as a result of the controlled release formulation. The 2,4-D residue concentrations in the water at posttreatment day 1 ranged from 0.071-0.130 mg/l in the higher BEE application and 0.040-0.066 mg/l in the lower BEE treatment. Of the two application rates applied, the highest produced 75-80 percent watermilfoil control, whereas the lowest produced 60-70 percent control. However, neither treatment produced total watermilfoil control.

Hoepfel and Westerdahl (1983) conducted another field experiment in Lake Seminole, Georgia, where two different formulations of 2,4-D (dimethylamine, DMA and BEE) were applied at two different rates (45-kg ae/ha and 22.5-kg ae/ha). Again, the targeted plant was watermilfoil. The 2,4-D exposure time (persistence) in the water was between 5 and 7 days. The half-life of 2,4-D residue persistence ranged from approximately 3.7 to 4.4 days. The high rate DMA application produced aqueous 2,4-D residue concentrations at posttreatment day 1 ranging between 1.3 and 3.8 mg/l. The other three applications produced residue concentrations of 0.28-1.3 mg/l. Residues were still higher in the high rate DMA treatment than the other three at posttreatment day 4. The high-rate DMA treatment with the high aqueous residues produced complete watermilfoil control for more than 70 days and continued for the remainder of the growing season. Regrowth and reinfestation occurred by posttreatment day 70 in the other three treatments with the lower aqueous residue concentrations.

## MATERIALS AND METHODS

The present study was developed and designed to better resolve 2,4-D concentration and exposure time relationships for the control of watermilfoil. The concentrations examined were similar to aqueous residue levels found in previous field

applications. Exposure times tested were also based on herbicide persistence and dissipation rates developed from field applications.

The laboratory system used for this study was a modification of the diluter system used by Hall et al. (1982) and Westerdahl et al. (1983). This system was located in a controlled environment greenhouse and consisted of twenty-four 55-ℓ vertical aquaria, approximately 1 m tall by 0.3m<sup>2</sup>. Supplemental lighting was provided by a light bank suspended above the aquaria setup. The plants received 13 hr of light and 11 hr of dark per day. The mean photosynthetically active radiation received by the aquaria was 1600 uE/m<sup>2</sup> (Hall et al. 1982), which corresponds to 75 percent solar noon sunlight received at this altitude. The aquaria setup was surrounded by vinyl curtains to prohibit exposure to the sunlight filtering through the greenhouse ceiling and walls. The color of inside vinyl liner was white to diffuse the supplemental lighting. Each aquaria was independently supplied with a continuous flow of reconstituted hard water (Hall et al. 1982, USEPA 1975). The water volume (55 ℓ) of each aquaria was displaced continuously with fresh, reconstituted hard water every 24 hr.

Watermilfoil was purchased from and supplied by Suwannee Laboratories, Inc., Lake City, Florida. The watermilfoil was separated into 15-cm apical propagules and planted in 250-ml beakers containing sediment. Four propagules were planted 5 cm deep in each beaker, and 11 beakers were placed in each aquaria. The sediment used was collected from Brown's Lake, Waterways Experiment Station. The sediment was enriched with nutrients (Ra-pid-gro with Forti-5™, Ra-pid-gro Corp.) to ensure proper macro- and micronutrient availability and to reduce the effects, if any, of nutrient limitation. Calculated elemental enrichments were approximately 78-mg(P)/beaker, 18-mg(N)/beaker and 41-mg(K)/beaker. The nutrient additions were approximately 21-46 percent of the nitrogen, 3.5 percent of the phosphorus, and 3.6 percent of the potassium contained in a beaker of the Brown's Lake sediment (Barko and Smart 1986). When the planted propagules grew to approximately 10 cm below the water surface (two weeks), the plants were then subjected to the test protocol. One beaker of plants was randomly removed from each aquaria just prior to 2,4-D application to provide an estimate of treated biomass.

In all, 14 experimental herbicide concentration/exposure time tests and 2 untreated references were conducted in triplicate. Due to lack of space and number of aquaria (24), the tests had to be separated into two independent test runs. Each triplicate was randomly distributed among the 24 aquaria in each run. The 2,4-D concentrations/exposure times and runs in which they occurred are presented in Table 1.

The 2,4-D stock solutions were prepared from analytical grade 2,4-D acid (>97 percent acid), supplied by Union Carbide Corporation. The 2,4-D acid (powder) was dissolved in ethyl alcohol and then diluted with distilled H<sub>2</sub>O to make 1-ℓ stock solutions. Calculated volumes of the 2,4-D stock solution were added to the aquaria to provide the treatment concentrations. The 2,4-D solution remained in the aquaria for the amount of the exposure time. Once the exposure time was reached, the water in the aquaria was then emptied and replaced with fresh water. Each aquaria was emptied and filled three times to rinse out 2,4-D residues. Residue samples were taken from each aquaria immediately after treatment to verify treatment concentrations, and just prior to the first rinse and

Table 1  
Experimental Protocol

<i>Concentration (mg 2,4-D ae/l)</i>	<i>Exposure Time (hours)</i>	<i>Experimental Run</i>
0.5	12	2
0.5	24	1
0.5	36	2
0.5	48	1
0.5	60	2
0.5	72	1
1.0	12	2
1.0	24	1
1.0	36	2
1.0	48	1
2.0	12	2
2.0	24	1
2.0	36	2
2.0	48	1
0.0	0	1
0.0	0	2

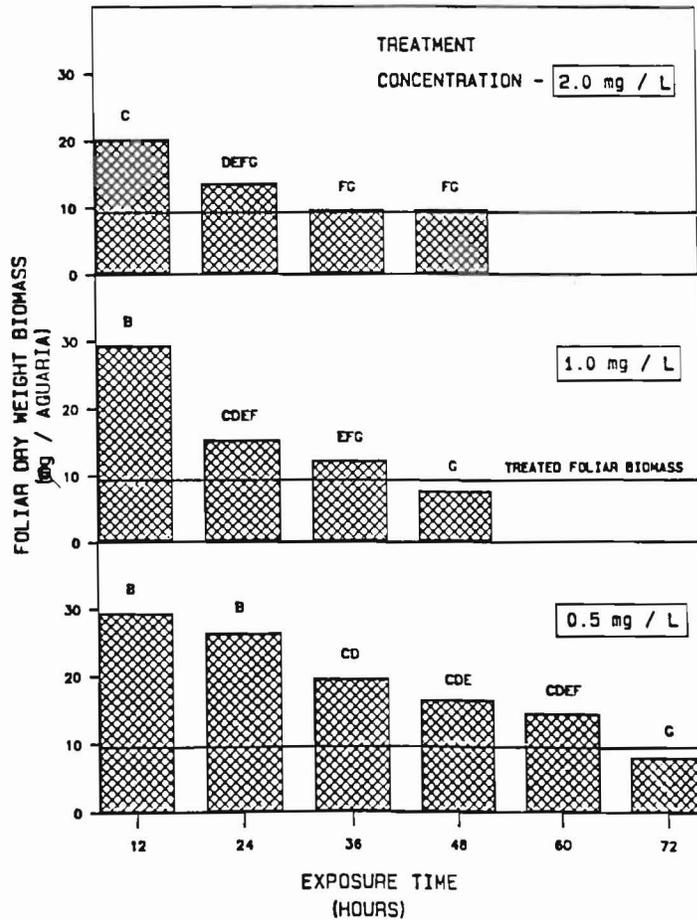
after the final rinse to verify the removal of the 2,4-D residues. Residue samples were analyzed by the Analytical Laboratory Branch, Tennessee Valley Authority.

The treated plants remained in the aquaria for four weeks to allow for the effects of the herbicide to be analyzed. The plants were then harvested from each aquaria, and the biomass was separated into foliage and roots. Adventitious roots outside the sediment were included with the foliage, and the original propagule stem placed in the sediment, if present, was included with the roots. The harvested biomass was then dried at 55° C and weighed. Quantitative results were determined based on dry weight biomass contained in each aquaria. Quantitative biomass determinations were converted into percent control (100 percent control being equivalent to total kill) by subtracting the harvested biomass from the pretreatment biomass estimation and dividing it by the posttreatment biomass produced in the reference treatment. Qualitative results were based on weekly visual evaluations of each aquaria. Visual estimations of control were based on a scale of 0-100 percent. Statistical analyses were conducted using SAS (SAS Institute, Inc., Cary, North Carolina). Analysis of variance was conducted to determine if the results for each treatment were statistically different.

## RESULTS AND DISCUSSION

The treated and harvested watermilfoil biomass in this study was similar in physiological condition and biomass of watermilfoil normally treated in the field. The watermilfoil at the time of treatment was young and rapidly growing. In the field, maximum seasonal watermilfoil biomass ranges from 32-360 g/m<sup>2</sup> (Grace and Wetzel 1978). The estimated treated watermilfoil biomass of the present study averaged 9.4 and 9.31 g per aquaria for the two runs. Converted to field concentrations, this biomass was equivalent to 104 g/m<sup>2</sup>. The biomass harvested (4-week posttreatment) in the reference aquaria averaged between 26 and 35 g. Converted to field measurements, this average would be equivalent to 293 and 389 g/m<sup>2</sup>.

Results from the two combined runs including the 14 treatments provide good evidence for the correlations between 2,4-D concentration and exposure time and the control of watermilfoil (Figure 1). Figure 1 graphically represents the mean biomass produced per aquaria for each treatment. The horizontal line just under the 10-g biomass per aquaria represents the estimated watermilfoil biomass treated. Harvested biomass similar to or less than the biomass treated indicates that watermilfoil biomass production was inhibited and the vegetation was completely controlled (killed).



Note: Each bar represents the treatment mean per aquaria. Means with the same letter are not statistically different according to Duncan's multiple range test.

Figure 1. Mean treatment biomass

Watermilfoil biomass production was not affected (no control) by 2,4-D in the 1.0-mg/ℓ to 12-hr exposure or the 0.5-mg/ℓ to 12- and 24-hr exposure treatments. In these tests, posttreatment biomass production was similar to that in the reference aquaria. The watermilfoil treated initially showed signs of injury but just for a short time, and recovery occurred within days.

Various degrees of watermilfoil injury and positive net posttreatment biomass

production (marginal control) occurred in the 2.0-mg/l to 12- and 24-hr exposure treatments, the 1.0-mg/l to 24- and 36-hr exposure treatments, and the 0.5-mg/l to 36-, 48-, and 60-hr exposure treatments. Percent control ranged from 36 to 84 within these treatments. For each concentration of 2,4-D, percent control increased proportionally with increasing exposure times.

Four 2,4-D treatments (2.0 mg/l-36 hr, 2.0 mg/l-48 hr, 1.0mg/l-48 hr, and 0.5 mg/l-72 hr) produced zero net biomass after treatment (complete control). The biomass harvested after the four-week posttreatment was equal to or less than the biomass treated. The plant tissue harvested in these aquaria was nonliving fragments and remnants of the original biomass treated, not yet decomposed. Roots were absent, and viable foliar tissue was visually evident in just a few of the originally planted beakers within the aquaria of these treatments.

Based on the results of these 14 concentration/exposure time tests, the estimated exposure time needed for the 2,4-D concentration of 2.0 mg/l to completely control watermilfoil is 34 hr. The estimated exposure times needed for 1.0 and 0.5 mg/l are 41 and 63 hr, respectively.

These results can be taken a step further and watermilfoil control relationships correlated for the entire range of 2,4-D concentrations (0.5-2.0 mg/l) and exposure times (12-72 hr) as shown in Figure 2. Also, in Figure 2, three degrees of watermilfoil control (no control, marginal control, and total effective control) are delineated within a "X-Y" coordinate system (exposure time on the X axis and concentration on the Y axis). It would be expected that 2,4-D treatments with concentrations and exposure times falling inside the dashed line (marginal control line, Figure 2) would have little or no effect on watermilfoil. Treatments with concentrations and exposure times falling between the dashed line and solid line (complete control line) would be expected to produce marginal watermilfoil control, with the degree of injury increasing away from the origin. Treatments resulting in 2,4-D concentrations and exposure times falling outside the solid line (line of control) would be expected to produce complete, effective watermilfoil control.

In the field, if it is known that a system being treated has "X" exposure time as a result of water exchange and expected residue dissipation rate, then at least "Y" concentration of 2,4-D where "Y" intersects the control line at "X" must be applied to achieve complete control. Conversely, if "Y" concentration of 2,4-D is to be applied, then the minimum amount of exposure time ("X") required to achieve complete control will fall on the line of control intersecting "Y." Any herbicide concentration/exposure time correlation falling inside the line of control will result in marginal control or have no effect at all.

Previous field applications of 2,4-D support these concentration and exposure time relationships. Crude 2,4-D water-residue dissipation rates that were calculated from the results of Getsinger and Westerdahl (1984) and Hoepfel and Westerdahl (1983) and converted to concentration and exposure time points of reference, along with the efficacy results from these applications, correlate well with the no effect, marginal control, and total effective control developed in this research (Figure 2).

Aqueous 2,4-D concentrations in Getsinger and Westerdahl (1984) were low, barely above the threshold levels determined by Hall et al. (1982) and Westerdahl et al. (1983).

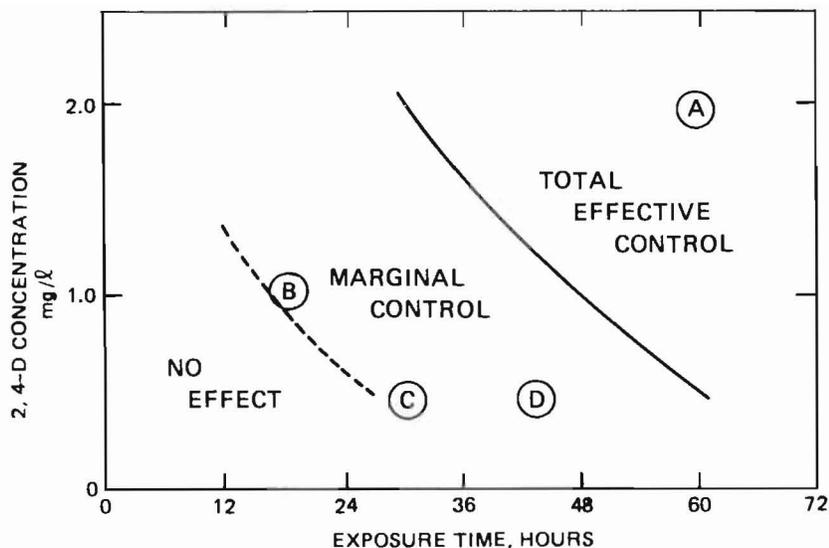


Figure 2. 2,4-D concentration/exposure time relationships for the control of watermilfoil

Exposure times required at these low concentrations to completely control watermilfoil are extremely long. The estimated concentration/exposure time values calculated from these field applications would be expected, using the results of the present research, to produce marginal watermilfoil control. As expected, marginal control occurred in the field where the treated vegetation was controlled at approximately 60-85 percent, followed by vegetative regrowth and reestablishment (Getsinger and Westerdahl 1984).

Concentration and calculated exposure times resulting from the field applications of Hoepfel and Westerdahl (1983) better resolve the developed relationships of the present research. Three of the four field applications resulted in watermilfoil injury, but the plants recovered and reestablished themselves. One of these marginal control applications (B, Figure 2) maintained a calculated minimum 2,4-D concentration of 1.0 mg/l for a maximum exposure of 18 hr. The highest aqueous 2,4-D residue concentration collected within this treated plot was 1.3 mg/l. The other two marginal control applications (C) and (D) in Figure 2 maintained a calculated minimum 2,4-D concentration of 0.5 mg/l for a maximum exposure of 30 and 40 hr, respectively. The highest residue concentrations collected in these two treated plots were 0.68 and 0.65 mg/l. The one effective field application (Hoepfel and Westerdahl, 1983) maintained a calculated minimum 2,4-D concentration of 2.0 mg/l for a maximum exposure of 60 hr (A, Figure 2). The maximum residue concentration collected in this test plot was 3.8 mg/l. Based on the relationships developed in the present study, this field application would be expected to completely control (kill) the exposed watermilfoil. The watermilfoil exposed to this field application of 45-kg DMA ae/ha (Hoepfel and Westerdahl, 1983) was completely controlled for the entire growing season.

## CONCLUSIONS

Fourteen different herbicide concentration and exposure time tests were conducted

using 2,4-D in order to develop concentration/exposure time relationships for the control of watermilfoil. At concentrations of 2.0-, 1.0- and 0.5-mg 2,4-D/l, watermilfoil must be exposed an estimated 34, 41, and 63 hr, respectively, to achieve complete control. Three degrees of watermilfoil control (no control, marginal control, and total effective control) were delineated within a "X"- "Y" (exposure time, concentration) coordinate system. Previous field results relate well with the results of this study indicating that the laboratory concentration and exposure time relationships have validity and can be used in developing submersed aquatic plant management strategies.

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# Feasibility of Using Plant Growth Regulators for Managing Aquatic Plants

by  
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## INTRODUCTION

Plant growth regulators (PGRs) have many uses in terrestrial plant management systems. They are used to increase (or decrease) growth rates, enhance (or slow) flowering or fruit ripening, and modify properties such as color or texture. One group of PGRs acts as an “antigibberellin” by blocking three essential steps in the gibberellin synthesis pathway. Without the production of this natural hormone, plant elongation is reduced. These antigibberellins are used to reduce the number of mowings in turf, the number of stem prunings in fruit trees, lankiness in potted plants, and lodging in grass crops. On a hypothetical basis, the use of an antigibberellin for aquatic plant management would seem ideal: retain a functional plant but at a shorter height.

The specific objectives of this project are to determine:

- The effects of antigibberellins on stem length and other associated length and biomass parameters (growth responses).
- The effects of antigibberellins on the physiological competency of the plants, with emphasis on oxygen production (physiological responses).
- Length of time in which antigibberellin effects persist.
- Effective exposure times for antigibberellin effects to be expressed.

The tests are being conducted in the laboratory in a bioassay system in which 4.0-cm apical stem segments of hydrilla (*Hydrilla verticillata*) and watermilfoil (*Myriophyllum spicatum*) are grown and exposed to the antigibberellin in sterile artificial media under controlled environment conditions (25°C, 200  $\mu\text{E}/\text{m}^2/\text{sec}$ , 16:8 hr light/dark photoperiod). We also have conducted some preliminary small-scale field tests in which plants were grown in 13-gal barrels outdoors.

## RESULTS

The following summarizes our results on the effects of paclobutrazol, primarily on hydrilla. At the time of this report we had begun testing a second compound, uniconazol. Flurprimidol is another compound that will be screened before the project period is completed.

### Species differences in response to dose

In the bioassay system watermilfoil was more sensitive to paclobutrazol than hydrilla. No increase in main stem length over a 4-week exposure period was obtained at 75  $\mu\text{g}/\ell$  ai

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on milfoil, whereas it took 3,000  $\mu\text{g}/\ell$  ai to achieve the same result on hydrilla. Growth suppression of the main stem was obtained on hydrilla at the lowest concentration tested (7.5  $\mu\text{g}/\ell$  ai). An effective working range for hydrilla is approximately 7.5 to 750  $\mu\text{g}/\ell$  ai (providing 50 to 30 percent of untreated control stem length increase, respectively), whereas on milfoil it is 7.5  $\mu\text{g}/\ell$  ai or lower. The effects of paclobutrazol on hydrilla in bioassay are remarkably consistent. This experiment has been repeated three times, and growth responses are similar each time.

### **Effective exposure time**

Effects on treated plants were visible as soon as untreated plants began growing. Differences in length can usually be monitored within one week of treatment, thus indicating that as soon as the gibberellin synthesis pathway is shut down, new growth will be immediately affected.

### **Effects on lateral stem production and elongation**

At "low" paclobutrazol concentrations (75  $\mu\text{g}/\ell$  or less) after a 4-week exposure, lateral stem production was stimulated (a mean number of 6 per stem in contrast to 0 per stem on the untreated controls). However, the mean length per lateral (3 cm) at these concentrations was lower than in the untreated controls (7 cm). If all stem (main + laterals) lengths are added, overall length in the treated plants was approximately the same as in the untreated plants. This condition is also reflected in slightly higher (but not significantly different at the 0.05 level) fresh weights in the treated versus untreated plants. A large number of short roots are also produced on treated plants. These results suggest that plants treated with low concentrations in the field might take on a bushy, stoloniferous type growth form, i.e. short, but with numerous lateral stems and a horizontal spreading habit. The retention of biomass and surface area in treated plants could be construed as beneficial by providing habitat for epiphytic and other plant-associated organisms similar to that of untreated plants but on a significantly shorter main stem.

### **Physiological effects**

Net photosynthesis on a dry weight basis was significantly inhibited (50 percent of untreated controls) in hydrilla at concentrations as low as 375  $\mu\text{g}/\ell$  paclobutrazol. However, percent dry weight increased with an increase in paclobutrazol concentration due to an increase in starch. The result was lower amounts of chlorophyll in treated plants on a dry weight basis and indicated that photosynthetic competence per unit chlorophyll was not greatly affected, i.e. the decrease in net photosynthetic rate was probably related to the amount of chlorophyll present in the tissue.

### **Duration of effect**

Preliminary data suggest that as soon as paclobutrazol is removed from solution, the plants reestablish normal growth patterns. This condition corroborates the mode of action of these compounds, i.e. they do not have a toxic effect (except at high dosages) but simply inhibit gibberellin synthesis. Once the antigibberellin is removed from solution, synthesis resumes.

### **Small-scale field tests**

These studies were conducted on hydrilla and showed paclobutrazol to significantly reduce plant height at concentrations as low as 7.5  $\mu\text{g}/\ell$ . In addition, a stoloniferous growth, as predicted from the bioassay tests, was achieved. An interesting finding was that starch did not build up in these plants with increasing dosage as it had in the bioassay. We believe this finding was due to the utilization of photosynthate in active growth and biomass increase in the field test rather than its sequestration into storage product which was common in the culture flasks.

### **Preliminary results on uniconazol**

Uniconazol appears to produce the same effects as paclobutrazol but to be more active at lower dosages. This result is expected as both compounds are from the pyrimidine group of chemistries. Flurprimidol is a triazole, and it will be interesting to see if plant responses to this chemistry are different.

In general, the potential for using antigibberellins in an aquatic plant management situation looks promising. They are effective at very low concentrations in reducing stem elongation, their effects are immediate, they do not disrupt physiological competence, and they promote a stoloniferous low-growing habit. The downside of these chemicals may be their lack of holding power, but this condition may be remedied by a delivery system (controlled release and soil application are two possibilities) in which the antigibberellin is delivered to the plant over prolonged periods of time.

# Plant Growth Regulators: Strategy for Use in Aquatic Plant Management Schemes

by  
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## INTRODUCTION

Manipulation of plant growth with both natural and synthetic chemical regulators has been a very common practice in agriculture for more than a decade. Indeed, many herbicides are plant growth regulators (PGRs) at sublethal concentrations. The discovery of 2,4-D (2,4-dichlorophenoxyacetic acid) and the subsequent development of the herbicide industry can be traced back to auxin research during the 1930's (Hamner and Tukey 1944). This research also led to the use of auxins to promote rooting (Thimann and Went 1934). Another plant hormone, ethylene, has been used to degreen oranges since the 1920's (Denney 1924). In general, plant hormones or synthetic hormone analogs are currently used for seed germination, rooting, dwarfing, branching, the induction of flowering, the development of different sizes and shapes of fruit, defoliation, delaying fruit drop, promoting fruit maturity, promoting latex flow, promoting malting, and preventing sprouting (Morgan 1980).

Interest in PGRs and aquatic angiosperms developed slowly through the 1970's (Anderson 1982a). Much of this interest developed from the need to better understand the physiology and biochemistry of nuisance aquatic plants, in order to facilitate the development of better control methodologies and strategies. Nuisance aquatic plants typically hold some competitive advantage over those aquatics not usually troublesome. This is particularly true with exotic species accidentally or intentionally introduced into an aquatic ecosystem with indigenous aquatic plants. Competitive advantages such as low light compensation point, ability to utilize bicarbonate as a carbon source, prolific production of dormant overwintering structures, and differential growth strategies allow many exotic species to grow to monocultures in infested waterways. Understanding of the physiological and biochemical control of the internal plant mechanisms necessary for these competitive advantages will not only allow for the development of better aquatic plant management strategies but also provide a better understanding of processes such as flowering and dormancy, so vital to agriculture.

Recent research on the understanding of the role of plant hormones in the growth and physiology of aquatic angiosperms has indicated that very little is known about these processes, but potential for new plant management methodologies does exist. Gibberellic acid, for example, has been shown to inhibit float formation in *Eichhornia crassipes* (waterhyacinth) (Pieterse, Aris, and Butter 1976). The waterhyacinth is one of the most troublesome aquatic plants of tropical and subtropical waterways and lakes. Gibberellic acid, alone or in combination with other management methods, might prove effective in controlling the waterhyacinth population. More recently it has been shown that

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gibberellic acid synergistically enhances the potency of the herbicide 2,4-D; the lethal concentration for waterhyacinths can be lowered an order of magnitude if the 2,4-D is mixed with gibberellic acid (Pieterse and Roorda 1982).

Classic chemical approaches to aquatic plant management have concentrated on developing and screening new and better herbicides. PGRs represent a different approach and, as such, require a different development strategy for the development of PGRs as tools in the management of nuisance aquatic plants. The strategy is illustrated through an example based on research to control vegetative dormant propagule formation in *Hydrilla verticillata* Royle.

## PLANT GROWTH REGULATOR DEVELOPMENT STRATEGY

Aquatic plants are very beneficial to aquatic ecosystems. Along with their role as primary producers, aquatic plants serve as habitats for fish and invertebrates as well as food and nesting material for waterfowl. A management strategy that results in eradication of the plants is not desirable from an ecological point of view. Therefore, a management strategy needs to strive for a balance between uses of the water system and ecosystem stability.

The strategy for the use of PGRs in aquatic plant management schemes is dependent on an understanding of the biochemistry and physiology of the plant. The approach is to manipulate a physiological process such as flowering, dormancy, or vegetative growth such that the benefits of the plant to the aquatic ecosystem are realized but the plants do not become troublesome. This necessitates knowledge of the life cycle of the plant as well as both environmental and biochemical controls of that life cycle. A thorough understanding of the interactions of different plant hormones on the controls of growth and reproductive processes is critical. Once these results are generated they can be merged with an understanding of PGRs and their actions. At this point, a susceptible portion of the life cycle may be identified for interruption or a particular growth characteristic may be targeted for alteration.

Treatment of the plant with growth regulators suspected of causing the desired change or interruption may be an educated trial and error process at best. Presumably, the more information accumulated about the plant processes involved and the PGRs available, the better the chance of success. If treatment is effective and the desired growth characteristics are achieved, it is extremely important to observe any secondary effects present. These may or may not be desirable and must be factored into the efficacy evaluation of the PGR. Secondary effects of life cycle interruption, for example, may be altered vegetative growth characteristics.

Since aquatic plant management is necessary in both static and flowing waters, it is important to determine the minimum contact time required for efficacy. Plant uptake and translocation studies not only provide contact time information but may also provide details concerning the site and mode of action of the PGR. Dissipation studies are necessary at this point to determine if the chemical remains available to the plant for a time consistent with the contact time studies. These studies should include tests with suspended solids to determine if sorption to these solids significantly decreases the

bioavailability of the PGR. Results from these tests will also be useful in determining ultimate fate of the chemical in the aquatic environment.

Environmental fate and effects studies will be required in order to obtain Federal registration and licensing of the chemical. Chemicals applied directly to water systems used for drinking water supplies are particularly scrutinized for any environmental hazard. These studies involve the assessment of microbial, physical, and chemical degradation of the chemical; the determination of the bioavailability of the chemical after introduction into the aquatic ecosystem; and the evaluation of the effects, including toxicity and mutagenicity, of the chemical on nontarget organisms including mammals. Based on accumulated information, field trials may then be approved.

## APPLICATION OF THE STRATEGY

Research initiated in the early 1980's by this investigator has examined the potential for management of populations of *H. verticillata* through life cycle interruption. Current management strategies are hindered by the resiliency of this organism. Areas infested by *H. verticillata* may have as high as  $3 \times 10^6$  vegetative dormant propagules per hectare (Haller and Sutton 1975). These propagules represent several potential populations of plants and, as such, repopulate areas cleared through herbicidal treatment or mechanical removal of the vegetation. Reinfestation necessitates retreatment within 1 to 2 months. This treatment may be expensive, both monetarily and environmentally. If production of these propagules could be prevented or even significantly reduced, current management strategies might be more effective.

*Hydrilla verticillata* undergoes a well-defined vegetative life cycle. The mature plant produces two types of vegetative dormant propagules: in the leaf axils (turions), and on the end of subterranean stolons (tubers). Both are produced in the fall of the year. These propagules overwinter and subsequently sprout in the spring to produce new plants.

The environmental stimulus for propagule formation is photoperiod. Photoperiods of less than 12 hr produced propagules over a 15° to 30° C temperature range (Klaine and Ward 1984). A slight increase in propagule formation was seen at higher temperatures, probably due to higher productivity. Night interruption experiments on plants under a short photoperiod indicated the involvement of the phytochrome system. Endogenously supplied plant hormones did not inhibit this process. Abscisic acid, however, did significantly increase propagule formation. Measurement of endogenous levels of abscisic acid indicate the short (<12 hr) photoperiod plants have 80 times higher abscisic acid concentrations than do long (>12 hr) photoperiod plants.

These results allowed for the formation of a flow chart for propagule formation. This consisted of: Environmental stimulus of a photoperiod <12 hr → Plant receptor involving the phytochrome system → Message relayed on some biochemical basis to cells → Increase in abscisic acid production → Initiation of vegetative propagule formation. An attempt was then made to interrupt the process of propagule formation.

Night interruption experiments both in the laboratory and in the field were successful in eliminating or reducing propagule formation (Klaine and Ward 1984). Additional research and testing indicated that propagule formation can be significantly reduced

through night interruption (Anderson and Spencer 1987). While night interruption appears successful, it is not always practical. Cost and availability of a power source make interrupting the night with a light source often infeasible.

Interruption of the propagule formation through interruption of the phytochrome system is not feasible at this time. Indeed, known antagonists of the phytochrome system are phytotoxic themselves. The lack of complete knowledge regarding abscisic acid biosynthesis does not allow for the interruption of this process (Walton 1980). Instead, research focused on using plant growth regulators known to be antagonistic to the action of abscisic acid. The goal of this effort was to eliminate propagule formation without killing the plant. Thus, the benefits of aquatic vegetation could be realized but the competitive advantage of *H. verticillata* would be reduced, thus allowing more desirable native species to better compete.

Ethylene, both alone and in combination with gibberellic acid and kinetin, has been shown to be antagonistic to abscisic acid (Hallion 1976, Dunlap and Morgan 1977). Abscisic acid has also been shown to reduce ethylene levels (Gamborg and LaRue 1971). Ethylene applied as ethephon (2-chloroethylphosphonic acid) significantly reduced propagule formation in laboratory and greenhouse systems when applied repeatedly in small doses (0.1 to 1.0 mg/l applied every other day) (Klaine and Ward 1984). Significant reduction of propagule formation was also obtained with 1-aminocyclopropane carboxylic acid, the immediate precursor to ethylene. This reinforced the hypothesis that ethylene was in fact responsible for the effect.

Several currently used agricultural chemicals stimulate endogenous ethylene formation. Treatment of etiolated mung bean hypocotyl segments with 30 nanomolar thidiazuron (N-phenyl-N'1,2,3-thidiazol-5-ylurea) stimulated an increase in ethylene evolution over controls (Suttle 1984). Treatment of sunflower seedlings with chlorsulfuron (2-chloro-N-[4-methoxy-6-methyl-1,3,5-triazin-2-yl]aminocarbonyl] benzenesulfonamide) stimulated ethylene evolution over control levels 1 day after herbicide application and reached a maximum 2 to 3 days after treatment (Suttle 1983). Applications of cycloheximide induced ethylene production in intact citrus (*Citrus sinensis*) fruits (Riov and Yang 1982).

Propagule production in *H. verticillata* was inversely proportional to thidiazuron concentration in laboratory and greenhouse culture systems (Klaine 1986). Furthermore, a single  $10^{-6}$  M treatment during the fall of the year completely inhibited propagule formation throughout the short photoperiod season. Ethylene production by treated plants was proportional to thidiazuron concentration. Thus, it appeared that vegetative propagule formation could be eliminated with a single thidiazuron treatment, and that the mode of action was through endogenous ethylene evolution by the treated plant.

Secondary effects observed during these experiments indicated that thidiazuron exerted a cytokinin-like effect on plant growth. Concentrations  $\pm 10^{-6}$  M caused statistically significant increases in the number of branches of treated plants (Klaine 1986). Thidiazuron has been shown to have cytokinin-like activity in a number of bioassay systems (Baskakov et al. 1981, Kulaeva et al. 1983, Thomas and Katterman

1982). Other cytokinin-like compounds have been shown to stimulate ethylene production (Lau and Yang 1974, Yu et al. 1981).

Both *Spirodela polyrhiza*, a floating aquatic, and *Myriophyllum verticillatum*, a rooted aquatic, were stimulated to produce vegetative dormant propagules by the addition of abscisic acid; this propagule production was inhibited by the addition of cytokinins (Perry 1968, Weber and Nooden 1976). These results indicate some consistency between species concerning the role of these plant hormones in propagule formation. Anderson (1982b) investigated the effects of exogenously applied hormones on heterophylly in *Potamogeton nodosus*. Abscisic acid induced formation of "floating-type" leaves while the cytokinins benzyladenine and kinetin counteracted this effect.

It is clear that treatment of *H. verticillata* with  $10^{-6}$  M thidiazuron has a long-term effect. Cytokinin-induced branching continues for up to 4 months. In our laboratory systems, daily treatments with  $10^{-6}$  M abscisic acid seemed to counteract the thidiazuron effects. No atypical branching occurred, and propagules were produced rapidly. After 6 weeks, however, the plants appeared to be senescent. When abscisic acid treatments were discontinued, new growth appeared. This new growth, however, had the typical thidiazuron-induced growth characteristics seen previously. It appeared that abscisic acid merely inhibited the thidiazuron action. Thidiazuron, or some active metabolite, was still present in sufficient quantity to exert an effect.

Natural cytokinins have been tested previously on *H. verticillata* but had no effect on propagule formation (Van, Haller, and Bowes 1978). This may have been due to rapid metabolism of the cytokinin by the plant, thus eliminating the effect on a long-term process such as propagule formation. Thidiazuron appears to have a short half-life in water but is not rapidly metabolized by the plant after uptake (Suttle, USDA, North Dakota, personal communication). This may explain the success of a single treatment with this compound. Natural plant hormones are metabolized relatively fast by plants. This may account for the lack of activity seen previously with cytokinins. The naturally occurring cytokinin, 2-isopentenyladenine (2-IP), was tested using multiple low doses to determine if the same effect seen with synthetic cytokinins could be obtained with multiple doses of a natural cytokinin. *Hydrilla verticillata* plants were treated every other day with a dose of 2-IP necessary to bring the aqueous concentration to  $10^{-6}$  M. After 8 weeks no vegetative propagules were observed, and the typical branching associated with cytokinin treatment was observed. This is further evidence that cytokinins act antagonistically with abscisic acid in this system possibly through the stimulation of high levels of endogenous ethylene formation.

Since aquatic plant management is necessary in many types of aquatic systems, it is necessary to determine the minimum contact time necessary for elimination of propagule formation. Contact times of 1 to 56 days were examined using  $10^{-6}$  M thidiazuron. All contact times inhibited propagule formation and induced extensive branching in the plant. These results illustrate the rapid uptake of thidiazuron by *H. verticillata*. Experiments examining the translocation of thidiazuron within the plant are in progress.

In order to field test a chemical it is important to know certain information about the environmental fate and effects of the chemical. Since thidiazuron is currently registered

as a cotton defoliant, much of this information is available. A 7-day static renewal bioassay was conducted with *Ceriodaphnia dubia* in order to examine the chronic toxicity of thidiazuron. Methods were as prescribed and standardized by the US Environmental Protection Agency (Horning and Weber 1985). A no observable effect concentration (NOEC) of  $1 \times 10^{-5}$  M and a lowest observable effect concentration (LOEC) of  $5 \times 10^{-5}$  M were generated for thidiazuron. These values are significantly higher than the  $10^{-6}$  M concentration necessary to inhibit propagule formation in *H. verticillata*.

## DISCUSSION

The strategy for PGR development for use in aquatic plant management schemes relies heavily on knowledge of aquatic plant physiology and biochemistry. A better understanding of the aquatic plants will not only increase the chances of success for PGR research, but will also provide valuable information for other methods of aquatic plant management such as biological control. It is unlikely that one single control method will be both effective and economical. Rather, some combination of techniques will form an integrated management strategy that should prove to be a more effective management scheme. PGR treatments are perfect for such integrated management schemes. Examples of such integrated approaches might be the following: combination of PGRs with herbicide treatment to reduce total herbicide usage; combination of PGRs with insect predators to make the treated plant more susceptible to predation; and combination of PGRs with herbicide treatment to reduce plant regrowth from herbicide-resistant dormant vegetative structures.

The PGR development strategy presented here worked well for the interruption of vegetative dormant propagule formation in *H. verticillata*. It is hoped that this strategy will work for interruption of other processes such as flowering and root crown development, as well as the alteration of growth characteristics. A major concern for the potential use of PGRs in aquatic systems is the large quantity of environmental data required to secure use registration. Long-range PGR development efforts should include consideration of the generation of environmental fate and effects data required for registration.

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# Bensulfuron Methyl: A New Aquatic Herbicide\*

by

Lars W.J. Anderson\*\* and Nathan Dechoretz\*\*

## INTRODUCTION

Even though aquatic weed problems continue to increase throughout the United States, there has been a sharp decline in new, registered aquatic herbicides since the late 1960's (Anderson, 1986).† Few herbicides have the toxicological, physiochemical, and efficacy characteristics required for safe use in aquatic environments. Bensulfuron methyl (BSM) (Figure 1) has been under extensive investigation and development by Du Pont for use in rice. (For a comprehensive review of the sulfonylurea herbicides, including bensulfuron methyl, see Beyer et al. 1988).†† Among the beneficial attributes of BSM are: (a) its relatively short half-life, from a few weeks to a few months depending upon field conditions; (b) its activity in the soil; (c) its low fish toxicity (e.g.  $LC_{50} > 150$  ppmw for trout and bluegill sunfish); (d) the low concentrations required for control (ca. 10 to 100 ppbw); and (e) the potential to use it for preemergence control or suppression of growth as well as for late postemergence suppression of reproductive capacity. Du Pont has submitted a petition for an Experimental Use Permit for 1988.

## METHODS

### Greenhouse exposures

Preemergent exposures to *Potamogeton pectinatus*, *P. nodosus*, *Myriophyllum spicatum*, and *Hydrilla verticillata* were conducted by planting either propagules (tubers or winter buds) or apical cuttings in U.C. mix in 5-cm plastic pots immersed in 20-l glass jars. BSM was added once to produce concentrations from 1 to 100 ppbw depending upon the species. After 4 weeks, shoot lengths and weights were determined. In some experiments, propagules were exposed for 24 hr to higher concentrations (0, 0.1, 1.0, 10.0 ppmw), and responses were assessed 4 weeks later.

To assess the soil-activity of BSM, simulated soil-surface applications were made as follows: *Potamogeton pectinatus* tubers and *P. nodosus* winterbuds were planted in 1-l plastic containers filled with clay-loam soil. The herbicide was applied on the surface at

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\*Research conducted with the US Department of Agriculture/Agriculture Research Service (USDA/ARS) funding only. Use of herbicide common or trade names does not imply recommendations or endorsements of any kind.

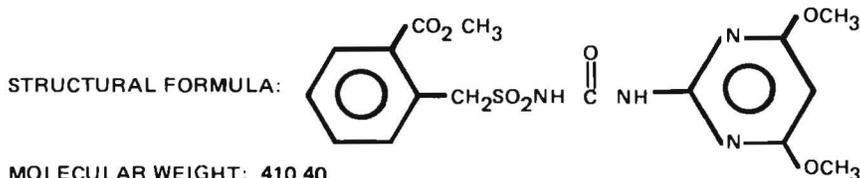
\*\*Research leader and biologist, USDA/ARS Aquatic Weed Control Research Laboratory, Botany Department, University of California, Davis, California. (N. Dechoretz is currently a Biologist with the California Department of Food and Agriculture.)

†Anderson, L.W.J. 1986. "Recent Developments and Future Trends in Aquatic Weed Management," *Proceedings, EWRS/AAB, 7th Symposium on Aquatic Weeds*, pp 9-16.

††Beyer, E.M., Duffy, M.J., Hay, J.V., and Schlueter, D.D. 1988. "Sulfonylurea Herbicides," *Herbicides: Chemistry, Degradation, and Mode of Action*, Marcel Dekker, Inc., Vol 3, pp 117-189.

COMMON NAME: BENSULFURON METHYL

CHEMICAL NAME: METHYL 2-[[[(4, 6-DIMETHOXYPYRIMIDINE-YL) AMINO] CARBONYL] AMINO] SULFONYL] METHYL] BEZONATE



MOLECULAR WEIGHT: 410.40

MELTING POINT: 185-188°C

PHYSICAL FORM: WHITE TO YELLOW, ODORLESS, SOLID

VAPOR PRESSURE:  $2.1 \times 10^{-14}$  MG HG AT 20° C

AQUEOUS SOLUBILITY: PH 4.8 - 2.9 PPM  
PH 5.8 - 12.0 PPM  
PH 6.9 - 120 PPM  
PH 7.8 - 1200 PPM

PKA = 5.2

STABILITY: IN AQUEOUS SOLUTIONS, "LONDAX" IS MOST STABLE UNDER SLIGHTLY ALKALINE CONDITIONS (pH 8.0) AND DEGRADES SLOWLY UNDER ACIDIC CONDITIONS.

Figure 1. Structure and some physical characteristics of bensulfuron methyl

rates of 0.05, 0.1, 0.25, and 0.5 kg/ha. Other treatments were made by applying the herbicide above the planted propagules but layered at a depth of 2.5 cm.

The containers were then immersed in 18-l jars containing well water and grown in the greenhouse under natural lighting. Containers with plants and untreated soil were placed in the same jars with the treated containers as well as in jars with no treated containers. For all treatments three propagules per container were used, and two treated and two untreated containers (except in control jars, which contained only untreated containers) were placed within 18-l jars, with three jars per treatment. The untreated containers placed in the jars with treated containers served as bioindicators for BSM that may be released from the soil. Shoot length, dry weight, and root dry weight were determined 4 weeks after treatments.

Other experiments included early postemergent exposures of one-week-old *P. pectinatus* (from tubers) and *Hydrilla verticillata* (from cuttings) from 1 to 24 hr to 10- or 100-ppbw BSM followed by grow-out in 90-l tanks. To determine the effect of plant age on susceptibility to BSM, both *Potamogeton* species were exposed to 100-ppbw BSM at the following times from day of planting: 3, 7, 10, 14, 21, and 28 days. Responses of these plants were determined 4 weeks after the treatment date by obtaining their dry weights and shoot lengths.

### Outdoor cultures

The response of mature (4-5 week old) *P. pectinatus*, *P. nodosus*, and *H. verticillata* (monoecious) to two split applications of 50-ppbw BSM was determined 6 weeks after applications under short photoperiods (12 hr). Two experiments were performed, one in April and one in September. These conditions are conducive to vegetative propagule

formation in all three species (Spencer and Anderson 1986, 1987).\*,\*\* Plants were grown individually from propagules in 1-l plastic pots containing U.C. mix under long days in a greenhouse for 4 weeks. Upon transfer to 90-l outdoor culture tanks, plants were arranged in groups (replicate treatments) of four plants, six groups per treatment. Applications of BSM were made 1 week after transfer to outdoors and 2 weeks later. Water levels were maintained in all containers. At harvest, plants were separated into shoots, roots, and propagules, then weighed.

### Field drawdown canal applications

In April 1986, bensulfuron methyl was applied in two canals that had been dewatered since fall of 1985 (Richvale and Byrnes canals). Rates of 0.1 kg/ha (Richvale) and 0.1 and 0.2 kg/ha (Byrnes) were used on replicated 100- by 4-m plots. Before applications, 15- by 20-cm sediment core samples were taken to determine propagule density: 3 cores from four sites within each plot were sampled (i.e., 12 cores per plot). After canals were filled, aboveground weed biomass was sampled from six 0.25-m<sup>2</sup> quadrats within each plot in June, August, and September or October. When canals were dewatered after the 1986 growing season, additional sediment cores were taken for propagule density as described above.

## RESULTS

Significant reductions in biomass and shoot lengths were observed in all species at concentrations above 2.5 ppbw when BSM was applied pre- or early postemergent (Figures 2-4). Hydrilla cutting were affected by 1-ppbw BSM, and the root production was most reduced (Figure 5). This is consistent with BSM's mode of action in blocking cell division, which particularly affects roots. At 50 ppbw, the pondweed growth was reduced by ca. 80 percent. Hydrilla shoot length and shoot weight was reduced by ca. 60 percent at 50-ppbw BSM.

Table 1 shows that propagules of both pondweeds exposed for 24 hr to 0.1-ppmw (100 ppbw) BSM produced plants with significantly less shoot weight and for sago pondweed (*P. pectinatus*), less shoot length. At 1.0 ppmw, shoot lengths of both species was reduced by ca. 95 percent. The soil surface applications all reduced shoot length and weights of both species (Tables 2, 3). The reductions in the growth of plants in the untreated containers placed in the vessels containing treated soil indicate that with the soil used here, BSM was leached or otherwise mobilized from the soil surface. It should be noted that we have not seen any evidence of this mobility in the field applications to dewatered canals; a very distinctly defined border at the edges of canal plots has been consistently observed.

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\*Spencer, D.F., and Anderson, L.W.J. 1986. "Photoperiod Responses in Monoecious and Dioecious *Hydrilla verticillata*," *Weed Science*, Vol 34, pp 551-557.

\*\*Spencer, D.F., and Anderson, L.W.J. 1987. "Influence of Photoperiod on Growth, Pigment Composition, and Vegetative Propagule Formation for *Potamogeton nodosus* Poir. and *Potamogeton pectinatus* L.," *Aquatic Botany*, Vol 28, pp 103-112.

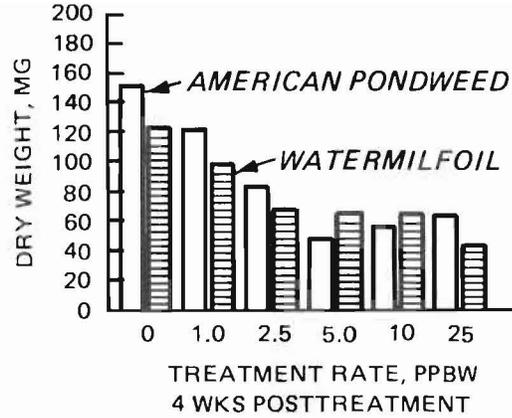
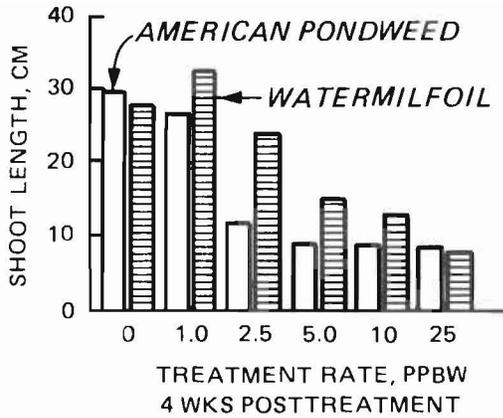


Figure 2a. Shoot length of *Potamogeton nodosus* and *Myriophyllum spicatum* 4 weeks after continuous exposure to bensulfuron methyl in water

Figure 2b. Dry weight of *Potamogeton nodosus* and *Myriophyllum spicatum* 4 weeks after continuous exposure to bensulfuron methyl in water

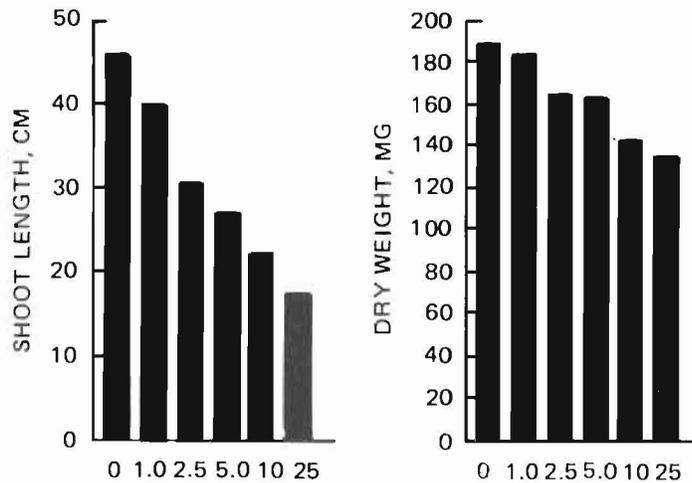


Figure 3. Effect of early postemergence application of bensulfuron methyl on *Hydrilla verticillata*. Measurements were made 4 weeks after start of continuous treatment

For the subsurface layered treatments (Tables 4, 5), the 0.01-kg/ha rates produced 50 percent reductions in shoot length, shoot weight, and root weight of sago pondweed. Significant reductions in growth were observed only at and above the 0.25-kg/ha rates in American pondweed. Note that, unlike the surface applications (Tables 2, 3), no apparent leaching or mobilization from the 2.5-cm herbicide depth was observed except for the 0.1-kg/ha rate in the sago pondweed assays.

The minimum contact time used (1 hr) caused reduction in shoot length in hydrilla and sago pondweed at 100 ppbw when measured 4 weeks after exposure (Table 6). This exposure also caused a significant reduction in sago pondweed dry weight; however, even 24-hr exposure did not cause significant reduction in hydrilla dry weight. At 10 ppbw,

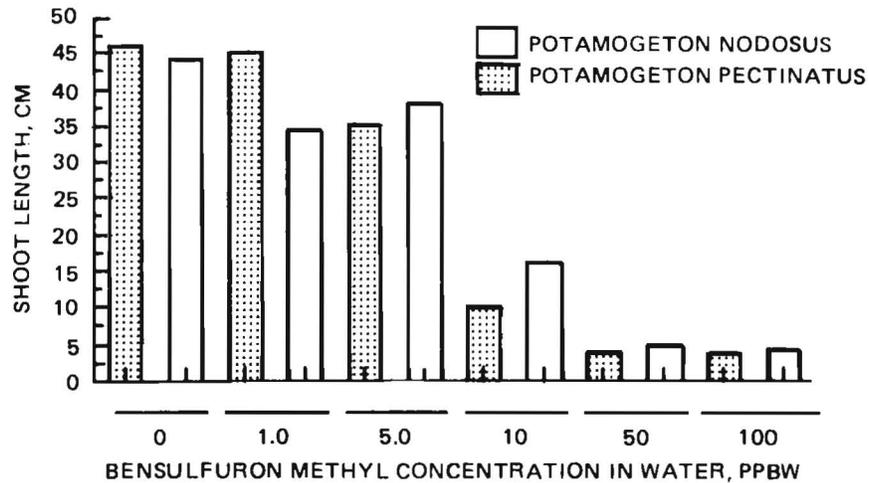


Figure 4a. Effect of preemergence water exposure to bensulfuron methyl. Shoot length measured 4 weeks after exposure

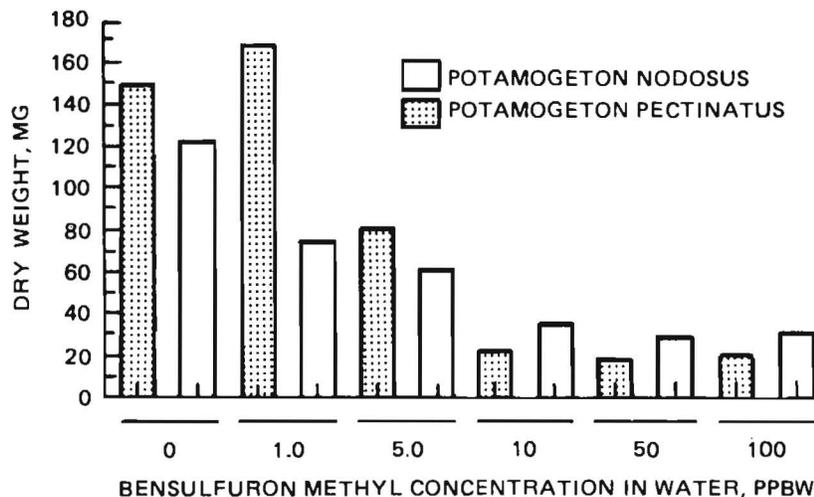


Figure 4b. Effect of preemergence water exposure to bensulfuron methyl. Dry weights, 4 weeks after exposure

BSM caused significant reduction in shoot length of both species with a 1-hr exposure; however, longer exposures caused variable response in sago pondweed. Exposures of 100 ppbw lasting 6 hr greatly reduced the growth of sago pondweed. Though these effects demonstrate the potency of BSM, these concentration/duration combinations are not sufficiently low to permit this herbicide's use as an in-water treatment in flowing waters typical of canals and drains in the Western United States.

The importance of preemergence or early postemergence application of BSM on reducing growth is clearly demonstrated in Table 7. Both *Potamogeton* species showed the greatest reductions in weight and length when they were 3 to 10 days old at the time of treatment. Plants that were 17 days old, or older, when exposed showed no significant reductions in biomass or length. It should be noted, however, that in unrestricted growth conditions such as lakes or canals where plants can continue to proliferate throughout

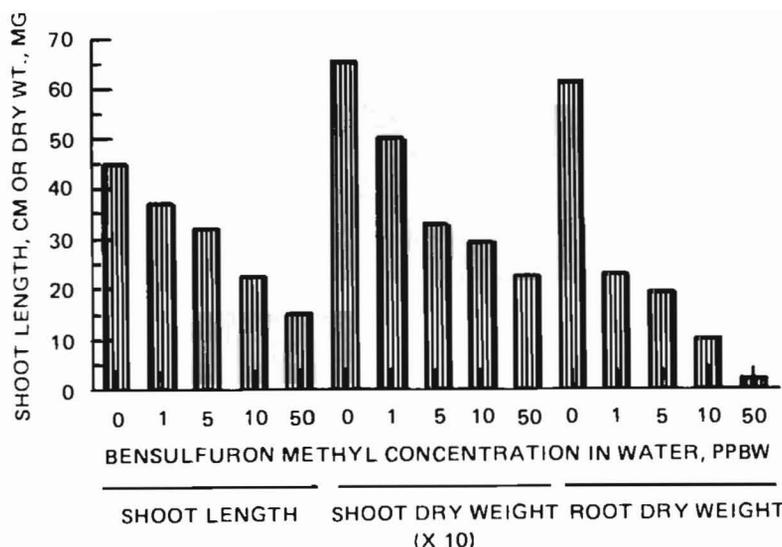


Figure 5. Effects of 14-day exposure of *Hydrilla verticillata* rooted apical cuttings to bensulfuron methyl. Measurements were made 4 weeks after exposures

Table 1

Shoot Length, Shoot and Root Dry Weight of American and Sago Pondweed 4 Weeks After 24-Hour Exposure of Vegetative Propagules to Bensulfuron Methyl

Treatment Rate (ppmw)	Shoot Length (cm)		Shoot Dry Weight (mg)		Root Dry Weight (mg)	
	American	Sago	American	Sago	American	Sago
0	45.9 ± 2.2	52.0 ± 2.0	106 ± 2	155 ± 14	56 ± 5	41 ± 1
0.1	33.0 ± 10.7	7.7 ± 1.2	58 ± 6	71 ± 17	35 ± 4	25 ± 4
1.0	4.7 ± 0.3	4.3 ± 0.3	22 ± 2	50 ± 9	30 ± 3	8 ± 2
10.0	2.9 ± 0.2	3.2 ± 0.5	21 ± 3	65 ± 10	24 ± 4	5 ± 1

Table 2

Growth of Sago Pondweed 4 Weeks After Preemergence Soil Surface Application of Bensulfuron Methyl

Treatment Rate (kg/ha)	Shoot Length (cm)		Shoot Dry Weight (mg)		Root Dry Weight (mg)	
	Treated Carton	Untreated Carton	Treated Carton	Untreated Carton	Treated Carton	Untreated Carton
Control	49.4*	50.7	122	130	12	18
0.05	7.4	7.0	33	30	2	8
0.10	6.6	4.8	42	27	0	3
0.25	6.5	6.1	46	42	0	2
0.50	5.9	7.9	42	56	0	3

\*Value represents mean of three replicates.

spring and summer, one would expect some effects from BSM on biomass even with midsummer applications.

Other very important effects from late-season treatments are also indicated from results presented in Table 8 and Figure 6. These data show that when relatively mature plants are exposed to BSM, some reductions in biomass can occur, but more importantly,

**Table 3**  
**Growth of American Pondweed 4 Weeks After Preemergence Soil Surface Application of Bensulfuron Methyl**

<i>Treatment Rate (kg/ha)</i>	<i>Shoot Length (cm)</i>		<i>Shoot Dry Weight (mg)</i>		<i>Root Dry Weight (mg)</i>	
	<i>Treated Carton</i>	<i>Untreated Carton</i>	<i>Treated Carton</i>	<i>Untreated Carton</i>	<i>Treated Carton</i>	<i>Untreated Carton</i>
Control	61.2*	60.4	179	200	64	56
0.05	9.8	10.5	120	99	0	14
0.10	6.1	8.5	83	81	0	8
0.25	6.1	5.1	89	65	0	3
0.50	6.3	5.5	66	89	0	2

\*Value represents mean of three replicates.

**Table 4**  
**Growth of Sago Pondweed 4 Weeks After Preemergence Subsurface Soil Application of Bensulfuron Methyl**

<i>Treatment Rate (kg/ha)</i>	<i>Shoot Length (cm)</i>		<i>Shoot Dry Weight (mg)</i>		<i>Root Dry Weight (mg)</i>	
	<i>Treated Carton</i>	<i>Untreated Carton</i>	<i>Treated Carton</i>	<i>Untreated Carton</i>	<i>Treated Carton</i>	<i>Untreated Carton</i>
Control	65.6A*	64.2A	88AB	68B	11A	12A
0.01	31.2C	62.3A	38C	71B	5B	10A
0.025	11.1D	63.1A	13D	99A	0C	11A
0.05	7.1D	60.8AB	15D	105A	0C	13A
0.1	4.8	51.9B	12D	77B	0C	10A

\*Value represents mean of three replicates.

**Table 5**  
**Growth of American Pondweed 4 Weeks After Preemergence Subsurface Soil Application of Bensulfuron Methyl**

<i>Treatment Rate (kg/ha)</i>	<i>Shoot Length (cm)</i>		<i>Shoot Dry Weight (mg)</i>		<i>Root Dry Weight (mg)</i>	
	<i>Treated Carton</i>	<i>Untreated Carton</i>	<i>Treated Carton</i>	<i>Untreated Carton</i>	<i>Treated Carton</i>	<i>Untreated Carton</i>
Control	52.7AB*	55A	112AB	119AB	30A	31A
0.01	42.6B	52.9AB	125AB	136AB	25A	28A
0.025	13.8C	52.5AB	71C	133AB	4B	28A
0.05	6.8C	51.0AB	77C	141A	3B	35A
0.1	6.7C	48.9AB	83C	145A	2B	35B

\*Value represents mean of three replicates.

the ability of the plants to produce vegetative reproductive structures is severely impaired. Both the spring treatments (Table 8) and fall treatments (Figure 6) resulted in complete or near-complete suppression of propagule formation. The impact of BSM on hydrilla aboveground turion formation is particularly important since these structures, by their location in the water column, provide monoecious hydrilla with excellent dispersal abilities. By comparing the number of gravitropic shoots in the pre-BSM treated subsampled plants (Figure 6C) to the number of tubers finally formed, it becomes clear that the latter had started forming before BSM was applied.

Significant reductions in biomass were achieved in the canals treated at 0.1 and 0.2 kg/ha (Figure 7 A, C). In the Byrnes Canal, the largest differences between control and

**Table 6**  
Shoot Length and Dry Weight of Sago Pondweed and Dioecious Hydrilla After Short Exposure to Bensulfuron Methyl (BSM)

Treatment	Exposure Period (h)	Hydrilla		Sago Pondweed	
		Shoot Length (cm)	Dry Weight (mg)	Shoot Length (cm)	Dry Weight (mg)
Control	1	43.7AB*	214A	45.2BC	214A
	3	40.1ABC	223A	48.7AB	144B
	6	49.0A	208A	40.0BC	142B
	24	45.4	217A	45.8BC	139B
BSM 10 ppbw	1	29.2DE	214A	44.0BCD	140B
	3	26.7DE	189A	48.2AB	198A
	6	35.2BCD	193A	57.9A	202A
	24	33.9CD	219A	41.2BCD	115CB
BSM 100ppbw	1	39.9ABC	253A	31.9DE	87CD
	3	29.4DE	263A	27.4E	74D
	6	28.4DE	210A	7.1F	44E
	24	24.1E	157A	8.6F	29E

\*Means followed by the same letter within each column are not significantly different at the 5 percent level according to Duncan's multiple range test.

**Table 7**  
Response of American and Sago Pondweed to Bensulfuron Methyl When Applied to Plants at Different Levels of Maturity

Age of Plant at Time of Treatment (days)	American Pondweed				Sago Pondweed			
	Dry Weight (mg)		Shoot Length (cm)		Dry Weight (mg)		Shoot Length (cm)	
	Control	Treated (100 ppbw)	Control	Treated (100 ppbw)	Control	Treated (100 ppbw)	Control	Treated (100 ppbw)
3	285BC*	92D	40A	16B	96B	25D	55A	8B
7	369AB	200CD	35A	24B	116AB	58C	56A	19B
10	437A	195CD	40A	20B	111AB	48C	55A	26B
14	326AB	338AB	42A	38A	107AB	87B	55A	25B
17	350AB	339AB	39A	36A	120A	104AB	59A	58A
21	332AB	349AB	44A	44A	126A	122A	59A	46A
28	358AB	327AB	45A	43A	129A	127A	58A	58A

\*Means followed by the same letter within same parameter column are not significantly different at 5 percent level based on Duncan's multiple range test.

BSM plots occurred in June several weeks after rewatering. Except for the August samples in this canal, there was no difference between the low and high rates. In addition, reductions in the winter bud density in the Byrnes Canal and to a lesser extent the Richvale Canal occurred in the fall-winter samples following BSM applications. This fact is consistent with both the reduction in biomass and the above-noted effects on propagule formation. Thus, the applications of BSM had a dual benefit in reducing standing crop as well as eliminating many propagules from which the next season's growth emerges.

Table 8  
 Effect of Two Sequential Bensulfuron Methyl Treatments (50 ppbw) on 4-Week Old Monoecious Hydrilla, Sago Pondweed, and American Pondweed\*  
 Dry Weight (mg/plant)

Species	Shoot**			Root			Propagules/Plant		
	Pre	Control	Londax	Pre	Control	Londax	Pre	Control	Londax
Sago pondweed	685a†	2268b	795a	176c	713a	248c	0	7.7	0
American pondweed	1119a	2482b	786c	471c	428c	395c	0	6.4	0
Monoecious hydrilla	1654a	4213b	2248c	116d	212d	81d	0	3.4	0

\*Plants grown from propagule for 4 weeks under 16-hr photoperiod in growth chamber at 25° C. After 4 weeks, plants were transferred to outdoor container under natural (55 percent shade) light and covered daily to provide a 10- to 12-hr photoperiod.

Treatments were: 6 tubs with 4 plants of each species untreated and 6 tubs with 4 plants each treated with 50-ppbw Londax on 4/6/87 and 4/20/87.

\*\*N = 4 plants for pretreatment for each species.

N = 6 tubs (=24 plants for 6-week posttreatment.)

†Values (means) within a row with the same letter are not significantly different at 5 percent level using Duncan's separation.

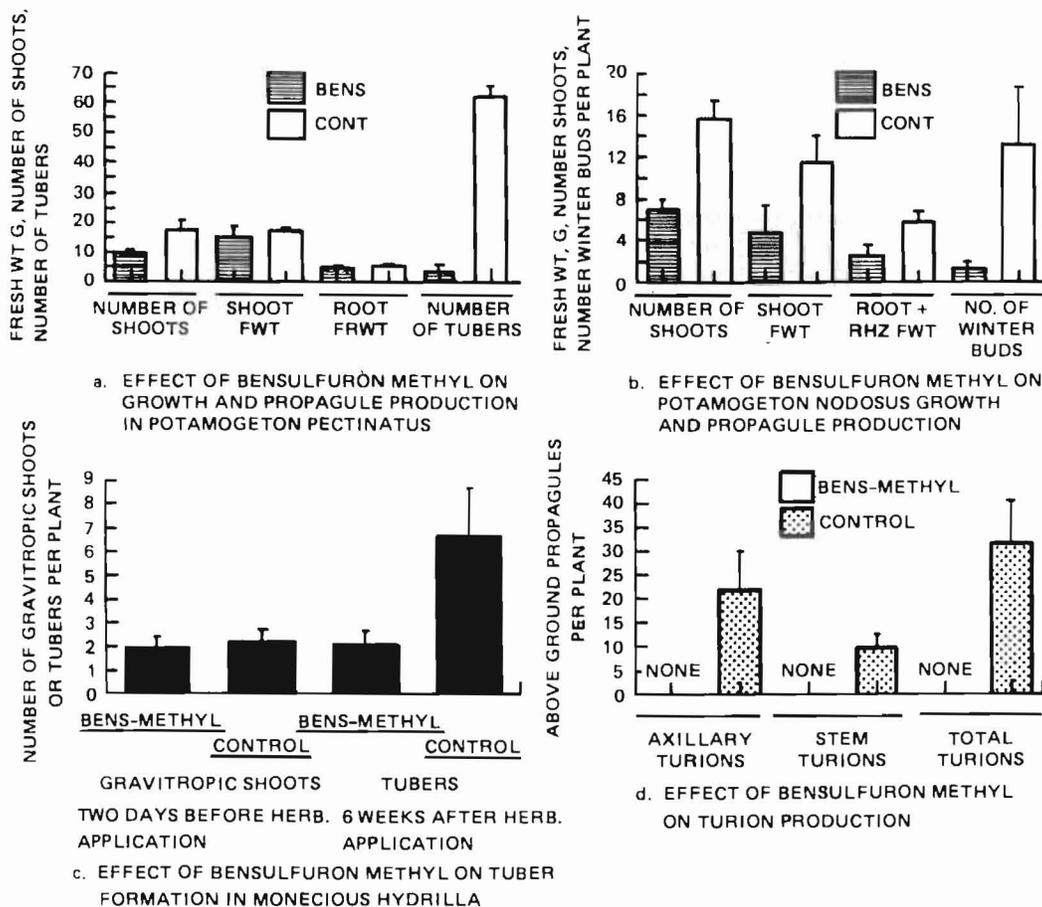


Figure 6. Effects of bensulfuron methyl on growth and propagule production in three aquatic weeds: *Potamogeton pectinatus*, *P. nodosus*, and monoecious *Hydrilla verticillata*. Two split-applications of 50 ppbw were applied 2 weeks apart when plants were 4-5 weeks old; plants were harvested 6 weeks after the last application. Values are means and standard deviations for six replicate treatments, four plants per replicate

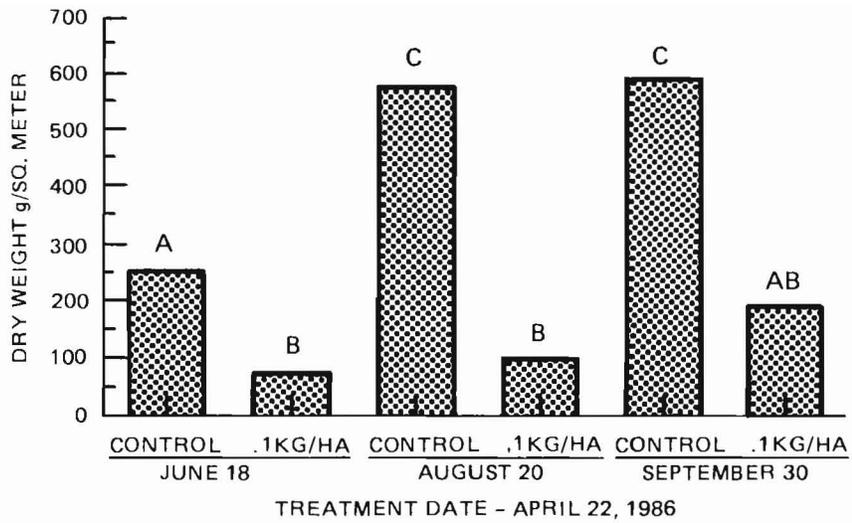


Figure 7A. Effect of londax on submerged aquatic weed growth (Richvale)

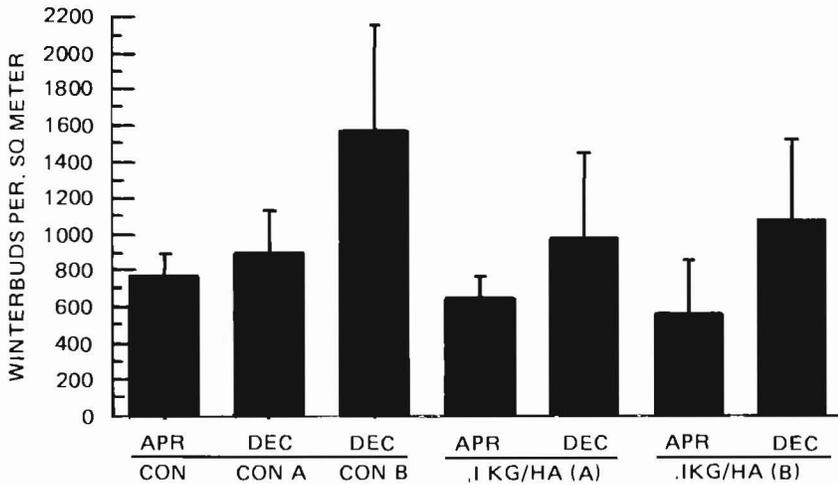


Figure 7B. Effect of londax (0.1kg/ha) on American pondweed winter-bud production (Richvale irrigation canals)

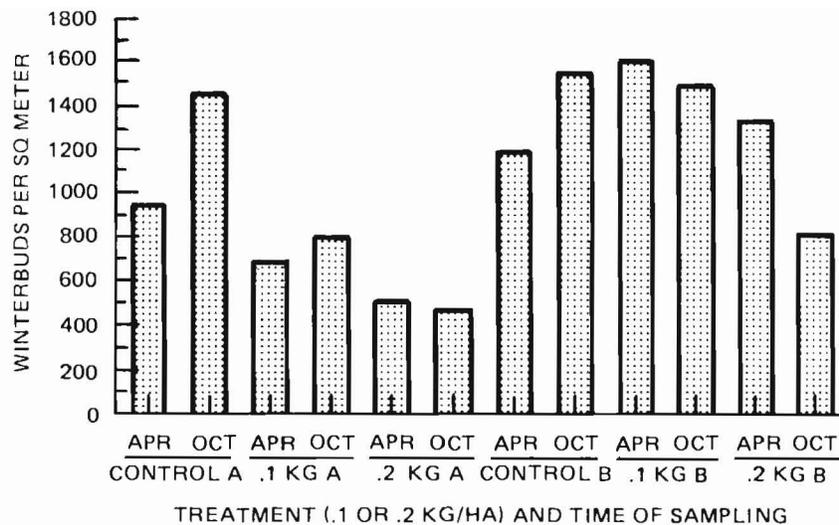


Figure 7C. Effect of drawdown londax application on American pondweed winterbud production (Solano irrigation district canals)

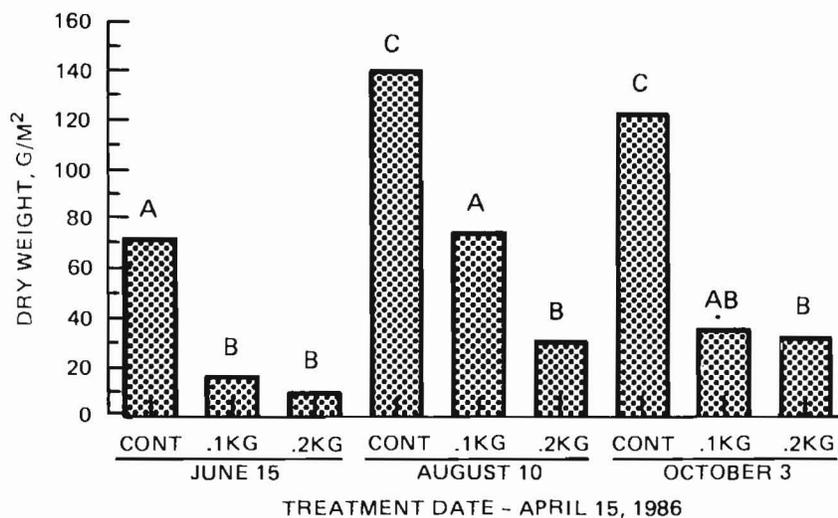


Figure 7D. Effect of londax on submersed aquatic weed growth (Byrnes)

## SUMMARY AND CONCLUSIONS

Taken together, these results strongly suggest that BSM has tremendous potential for development as an aquatic herbicide. More information is needed on field efficacy in both drawdown and ponded water sites. Preliminary dissipation studies suggest that full-season control or sufficient suppression of weed growth could be obtained with a single application at concentrations from 50 to 100 ppbw. Soil surface (drawdown) applications at rates of 0.5 to 0.25 kg/ha probably will provide excellent control also. However, since the sulfonylureas in general interact differently in different soil types, more work is

needed to determine optimal rates and optimal moisture conditions for drawdown applications. Similarly, pH affects BSM solubility and may also affect efficacy in water treatments.

The potential opportunities for BSM as an aquatic vegetation management tool are illustrated in Figure 8. This compound may provide several avenues for selective, well-targeted aquatic weed management, both from a herbicidal approach and from a growth-regulator approach in which plant suppression to an economically and environmentally acceptable level is achieved at very low rates.

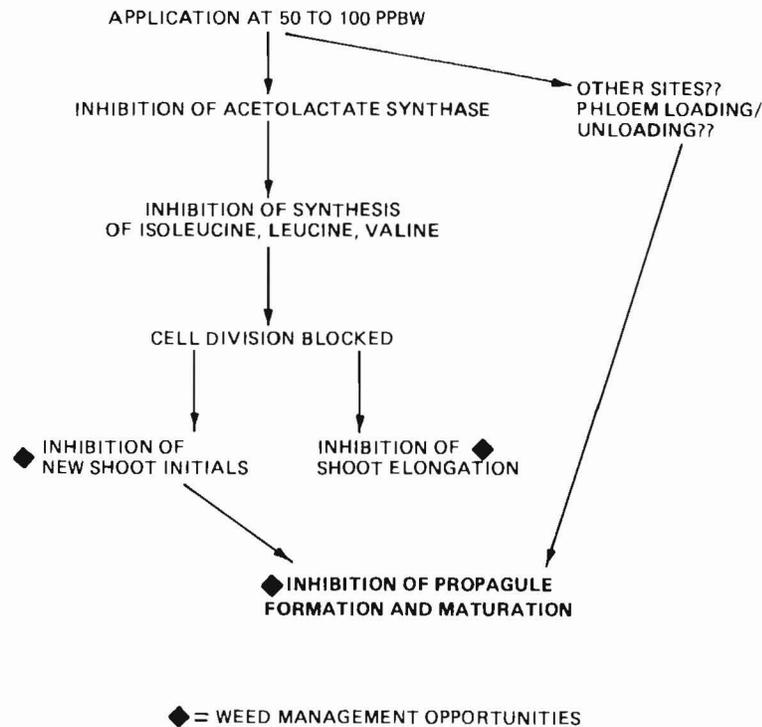


Figure 8. Physiological effects of bensulfuron methyl in *Hydrilla* and *Potamogeton*

## ACKNOWLEDGMENTS

We are greatly appreciative of the cooperation and enthusiasm from Dr. Robert Ackerson, Du Pont, and for Du Pont supplying bensulfuron methyl. The technical assistance of Ms. Karin Mock, Ms. Doreen Gee, Mr. Robert Pine, and Ms. Cathy Cowan is gratefully acknowledged.

# **Biological Control of Aquatic Plants**

# Plant Lectin Involvement In Host Specific Association Of Microflora With Submersed Aquatic Plants

by  
Edwin A. Theriot\* and Stewart Kees\*

## INTRODUCTION

Host specific microorganisms is the first prerequisite for genetically engineering a biocontrol agent. Specificity is the result of several different characteristics of an organism which are normally controlled by genes at different locations on a chromosome. Therefore, it would be very difficult, if not impossible, to genetically engineer specificity into an organism (Pennington 1986).\*\*

Lectins are proteins or glycoproteins that bind to cell surfaces via specific oligosaccharide components. Bacteria and fungi possess oligosaccharide components on their outer surfaces. Lectins are common to plants and some microbes. The involvement of plant lectins in host specific association with microbes has been demonstrated in the "Legume-Rhizobium" system (Dazzo and Hubbell 1975).† *Rhizobium* bacteria bind specifically to lectins on the root hairs of certain legume species. This specific attachment is required before the bacteria can form nodules and produce nitrogen for the host plant. The existence of lectins on the surface of hydrilla and Eurasian watermilfoil would mean that microorganisms which possess the complimentary oligosaccharides would attach to the surface of the plants. The association is specific if the lectins are unique to each plant species, as is the case with legumes.

We are conducting studies to isolate lectins from hydrilla and Eurasian watermilfoil in hopes that they will provide a means to determine specific association of microbes with the plants. This study is a portion of the first phase of an effort to genetically engineer microorganisms for the managements of submersed aquatic plants (Theriot 1987).†† The selection of a microbial candidate for engineering is the critical phase of the overall effort which hinges on our ability to identify microorganisms specific to the host plants.

The objectives of this study are to:

- Isolate lectins from hydrilla and Eurasian watermilfoil.
- Demonstrate agglutination of microorganisms with isolated plant lectins.
- Identify complimentary oligosaccharide units which bind to the isolated lectins.

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\*U. S. Army Engineer Waterways Experiment Station, Vicksburg, Mississippi.

\*\*Pennington, J.C., 1986. 'Bioengineering Technology Meeting,' Miscellaneous Paper A-86-1, U. S. Army Engineer Waterways Experiment Station, Vicksburg, Miss.

†Dazzo, F., and Hubbell, D., 1975. *Applied Microbiology*, Vol 30, pp 1107-1133.

††Theriot, E.A. 1987. 'Management of Aquatic Plants with Genetically Engineered Microorganisms; Phase I: Candidate Section,' *Proceedings, 21st Annual Meeting, Aquatic Plant Control Research Program*, Miscellaneous Paper A-87-2, US Army Engineer Waterways Experiment Station, Vicksburg, Miss.

## METHODS AND MATERIALS

Partially purified glycoproteins are isolated from watermilfoil and hydrilla. Shoots, roots, and tubers (in the case of hydrilla) are towel-dried to remove excess water, homogenized in a Waring blender, sonicated for 3 min, and centrifuged at 27,000 g for 30 min. The supernatant was collected and subjected to ammonium sulfate precipitation at 1°-5°C. The mixture was then centrifuged at 35,000 g for 30 min, and the protein precipitate was harvested. The supernatant from each ammonium sulfate cut was saved for subsequent precipitations. The protein precipitate was resuspended in ammonium sulfate extraction buffer at pH 7.2, then stored at -20°C until ready for further purification.

The stored protein extracts were dialyzed against 0.1-Molar phosphate buffer, pH 7.2, for 24 hr and tested for protein content using the Lowry method (Lowry et al. 1951).<sup>\*</sup> The most active fractions were pooled and further purified by affinity chromatography. Pooled protein was passed through seven affinity gel columns specific for as many oligosaccharides. Protein quantification before and after application to each affinity column identified instances of binding. In the event of binding, elusion of desired proteins was accomplished by step gradient techniques using different concentrations of the oligosaccharide for which the column is specific. The most active fractions were pooled and concentrated with Amicon ultrafiltration membrane cones.

The protein extracts in crude and purified form are tested for agglutination of fungal isolates using phase and fluorescence microscopy. Protein characterization is accomplished using polyacrylamide gel electrophoresis.

## RESULTS AND DISCUSSION

Crude protein complex was isolated from hydrilla and watermilfoil. Preliminary agglutination tests using hydrilla protein and fungal isolates collected from the natural microflora of the plant have resulted in aggregation of fungal hyphae observed in phase-contrast microscopy studies. Although not definitive these studies would seem to indicate that agglutinins of a protein nature exist in the tissues of hydrilla. These results have not yet been verified using purified lectin fractions.

Purified hydrilla protein demonstrated an affinity for  $\alpha$ -D-Lactose at very low levels when passed through affinity chromatography gels. It would seem that hydrilla may contain lectins which bind specifically to lactose units. Purified Eurasian watermilfoil protein bound specifically to  $\alpha$ -L-Fucose units in similar affinity chromatography studies. These results indicate that hydrilla and Eurasian watermilfoil possess glycoproteins that have affinities for specific oligosaccharides. However, the protein activity was low for both plants. It is believed that the phenolic compounds in the leaves and stems of both plant species are binding to the protein components soon after disruption of the plant tissues, reducing the protein activity.

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<sup>\*</sup>Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J. 1951. *Journal of Biological Chemistry*, Vol 193, p 265.

Electrophoretic analysis of active fractions, as identified from the affinity gels, failed to produce distinct bands of lectin. A faint band from the lactose gel for hydrilla was produced but was not well defined. The binding of protein with phenolic compounds prevented adequate binding with the affinity gels for isolation of the proteins.

### **SUMMARY AND CONCLUSIONS**

Glycoprotein components have been isolated from the stems and leaves of hydrilla and Eurasian watermilfoil which bind to specific oligosaccharides at very low levels. The existence of lectins in hydrilla and Eurasian watermilfoil has not been proven, but indications are that they exist and possess different specificities. Agglutination of microbes must be verified with purified glycoprotein fractions and observed on the surface of the plant using scanning and transmission electron-microscopy.

# Host Specificity of Microorganisms from Eurasian Watermilfoil

by

Craig S. Smith\*, Tara Chand\*\*, Robin F. Harris\*\*, and John H. Andrews\*

## Introduction

Historically, attempts to discover a naturally occurring biological control agent for Eurasian watermilfoil have not been particularly successful, and an effort was initiated by the Aquatic Plant Control Research Program to use genetic engineering techniques to produce a virulent, host-specific pathogen for use in milfoil control. In the initial proposal (Pennington 1984),† engineering for host-specificity was rejected because the basis for specificity is complex and often unknown and would therefore be virtually impossible to engineer. Instead, priority was given to the discovery of nonpathogenic or weakly pathogenic microorganisms that are host-specific for Eurasian watermilfoil. If one or more suitable host-specific candidates are found, later efforts will seek to convert them into virulent pathogens by introducing genes for the production of a toxin, a lytic enzyme, or possibly a plant hormone. Our role in the project is to develop techniques for evaluating the host specificity of microorganisms.

## Definition of Specificity

A microorganism is host-specific if it interacts to a different degree with different potential hosts. Levels of interaction meaningful in this context are:

- Attachment of the microorganism to the host.
- Growth of the microorganism in association with the host.
- Penetration of the microorganism into the body of the host plant.
- Production of symptoms in the host.

Each step in the list represents a greater degree of interaction than the previous one, i.e., a microorganism that grows in association with a host can be said to have a closer association with the host than one that merely attaches to it. Rather than defining specificity solely in terms of any one of the above levels of interaction, we define it by considering which of the interactions takes place. According to our definition, a microorganism is host-specific if it participates in more of the above interactions with the intended host than with other plants. For example, a microorganism exhibits a degree of host specificity if it attaches to milfoil, grows there, and penetrates into the plant body, while merely attaching and growing superficially on other plant species. Using this definition, specificity is not an all-or-none phenomenon; rather, it is possible to rate microorganisms according to their interaction with various plant species.

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\*\*Department of Soil Science, University of Wisconsin, Madison, Wisconsin.

†Pennington, J.C. 1984. "Feasibility of Applying Genetic Engineering Technology to Aquatic Plant Control," *US Army Engineers, Aquatic Plant Control Research Program Information Bulletin*, A-84-2, US Army Engineer Waterways Experiment Station, Vicksburg, Mississippi.

## Host Specificity Assay

We use a three-step laboratory assay to evaluate the interaction of microorganisms with watermilfoil and other aquatic plants (see Figure 1). The first step is an association phase, in which nine plants (per replicate) are placed into a suspension of the test microbe for 24 hr to allow the microorganism to become associated with the plant. Plants are then washed thoroughly, and plants, media, and washes are sampled as follows:

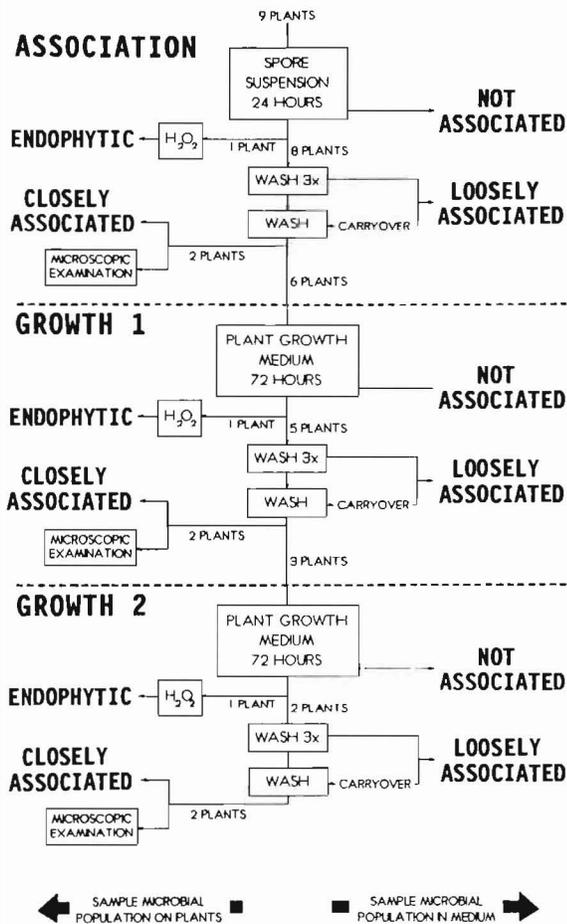


Figure 1. Flowchart describing the microbe-plant interaction assay. The three stages of the assay (association, growth 1, and growth 2) are shown, as are the points at which plants (left) and media and washes (right) are sampled. Samples are labeled with the name of the microbial component they measure (not associated, loosely associated, closely associated, endophytic or carryover). Plants reserved for microscopic examination are not used to evaluate a specific microbial component, but will be used to validate plate count assays of other components (see text)

- Samples of the medium are plated on an appropriate culture medium to determine how many colony forming units (cfu) of the microorganism are *not associated* with the plant.
- One plant is surface sterilized for 5 min with 15 percent H<sub>2</sub>O<sub>2</sub>, then macerated. The macerate is plated to determine presumptively the number of microorganisms that have penetrated into the plant body and become *endophytic*.
- The remaining plants are washed four times to remove any loosely attached microorganisms. Samples from all four washes are pooled and plated to determine how many cfu were *loosely attached* and therefore removed by washing. A sample from the final wash is plated to determine how many cfu remain as *carryover* in the surface film on the plants.

- Two of the washed plants are removed, one of which is macerated and the macerate plated to determine how many cfu are *closely associated* with the plant, and the other is fixed for *microscopic examination* to validate plate count results. The plants reserved for microscopy will be examined for the presence of endophytic fungal growth when plating assays detect endophytic cfu, and the results of semiquantitative microscopic sampling of fungal growth on the plant will be compared with plate counts of the closely associated microbial component.

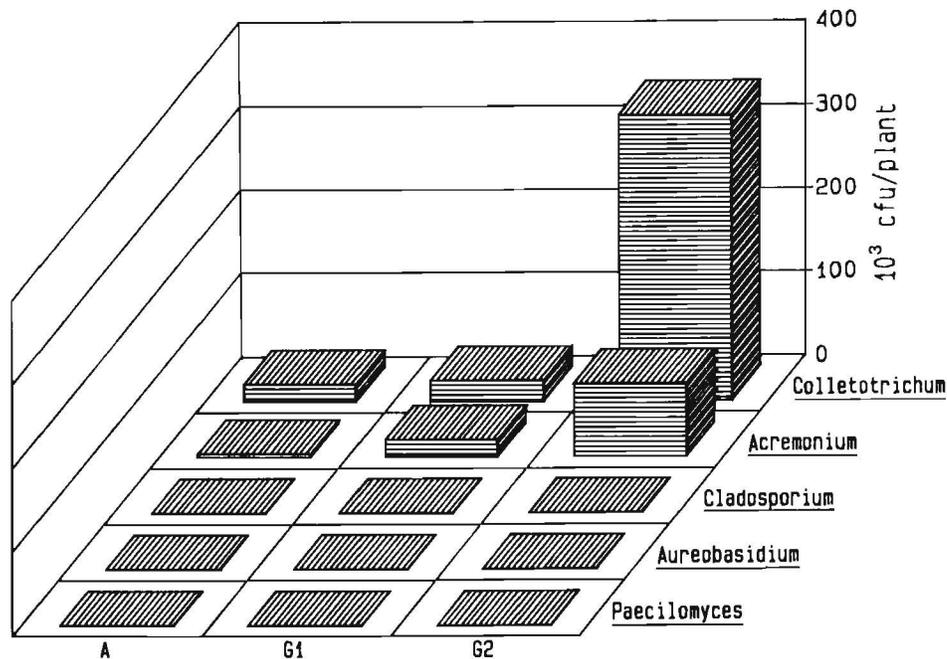
The remaining six plants are then transferred to the second phase of the assay, the first growth period (growth 1). In this step, plants are placed into plant growth medium for three days to provide an opportunity for the growth of any microorganisms that remain attached. At the end of the first growth period, plants are washed and samples collected as described above, and the remaining three plants are transferred to fresh growth medium for a second, three-day growth period (growth 2). After the second growth period, the medium, washes, and plants are again sampled in exactly the same fashion as before.

The present assay is considerably more complicated and time-consuming than will ultimately be necessary to screen microorganisms for host specificity. By sampling so many microbial fractions at each stage of the assay we hope to gain a thorough understanding of microbial dynamics in the assay system. When we have tested the assay with a range of microorganisms, we will examine the results and recommend appropriate simplifications.

To date, the assay has been used to evaluate the colonization and growth of fungi on watermilfoil. The ability of the assay to distinguish differences in microbial performance was assessed using five fungi: a weakly virulent pathogen of milfoil (*Colletotrichum gloeosporioides*), a common epiphyte of milfoil (*Acremonium* sp.), and three fungi that have occasionally been isolated from field-collected watermilfoil, but only in low abundance (*Cladosporium herbarum*, *Aureobasidium pullulans*, and *Paecilomyces* sp.). Differences in the number of fungal individuals closely associated with milfoil illustrate the success of the assay in detecting differences in fungal interaction with the plant (Figure 2). By the end of the 24-hr association period, the number of fungal cfu closely associated with the plant already differed among fungi. There were considerably greater numbers of *C. gloeosporioides* than *Acremonium*, and of *Acremonium* than the other fungi. As the experiment progressed through the two growth periods, *C. gloeosporioides* and *Acremonium* grew in association with the plant, but the other fungi did not. The assay also detected a difference in the ability of the fungal species to penetrate the plant body, since only *C. gloeosporioides* produced detectable numbers of endophytic individuals (data not shown).

## Future Goals

Future work will concentrate on assessing the host specificity of promising microorganisms and examining the effect of differing resident microbial communities on colonization. The host specificity of microorganisms that are good colonists of Eurasian watermilfoil will be evaluated by determining the nature of their interaction with other plant species. Plants for host-range studies will be selected from two groups: species



**Figure 2.** Numbers of fungi closely associated with Eurasian watermilfoil at the end of each stage of the interaction assay (A = association, G1 = growth 1, G2 = growth 2). Each bar is the mean of two replicate measurements

taxonomically related to Eurasian watermilfoil, and species that frequently co-occur with it in nature. Later experiments will repeat the assay with plants having varying resident populations of microorganisms. So far, plants used in the assay have been grown in culture and have had substantial epiphytic bacterial populations. We are developing techniques for producing axenic (bacteria-free) plants and will eventually be able to experiment with plants ranging from axenic to heavily colonized (e.g. field-collected plants).

# Genetic Engineering Technology Development: Release of Engineered Organisms into the Environment

by  
Dr. S. E. Lindow\*

While I also work on weed pathogens, I will be addressing a totally different topic: the development of bacterial biological control agents on leaves of terrestrial plants. If you simply consider this terrestrial habitat as underwater as an aqueous environment, it will be very pertinent to aquatic weed control agents. The studies we have had to do, and the questions we have had to ask will be the very same ones that will be asked regarding aquatic weed pathogens.

Genetic engineering technology is advancing rapidly. At this time its use is most advanced in bacteria. Fungal techniques are also developing quickly, however. There are a number of important physiological traits which can already be manipulated readily. The genes for a number of these important traits are already identified and can be moved into various types of organisms. Several of these genes would be very important and pertinent to the studies of aquatic weed diseases. For example, genes determining production of various macerating enzymes, toxin production, antibiotic sensitivities, and other traits are already in our hands, and we can start to manipulate them. Techniques are now available to allow us to move them from one organism to another. A new gene "B" can be inserted inside of another deleterious gene "A". The deleterious gene "A" is inactivated in this process, such as a gene that might cause it to have too broad a host range. An important gene "B" which might make it more able to produce a macerating enzyme, for example, is then inserted inside the deleterious gene *in vitro* and replaced in the target organism by homologous recombination. The techniques for such manipulation are being developed at a rapid rate.

What I would like to primarily address in this report is the fact that we can now release recombinant organisms. There have been several cases of deliberate introductions of recombinant organisms as of this year. I will address general features of one particular case of deliberate release, as well as some of the other organisms that have now been released, and then generalize requirements for future release of recombinant organisms. Are we opening the floodgate to easy release of recombinant organisms to the environment? I think not, but I will go through the protocols which are now developing that will allow us to release organisms on a rational, case-by-case basis.

An example that I will address frequently is the bacteria that live on leaves. Plants having leaf surface bacteria that cause ice formation freeze, and plants that do not have these ice-forming bacteria on their leaves do not freeze above  $-5^{\circ}\text{C}$ . Only four species of bacteria have the property of ice nucleation. *Pseudomonas syringae* is the bacterial species most important in triggering ice formation on plants. Plants have large numbers

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of associated microorganisms on their leaves and roots. Many millions of bacteria per square centimetre are very common on most plants. Such microbes are probably more numerous on aquatic plants than on terrestrial plants. Because of their great numbers associated with plants, and our evidence of their rather high specificity to the plants on which they live, we can expect that they can have some considerable importance to the plants on which they do live. In the case of ice nucleation active bacteria, ice moves away from the site where it is triggered by bacteria and damages sensitive plants. We can have considerable control over microorganisms. They can usually be rather easily cultured and can be genetically manipulated rather simply, especially in the case of bacteria. In the particular example addressed here, we can easily culture naturally occurring nonice nucleation active bacteria and apply them to plants to change the microbial balance on plants. Changes in the numbers and type of bacteria on leaves can be made to avoid populations of ice-nucleation active bacteria. The population size of a deleterious organism such as a weed pathogen could also be manipulated in a very similar way by direct application.

Molecular genetic techniques allow the identification of individual genes important in biological control agents. Techniques are now readily available to physically isolate individual genes from microorganisms, particularly bacteria. We simply isolate deoxyribonucleic acid (DNA) from our bacterium, cut it with restriction enzymes, ligate into appropriate cloning vectors such as the cosmid pLAFR1, and introduce the recombinant DNA such as by the phage  $\lambda$ , into *E. coli*. Individual clones containing the gene conferring the desired phenotype (such as ice formation in our case) are then identified by appropriate screening procedures. The ability to identify the gene conferring ice nucleation and its further analysis was rather straightforward. Our ice nucleation gene is about 4,200 nucleotides in length. This gene confers the production of a single, very large protein. The gene product was eliminated by modification of the gene with a large internal deletion. The particular recombinant bacterial release that I will be addressing is a bacterial strain having about half of the internal part of this *ice* gene removed. We could also have inserted different genes into that same *ice* gene in the process of homogenization, where the original  $\text{Ice}^+$  gene is replaced with an  $\text{Ice}^-$  version by homologous recombination.

While genetic techniques for use in constructing biological control agents are rather straightforward, important ecological processes or communities in which such organisms will be introduced need to be examined. We have had to do a number of ecological studies of our organism prior to its re-release into the environment. Several questions must be asked regarding an introduced microbe, such as: To where will it disperse in the environment? What other plants or organisms might it affect? How will it be dispersed? What is its expected longevity in the environment? In our particular organism, we were concerned about aerial dispersal during and after spray application to plant surfaces. Dispersal of aquatic pathogens in water columns would probably follow a similar pattern. Knowledge of the extent of dissipation after release into the environment is required to describe the effects of such organisms. For example, how much dilution occurs as a function of distance away from where we spray such bacteria will affect the potential impact of our bacterium on other organisms with which it might come into contact. We found a great dilution of our organism as a function of distance from our

experimental site. A nearly logarithmic decrease in the concentration of recombinant Ice<sup>-</sup> *P. syringae* strains away from where we sprayed them on potato plants was observed. Will such dilution of organisms that disperse from a plot be a problem or a benefit to us? Phrased another way, are ecological processes population size dependent and is some threshold population size required before an introduced strain is likely to be important in impacting other organisms? What other organisms might they come in contact with? In our particular case, we examined 85 different plant species which these organisms might come into contact with via aerosol dispersal. Plants were observed to differ greatly in the efficiency with which they supported population sizes of Ice<sup>-</sup> *P. syringae* strains following aerosol incubation.

An important question to ask of risk assessment studies, such as laboratory measurements of application of Ice<sup>-</sup> bacteria to plants in the greenhouse, is whether they reflect what actually happens in field conditions. We have tested the reliability and predictability of our laboratory studies by establishing large field trials in which we treated all the same plant species that we had tested in the greenhouse with *P. syringae* strains as in greenhouse studies. We asked whether the strains which we had tested in the greenhouse behaved similarly on the plants in the field. The answer is a qualified yes. We related the numbers of bacteria that we had recovered from a collection of inoculated plant species in the field with the numbers of bacteria found on these same plant species in the greenhouse and found a reasonably proportional relationship. Two *P. syringae* strains responded similarly in the field as in the greenhouse. Such ecological studies will be similar to the kind of information that will be required for the release of any modified organism. Can we predict the behavior of modified microbes once we have released them? I believe our answer, so far, is a qualified yes. We can, at least, suggest the worst case scenario and set up situations in the greenhouse or the water tanks that would indicate the maximum impact that such an organism might have. Since most modified microbes to be released will be similar in most ways to various endemic microbes from which they were constructed or to which they are related, we need only describe *differences* in behavior of such modified strains from their natural counterparts.

The release of Ice<sup>-</sup> strains of *P. syringae* on potato occurred at the University of California Tulelake Field Station near the California-Oregon border in a remote site in northern California. There were established regulatory procedures for the case-by-case review of requests for recombinant organism release, outside of physical containment. Our petition for such a release was first reviewed by the National Institutes of Health (NIH). This agency has since largely delegated authority to review projects other than biomedical studies to other agencies. The Environmental Protection Agency (EPA) and the U S Department of Agriculture-Animal and Plant Health Inspection Service (USDA-APHIS) are now the chief agencies from which permission for such field studies must be obtained. My colleague, Dr. N. J. Panopoulos, and I were the first permittees for such release of recombinant organisms, having obtained NIH approval in 1983. The subsequent public and media attention on our project has been incredible. In our particular case, since we were the first to encounter the procedural aspects of the regulation of this technology, we have had to face a number of uncertainties in their implementation. While we first obtained NIH approval for our release in 1982-83, other agencies also now require permits. The EPA requires notification of such studies as of

November 1984. As of June 1987, the USDA-APHIS also has authority to regulate the use of organisms classified as pests, such as biological weed control agents. Our efforts to field test Ice-strains of *P. syringae* have better established the procedures that are going to be acceptable in future submissions for release of recombinant organisms. Because of the several legal challenges to these procedures that were made during the regulation of our particular experiment, these procedures are now becoming much more firmly established.

Several unique features were required of our experimental design in the field release of Ice- *P. syringae* strains. Our experimental plot had to be separated spatially from other plants in the area. While other crops grow in the area of our plot, we had to maintain a buffer zone of a minimum of 30 m across to minimize the dispersal of Ice-strains. In our first trials, we have been required to perform very extensive monitoring of soil, vegetation, water, air, and insects in and near the plot to generate information on the dispersal of Ice- strains outside of the plot area. The information that we have gathered hopefully will make it unnecessary for future projects to perform as much of this type of activity. The recombinant techniques that have been utilized to develop Ice- *P. syringae* strains also have been very useful in their assessment in the environment. We have determined the DNA sequence of a unique region of the *ice* gene that we modified in these *P. syringae* strains to allow us to unambiguously reidentify these strains in the field. The deletion within the *ice* gene caused a novel DNA sequence to be formed at this junction. The DNA sequence within 21 base pairs of this junction was determined and a radioactive labeled P32 synthetic oligonucleotide having this sequence was made for use in colony hybridization techniques to confirm rifampicin-resistant bacteria recovered from environmental samples as the recombinant Ice- strains used in the experiment.

Three separate field tests of recombinant bacteria have now been performed. Our Ice- *P. syringae* experiment at the University of California and a similar experiment of Advanced Genetic Sciences were initiated in April, 1987. Monsanto Corporation in conjunction with Clemson University released a *P. fluorescens* strain containing the *lac* operon in August, 1987. Because our Ice- experiment was the first of its kind, it drew a lot of media attention. I do not expect that subsequent experiments will receive the attention the first experiments had.

Other regulatory agencies became heavily involved in the conduct of the University of California Ice- *P. syringae* experiment. EPA had a large contingent of scientists present at our field site to assess the movement and dispersal of the organisms around the plot, in addition to our work in the same area. The EPA utilized several million dollars' worth of equipment and satellite communication equipment, etc., to assess the movement of these organisms around the plot using a very extensive array of microbiological monitoring equipment. The information they gain from a study of our experiment will be utilized in regulation of future releases. It is not expected that such extensive monitoring of experimental sites will be performed in every case, since initial studies such as ours will generate the information necessary to show the safety or lack thereof for future similar experiments.

The University of California Ice- experiment has generated considerable information, both of scientific and safety aspects of the release of microorganisms. The movement of

the Ice- *P. syringae* strains was very restricted around the plot. While large numbers of organisms were dispersed outside of the plot during spraying, the vast majority moved only 1 m away. Scientists can have more comfort in the better knowledge of where the organisms will disperse in these types of applications. Monitoring of various types of plants, animals, water, and soil in and around the plot has shown that a large number of total native organisms exist in such terrestrial environments and that recombinant Ice- bacteria are not detected outside of the immediate plot area. It is clear that we can design experiments for which the dispersal of bacteria will be very restricted.

Not only has there been concern in the lay public for recombinant releases, but some of our fellow scientists are also questioning what we do and do not know about the whole field of microbial ecology and whether sufficient knowledge exists to allow us to anticipate possible effects of these types of organisms. Members of various disciplines, such as plant pathologists, agronomists, geneticists, ecologists, and others with experience in this area, have been brought together in a number of forums to ask whether sufficient knowledge exists to make such predictions. The conclusion generally appearing from such forums is that we probably can make some reasonable predictions about the behavior of recombinant organisms in natural environments. In particular, *comparative* behavior of modified organisms with natural counterparts probably can be well predicted.

The questions raised not only by scientists themselves but also by lay public have led to inevitable increases in regulatory oversight. There has been a need to bring together the various agencies which have claimed authority to regulate biotechnology products. These agencies have tentatively been brought together in what is known as the Coordinated Framework for the Regulation of Biotechnology. An interagency committee, which has yet to function and be finalized, will presumably coordinate submissions that would be made by applicants to the Food and Drug Administration, USDA, EPA, and other agencies which will be dividing authority to regulate in this area. The USDA as well as the EPA will likely be involved in the regulation of the biological control agents of aquatic weeds. As of now, it is feasible to contemplate future release of aquatic weed control agents. There are definite guidelines being established for such activities. Now that guidelines are well established for the release of recombinant organisms plans can be made for implementing these requirements. While considerable supportive data will likely be required to obtain permission to field-test recombinant organisms, much of the data required will be ecological data, which will be useful in developing experimental protocols for the evaluation of the efficacy of biological control agents.

# Microbiological Control of Eurasian Watermilfoil

by

Halm B. Gunner\*, Yuthana Limpa-amara\*,  
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## ABSTRACT

A scaled-up and modified field application of *M. terrestris* produced a dramatic reduction of *M. spicatum* in an infested lake within 4 weeks. A five-fold increase in treatment area over previous trials, lack of plant sequestering and monoculture inoculum, in contrast to contained quadrants and dual cultures, still resulted in significant plant kill. A 71 percent reduction was observed in plant biomass samples taken from the treated quadrant compared with those from the control. Concurrent laboratory experiments emphasized the need for a maximum inoculum concentration applied at the time of highest water temperature for optimum control.

Bacterial populations isolated from *M. spicatum* tissues were 4-5 orders of magnitude higher than those found in the surrounding water. Phyllosphere bacterial populations were stable, while rhizosphere populations exhibited pronounced fluctuations throughout the experimental period. Fungal populations were also higher on plant tissues than in the surrounding water, although not to the same degree as bacterial populations, nor did they fluctuate in the rhizosphere.

Treated and control plants supported virtually identical microbial populations with discrete, transient exceptions. Although *M. terrestris* exhibited a distinct affinity for the phyllosphere, the plant reacted differentially to the fungal presence. Plant midsection tissues showed increased bacterial numbers subsequent to inoculation with *M.t.*, while this increase did not occur on tips and roots. Sustained monitoring of microbial population dynamics throughout this work indicated that no ecosystem disruption resulted from this biological control strategy.

## INTRODUCTION

Work previously reported (Gunner, Limpa-amara, and Bouchard, 1986) has demonstrated the efficacy of two microorganisms derived from *M. spicatum*, a pectinolytic bacterium, *Bacillus sp.* isolate P-8 (BSP8), and the fungus *Mycocleptodiscus terrestris* (Gerdemann) Ostazeski, to bring about a decline of this plant. In order to approach commercial application standards, and after laboratory experiments had confirmed the control efficacy of *M. terrestris* by itself, field trials were conducted with the fungus as the sole inoculum. Pilot scale fermentations of the fungus were also undertaken to provide insights into potential problems in scaling up production.

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Management methods for the control of Eurasian milfoil based on the manipulation of biological systems have shown great potential advantages over other methods (Schuyttema 1977). A variety of organisms have been tested for use as biocontrol agents against *M. spicatum*. A snail, *Pomacea australis*, and the manatee, *Trichechus manatus*, were reported to be potent control candidates (Blackburn, Sutton, and Taylor 1971), but no further report has been given of their use (Amundsen and Brenkert 1978). Among 25 insects found to be associated with *M. spicatum*, *Paraponyx stratiotata* was the only one with sufficient host specificity to be considered for use as a biological control (Spencer and Lekic 1974). Two species of fish, *Tilapia zillii* and the Grass Carp or White Amur (*Ctenopharygodon idella*), have been used for aquatic weed management (Blackburn, Sutton, and Taylor 1971; Amundsen and Brenkert 1978). The inability of the former to survive low temperatures and the potential for the upset of ecological balances by the aggressive Grass Carp are significant drawbacks which have restricted the use of herbivorous fish as biological control agents (Bates, Burns, and Webb 1986; Blackburn, Sutton, and Taylor 1971).

Plant pathogenic organisms have also been tested for their ability to infect *M. spicatum* (Hayslip and Zettler 1973). Two fungi, *Fusarium sporotrichoides* and *Acremonium curvulum*, were reported to attack *M. spicatum* (Patlak 1982; Andrews and Hecht 1981; Andrews, Hecht, and Bashirian 1982). Although *F. sporotrichoides* can cause localized symptoms and *A. curvulum* can kill the plant under specific environmental conditions which increase plant susceptibility (Patlak 1982), the limitations on the effectiveness of these organisms suggests that further study of the interactions of the plant, its microflora, and the environmental conditions is necessary.

Work conducted in our laboratory has taken an ecosystem approach to the development of microbial control agents in which the emphasis is on organisms naturally present in the plant environment. Microflora native to the *M. spicatum* phyllosphere have been screened for the production of enzymes destructive to selected plant components, cellulose and pectin, as well as for their ability to generally stress the plant either alone or in concert. The contention that this search among plant-associated flora can identify organisms which after appropriate growth procedures and inoculation back onto the plant, are capable of bringing about its decline and death has been confirmed (Gunner 1983). A series of experiments in the laboratory has resulted in the selection of two promising microbial control agents, a fungus, *Mycocleptodiscus terrestris*, and a pectinolytic bacterium, BSP8, for further investigation (Gunner 1983, 1984). Studies of plant-microbe interactions have demonstrated that the selected species occupy an ecologic niche determined by the competitive advantage provided by their resistance to inhibitory substances released by the plant (Gunner 1984).

Results reported on preliminary field studies (Gunner, Limpa-amara, and Weilerstein 1985; Gunner, Limpa-Amara, and Bouchard 1986) have now conclusively demonstrated the potential of the joint application of these two organisms to effectively control *M. spicatum* in nature. Furthermore, jar and pool experiments (Gunner, Limpa-amara, and Weilerstein 1985) have shown that inoculation with *M. terrestris* alone produced a level of plant decline comparable to application of the

fungus along with its companion bacterium, if over a somewhat longer period of exposure.

## MATERIALS AND METHODS

### Experimental plant material

*Myriophyllum spicatum* L. from Stockbridge Bowl, Massachusetts, was used in all experiments. For laboratory experiments, healthy tips were cut from plants in the field and brought back in lake water. Six 10-cm tips were planted in individual plastic cups (5.5 × 5.5 × 5 cm) with a bottom layer of sterile pond sediments overlaid with 4-cm coarse gravel. These cups were placed in 38-ℓ aquaria filled with deionized water. Each tank was aerated on one end through a filter containing glass wool and activated charcoal and through an air stone at the other end. Plants were maintained on an 8-hr light and 16-hr dark cycle.

### Fungal cultures

*Mycoleptodiscus terrestris* (Gerdemann) Ostazeski (Ostazeski 1967) (*M.t.*), a cellulolytic organism originally isolated from necrotic areas of *M. spicatum* (Gunner 1983) was used throughout these studies.

### Preparation of fungal inocula

Fungal inocula were propagated in Potatoe Dextrose Salts Broth (PDSB) (Gunner, Limpa-amara, and Wellerstein 1985). Small-scale cultures for jar tests and fermenter inoculum were grown at 28° C on a rotary shaker at 125 rpm for 96-120 hr in Erlenmeyer flasks. Jar test inocula were allowed to settle, and the supernatant was discarded. The remaining cultures were blended for 1 min at high speed in a 1-qt Waring Blender. Larger batch cultures were grown at the Tufts University Biotechnology Engineering Center in a 300-ℓ Chemap fermenter at 30° C for 72-96 hr at an agitation speed of 300 rpm. The cultures were then passed through a Sharples unit at 13,000 g to yield a cell paste. The paste was removed to sterile plastic bags, packed in ice during transportation, and stored at 5° C for up to 2 weeks. Prior to the inoculation, the cell paste (22.7 kg) was diluted to 250 ℓ in 3-ℓ batches. Each 3-ℓ batch, containing 273-g cell paste, was blended at high speed in a 4-qt Waring Blender for 3 min before being distributed evenly among five 50-ℓ carboys.

### Inoculum viability and enumeration

Inoculum viability was tested and propagules enumerated by plating samples of 10-fold serial dilutions onto Martin's Agar (MA) (Martin 1950). For jar tests, a culture sample was taken and plated out immediately after blending. For lake inoculum, a composite sample was taken from the five carboys and plated out prior to departure for the site.

### Cell counts

Microbial populations were enumerated by serial dilution of samples. Fungal counts were made on MA after 72-hr incubation. The presence of *M.t.* colonies was confirmed after 10 days on plates containing two or more *M.t.* colonies. Total heterotrophic bacterial

counts were made on Trypticase Soy Agar (TSA) (BBL Co., Dickeyville, MD), while pectinolytic organisms were enumerated on Pectin Agar (PA) (Gunner, Limpamara, and Weilerstein 1985). Bacterial counts were made after 36- to 48-hr incubation at 26° C in darkness.

### Jar experiments

Experiments were conducted in 960-ml (1-qt) canning jars to determine the optimum level of *M.t.* inoculum, the most effective temperature for *M.t.* to function as a control agent, and to confirm the effectiveness of the fermenter cell paste. Jars containing plant cups were allowed to acclimate for 24 hr prior to inoculation under the conditions of respective experiments. All tests were performed on quadruplicate plant samples. At the termination of the experiments, plants were removed, washed, and dried at 105° C to a constant weight (48-72 hr).

In order to determine an optimum inoculation dose of *M.t.*, a series of jars was inoculated with 1, 3, and 5 ml of culture at a concentration of  $3.3 \times 10^5$  propagules/ml. Jars were incubated at 18° C on an 8-hr light and 16-hr dark cycle for 21 days.

To establish the optimum temperature for *M.t.* effectiveness, a series of jars was inoculated with 10-ml *M.t.* culture at a concentration of  $4.4 \times 10^5$  propagules/ml and incubated at 18°, 21°, or 25° C, respectively, on an 8:16-hr light:dark cycle for 16 days.

To check the effectiveness of the *M.t.* cell paste, a 3-l batch was prepared as described above to yield a concentration of  $1.9 \times 10^5$  propagules/ml. Plants in jars were inoculated with 10 ml and incubated for 28 days as described in the dose response test above.

### Field experiment

Stockbridge Bowl is a hard-water eutrophic lake located in Berkshire County in western Massachusetts. The Bowl is infested with *M.spicatum* in areas less than 6-7 m in depth. *M. spicatum* growth was continuously removed by mechanical harvester from early summer to early fall.

Two 70-m<sup>2</sup> sites, one treated and one control, 1.5 to 2.5 m in depth, approximately 500 m apart were chosen in the southeastern area of the lake. Test sites were marked off with buoys attached to cement blocks around the perimeters. The plants in these designated areas were allowed to grow without cutting throughout the experimental period.

The inoculum was prepared as previously described and transported to the lake in five 50-l carboys packed in ice. Inoculation consisted of 250 l of *M.t.* at a concentration of  $6.5 \times 10^4$  propagules/ml applied using the spray manifold apparatus (Figures 1 and 2) previously described (Gunner et al. 1985) at a pressure of ca 5 psi just under the surface of the water.

### Microbial populations

Water and plant samples were collected weekly from experimental sites for 11 weeks in order to determine microbial populations. Water column samples were collected in sterile 250-ml polyethylene bottles from a depth of 1 m. Plants were divided into tips, mid-sections, and roots. Five 10-cm tips, five 20-cm portions from the plant midsection, and five entire root systems were collected from each site, put into sterile 250-ml polyethylene bottles containing 50-ml sterile distilled water, and packed in ice for transportation.

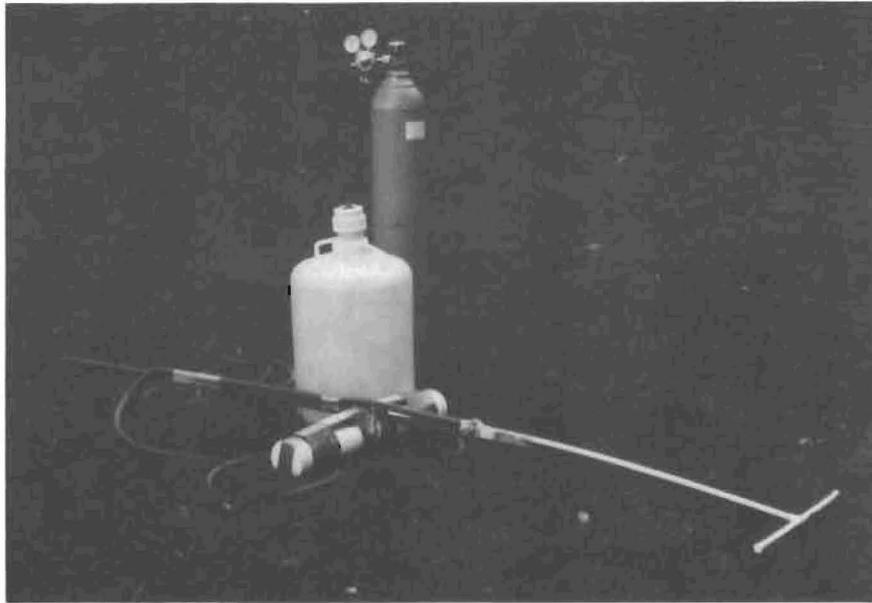


Figure 1. Spray manifold used for lake inoculation

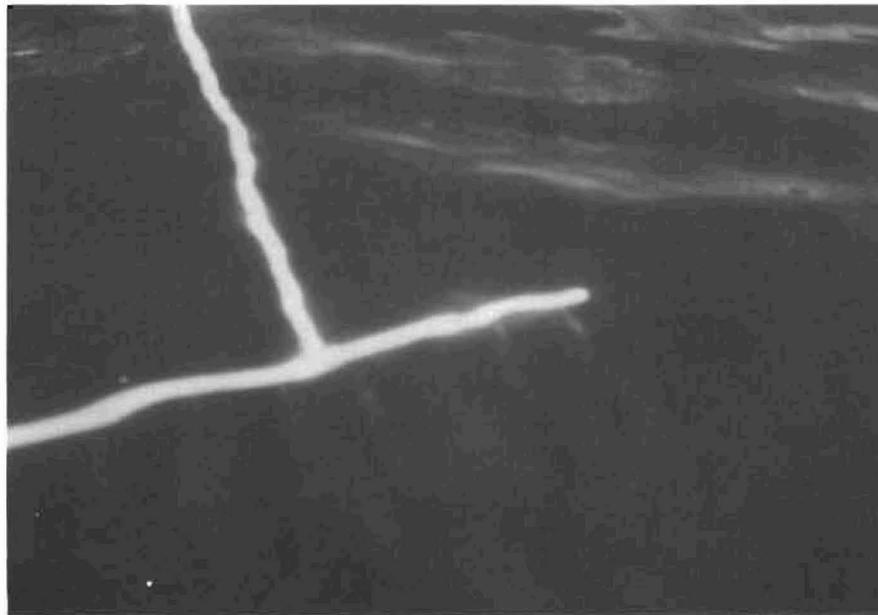


Figure 2. *M. terrestris* as delivered under the water surface by a spray manifold constructed of PVC tubing with eight 2-mm apertures

Plant samples were processed upon returning to the laboratory by transferring the contents of each bottle to sterile 250-ml blender jars, blending at high speed for 5 sec, and making 10-fold dilution series of each. Samples were plated out and incubated as previously described. The remaining plant samples were dried at 105° C to a constant weight (48-72 hr).

### **Biomass harvest**

At the termination of the experiment, eight samples of plant biomass were collected at random from each test plot, each sample representing an area of 0.25 m<sup>2</sup>. Samples were pulled from the marked area by a team of scuba divers. Excess sediment and foreign objects were washed from the plant samples before separation of stem and root sections. Each sample was oven-dried at 105° C until the weight remained constant.

### **Statistical methods**

Statistical analysis of data was performed using the F-test for the determination of overall variance and the t-test to determine if significant differences existed between treatments. Analysis was performed by computer application of BMDP statistical programs. The level of significance was determined within a 95 percent confidence interval ( $p \leq 0.05$ ).

## **RESULTS**

### **Jar experiments**

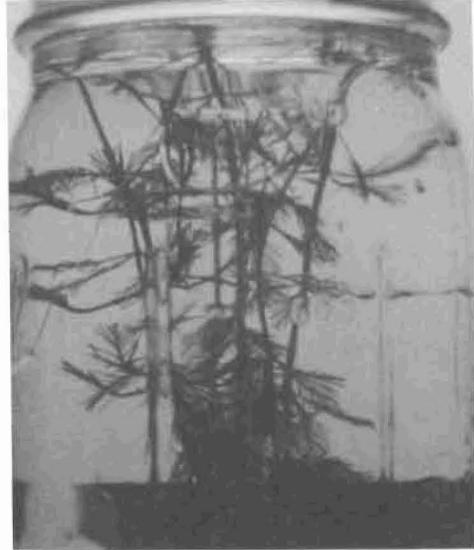
As reported previously (Gunner, Limpa-amara, and Weilerstein 1985), *M.t.* used alone as a control agent is capable of inducing *M. spicatum* death (Figures 3 and 4). Inoculation concentrations of 1 and 3 ml produced increasing levels of plant decline, while 5 ml resulted in complete degradation. Not only does viable plant tissue decrease (Figure 3), but the extent of root development diminishes (Figure 4) with increasing inoculum levels. The results of biomass determinations (Figure 5) support the visual evaluation, showing a decrease in plant dry weight with increasing inoculum levels. Though there was a 10 percent decrease in plant biomass as the inoculation dose rose from 1 to 3 ml, plant weight decline did not continue at the 5-ml dose level.

*M. spicatum* response to inoculation with *M.t.* also appeared to be conditioned by water temperature. As shown in Figure 6, when water temperatures increased from 18° to 25° C the *M.t.* efficacy, as measured by decrease in plant weight, rose. This condition was confirmed by the significant decrease in biomass weight between treated and control trials at 25° C ( $p$ -value 0.047), while no significant difference between treated and control plants occurred at either 18° or 21° C.

In Figures 7 and 8 are shown the results confirming the effectiveness of resuspended fungal cell paste in bringing about the decline of *M. spicatum*. Consistent with other jar experiments there was virtually no viable plant tissue or roots by 21 days after treatment. At this time, control plants had an average dry weight of 0.4270 g while treated plants averaged 0.3419 g. The  $p$ -value was 0.0373, indicating a significant difference between samples at the 95 percent confidence interval.



*a. Uninoculated control*



*b. Inoculated with 1-ml M.t. culture*



*c. Inoculated with 3-ml M.t. culture*



*d. Inoculated with 5-ml M.t. culture*

**Figure 3. Representative samples of *M. spicatum* from dose response test in jars 16 days after inoculation**

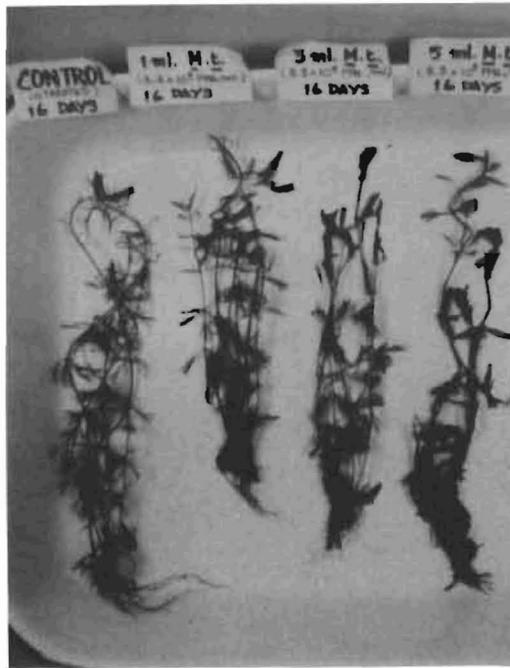


Figure 4. *M. spicatum* samples from dose response jar experiment removed from cups to expose roots

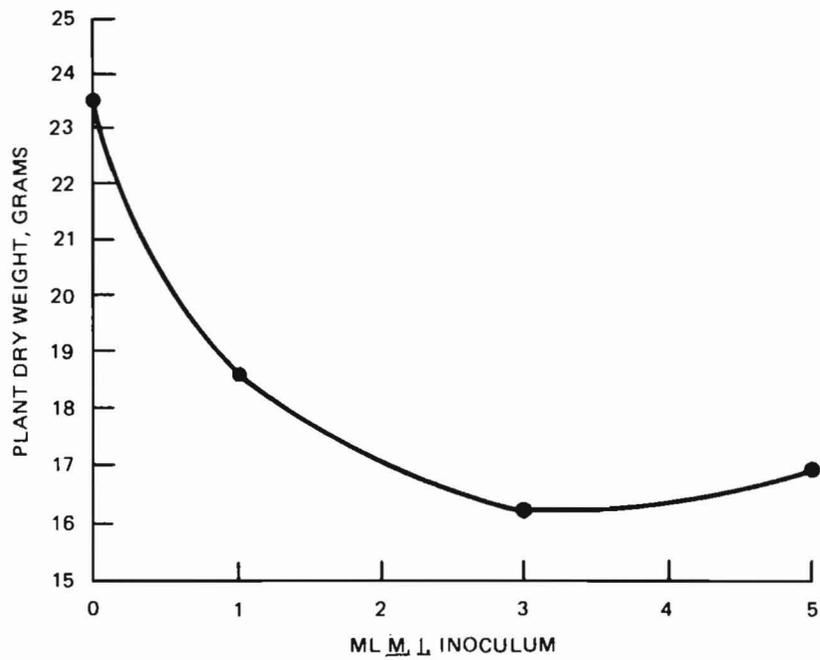


Figure 5. Biomass of *M. spicatum* from dose response jar experiment after 16 days

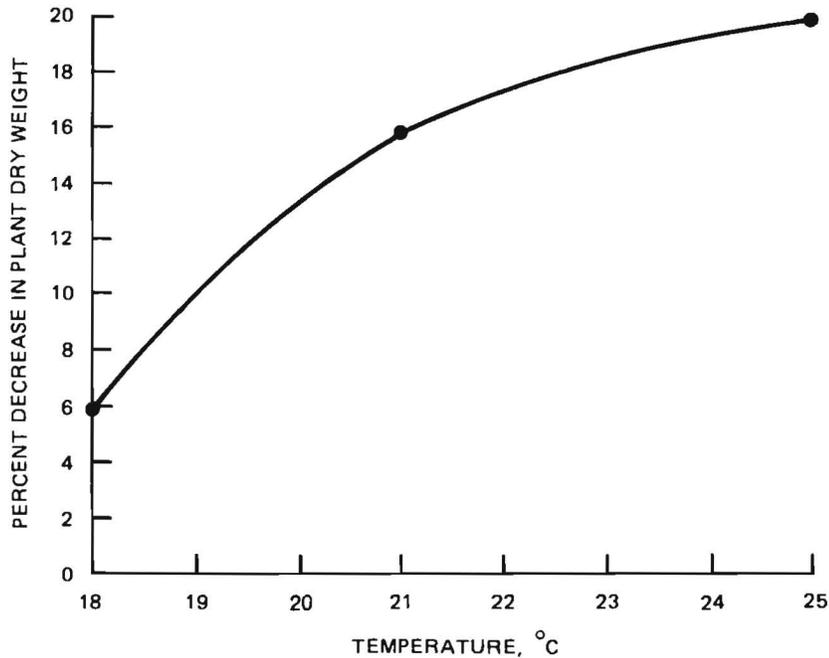


Figure 6. Biomass results (expressed as percent decrease in dry weight of treated versus control) of *M. spicatum* incubated at various temperatures 16 days after inoculation

### Microbial population dynamics

The numbers of total heterotrophic bacteria isolated from various *M. spicatum* tissues and from the adjacent water profiles are shown in Figures 9, 10, 11, and 12. The bacterial numbers from plant tissues remained 4 to 5 orders of magnitude higher than those from the water profiles, with root numbers an order of magnitude higher than tip and midsection numbers. Bacterial counts from the plant tips and water profiles remained virtually at a steady state throughout the experimental period (Figures 9 and 12). Bacterial numbers from the treated plant midsection increased during the second week after inoculation (Figure 10) and remained close to an order of magnitude higher than control numbers for 4 weeks before declining. Root populations (Figure 11) were not only an order of magnitude higher than tip and midsection populations, but also showed a distinct pattern of fluctuation not evidenced in other plant or water populations.

The numbers of strongly pectinolytic bacteria from plant tissues and water profiles (Figures 13, 14, 15, and 16) showed distribution patterns almost identical to those of the total heterotrophs in terms of population levels and stability. Strongly pectinolytic bacteria on the midsection of treated plants increased from a low at week 5 (Figure 14) to a maximum at week 8, the fourth week after inoculation. Indeed, they remained significantly higher than control numbers during these 4 weeks before declining to control levels.

As shown in Figures 17, 18, 19, and 20, the numbers of total fungi on plant tissues and in the water profiles followed the same general pattern shown by bacterial populations in that plant tissues supported populations 3 orders of magnitude higher than those found in the water profiles. Total fungal populations were, however, 1 to 2.5 orders of



Figure 7. Representative samples of *M. spicatum* in jars 21 days after inoculation with fermenter *M.t.* cell paste

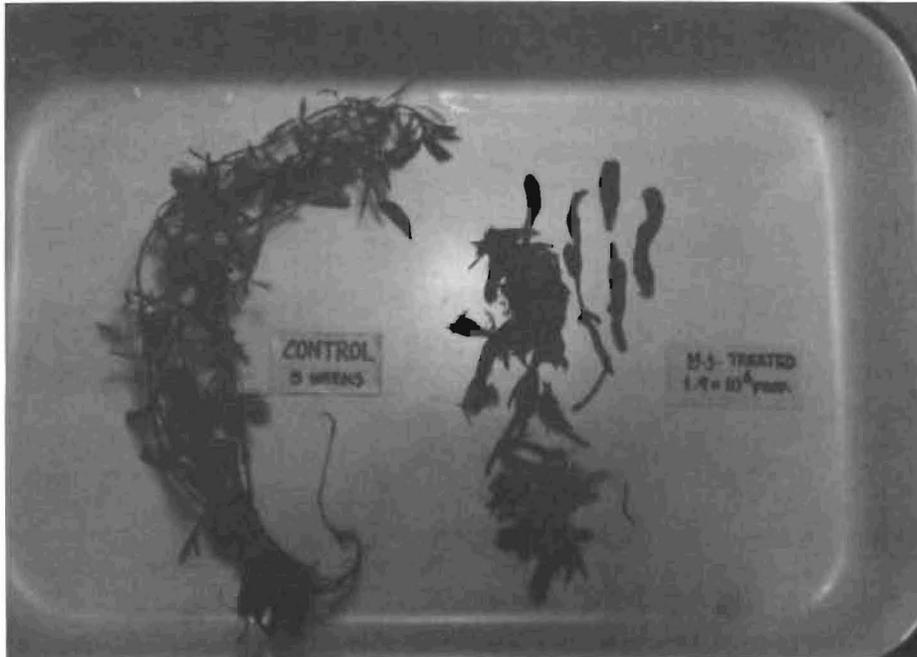


Figure 8. *M. spicatum* after treatment with fermenter *M.t.* cell paste removed from cups to expose roots.

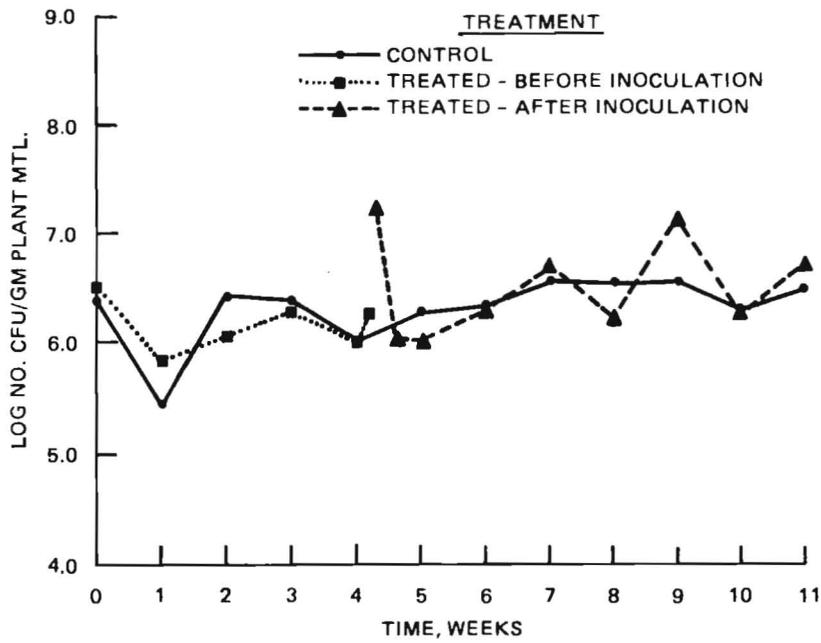


Figure 9. Microbial populations recovered on TSA medium from control and treated *M. spicatum* tips in field experiment

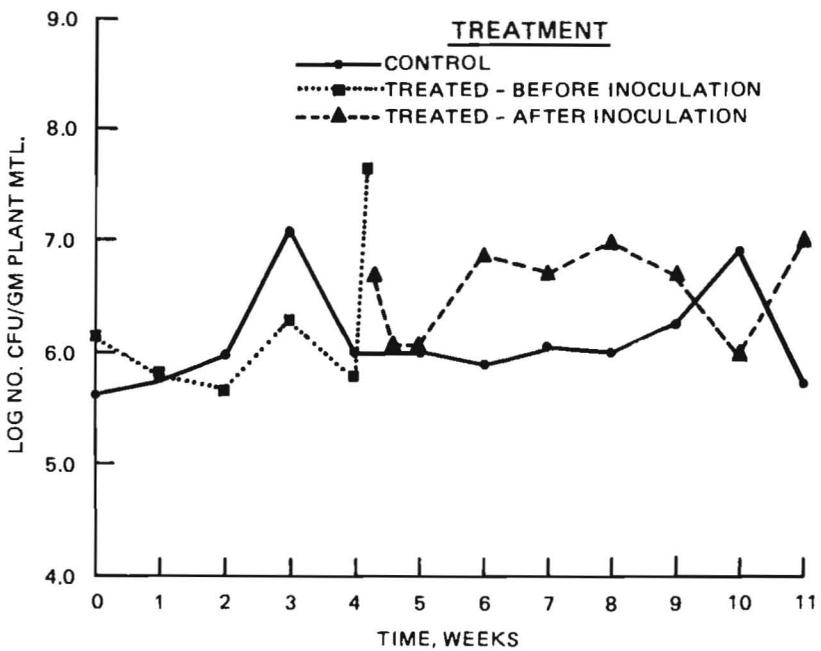


Figure 10. Microbial populations recovered on TSA medium from control and treated *M. spicatum* midsections in field experiment

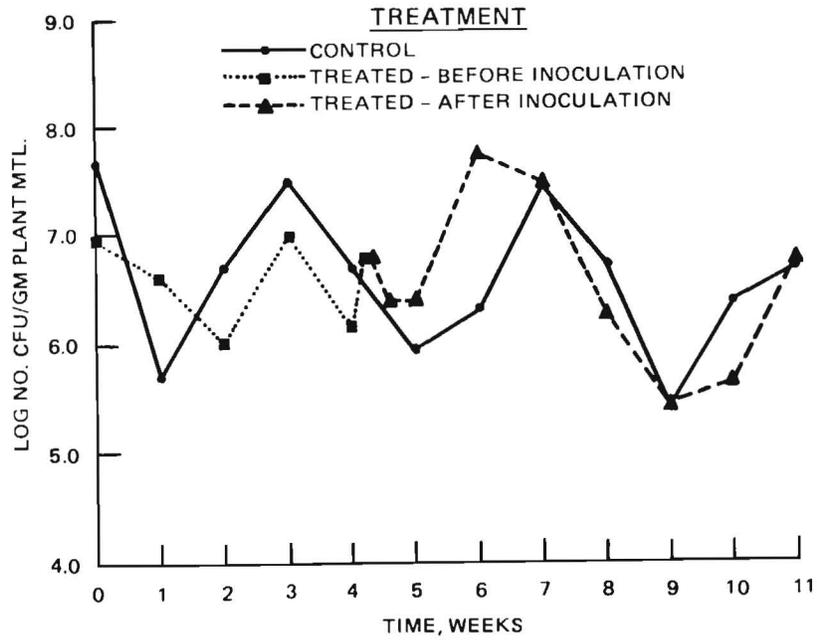


Figure 11. Microbial populations recovered on TSA medium from control and treated *M. spicatum* roots in field experiment

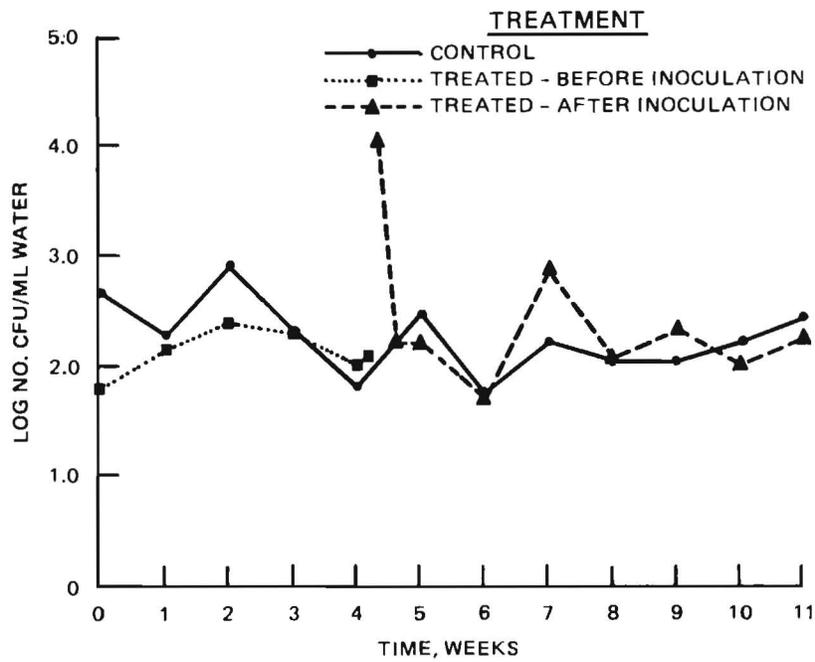


Figure 12. Microbial populations recovered on TSA medium from water profiles of control and treated plots of *M. spicatum* in field experiment

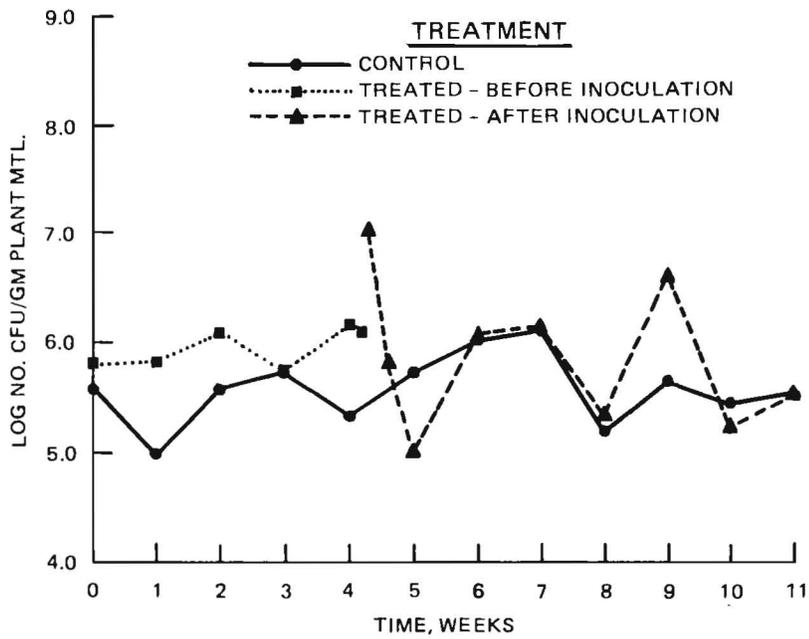


Figure 13. Populations of strongly pectinolytic bacteria recovered on PA medium from control and treated *M. spicatum* tips in field experiment

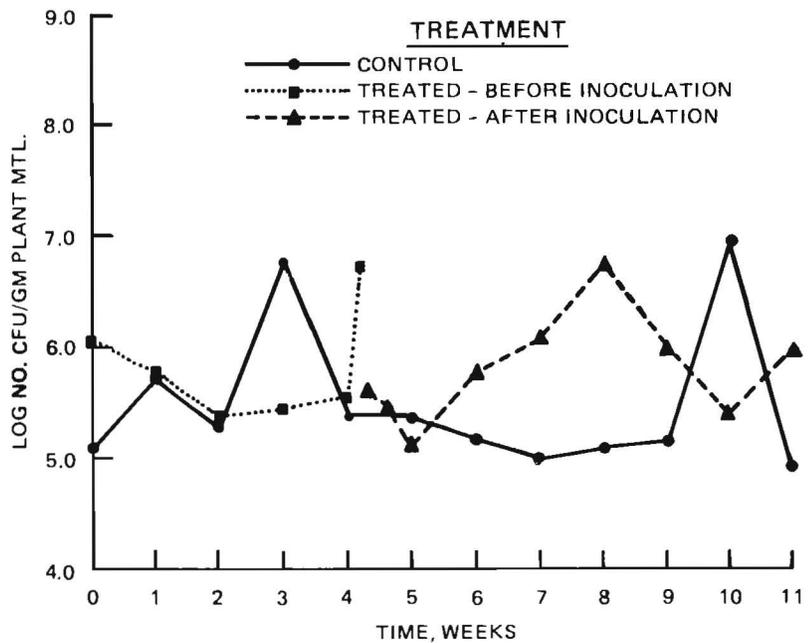


Figure 14. Populations of strongly pectinolytic bacteria recovered on PA medium from control and treated *M. spicatum* midsections in field experiment

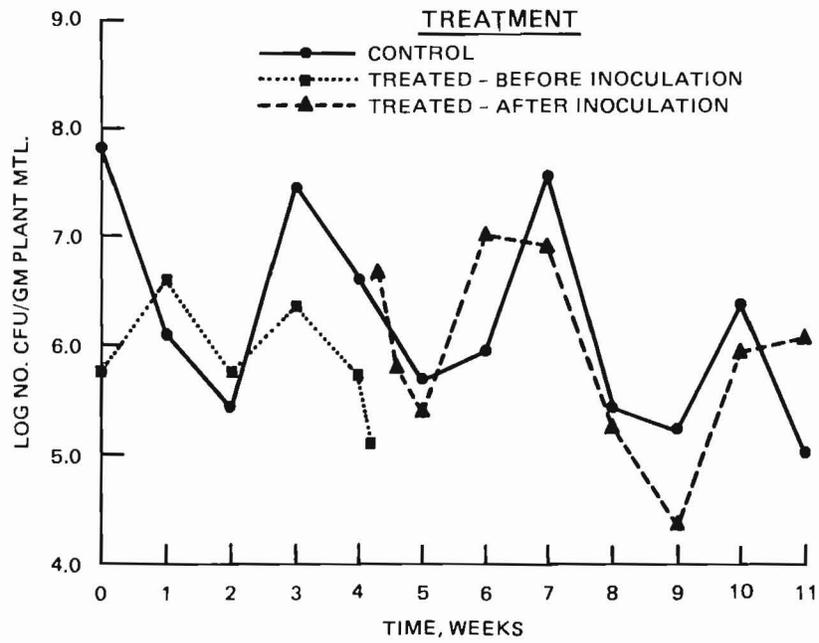


Figure 15. Populations of strongly pectinolytic bacteria recovered on PA medium from control and treated *M. spicatum* roots in field experiment

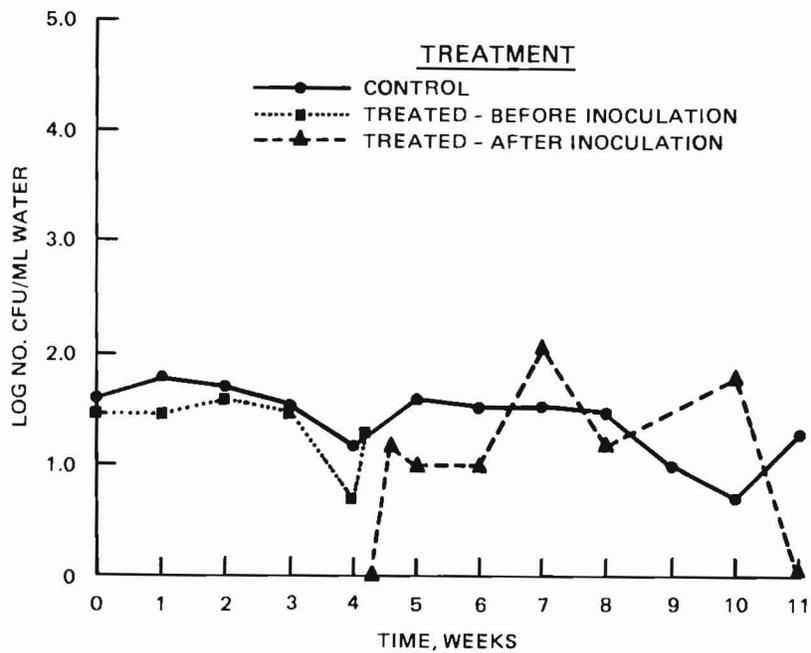


Figure 16. Populations of strongly pectinolytic bacteria recovered on PA medium from water profiles of control and treated plots of *M. spicatum* in field experiment

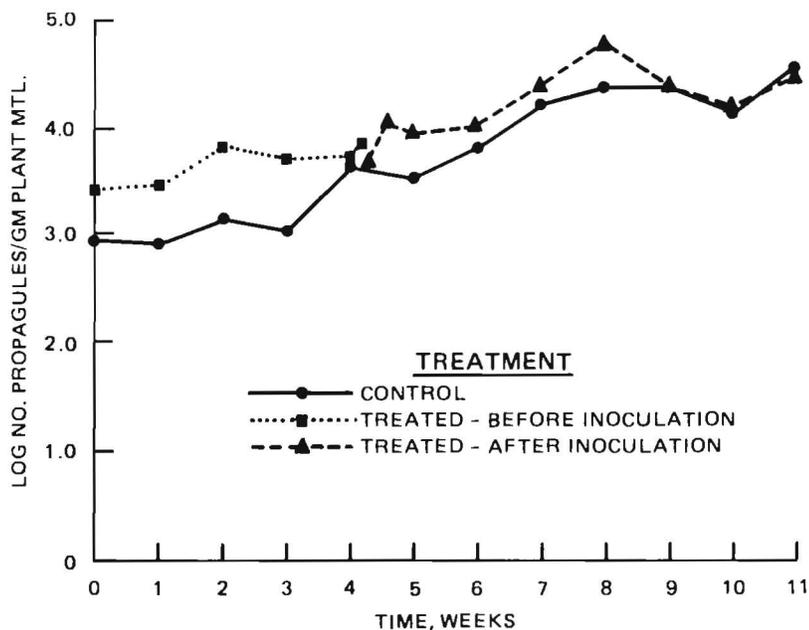


Figure 17. Total fungal populations recovered on MA medium from control and treated *M. spicatum* tips in field experiment

magnitude lower than corresponding bacterial populations. In contrast to bacterial populations, total fungal populations from plant tissues were stable throughout the experimental period, most notably root populations, while water profile populations fluctuated considerably (Figure 20).

The numbers of *M.t.* isolated from plant tissues during the lake trials are shown in Figures 21, 22, and 23. At no time during the experimental period was *M.t.* observed in the water profile. *M. terrestris* was observed sporadically on control tissues, most frequently on midsections. *M. terrestris* did occur after inoculation on treated tissues. In contrast to control numbers, treated tips (Figure 21) showed sustained *M.t.* population over the 4-week period immediately following inoculation, after which they declined to an undetectable level.

### Biomass harvest

The effect of treatment with *M.t.* on *M. spicatum* as measured by biomass decline is shown in Table 1. It will be noted that a 71 percent reduction in total plant biomass was observed. There was a four-fold reduction in stem-leaf biomass and a two-fold reduction in root material. The biomass of stem-leaf tissue in the control quadrant was 15.3 times greater than its root biomass, while only 7.4 times higher in the treated quadrant.

## DISCUSSION

Consistent with the results previously reported (Gunner, Limpa-amara, and Bouchard 1986), the trials described in the foregoing confirm the efficacy of the biocontrol strategy in which *M. terrestris*, originally isolated from *M. spicatum*, serves to bring about the

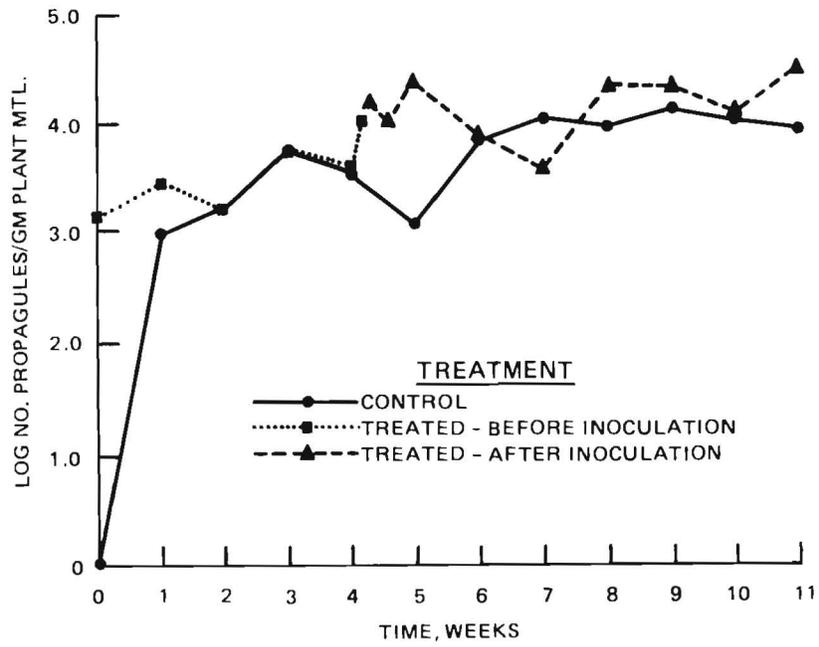


Figure 18. Total fungal populations recovered on MA medium from control and treated *M. spicatum* midsections in field experiment

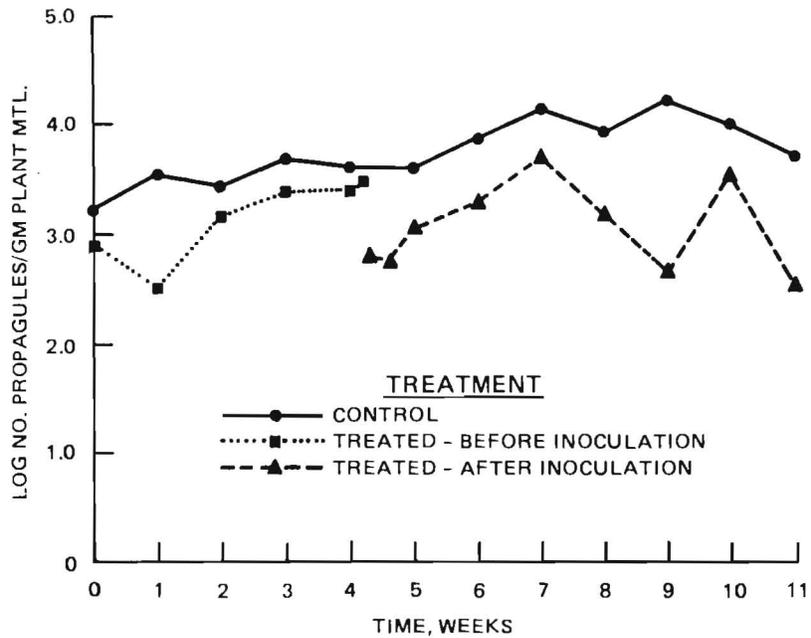


Figure 19. Total fungal populations recovered on MA medium from control and treated *M. spicatum* roots in field experiment

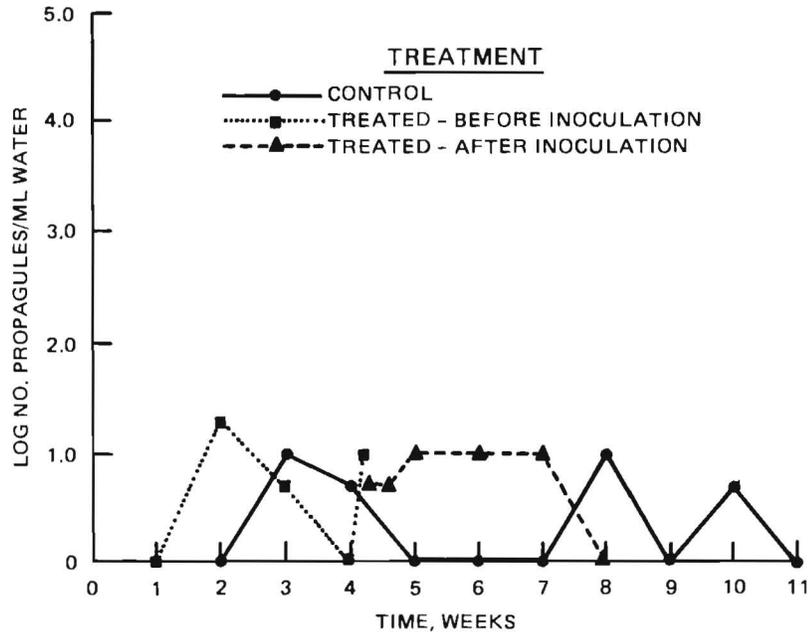


Figure 20. Total fungal populations recovered on MA medium from water profiles of control and treated plots of *M. spicatum* in field experiment

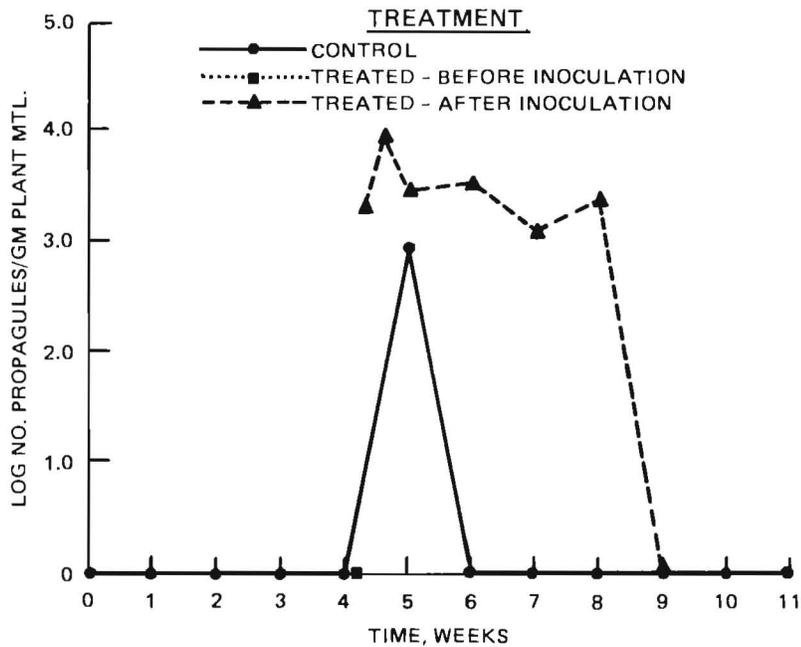


Figure 21. Populations of *M. terrestris* recovered on MA medium from control and treated *M. spicatum* tips in field experiment

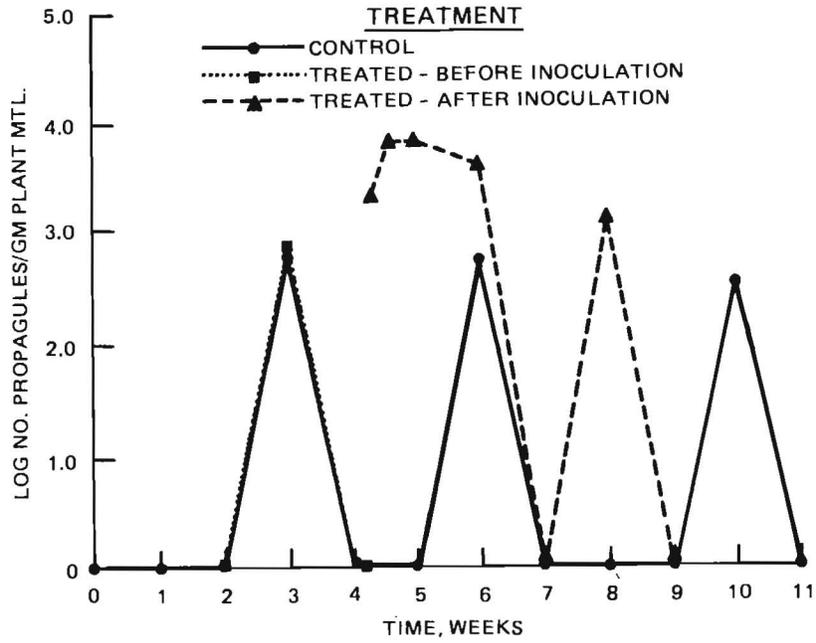


Figure 22. Populations of *M. terrestris* recovered on MA medium from control and treated *M. spicatum* midsections in field experiment

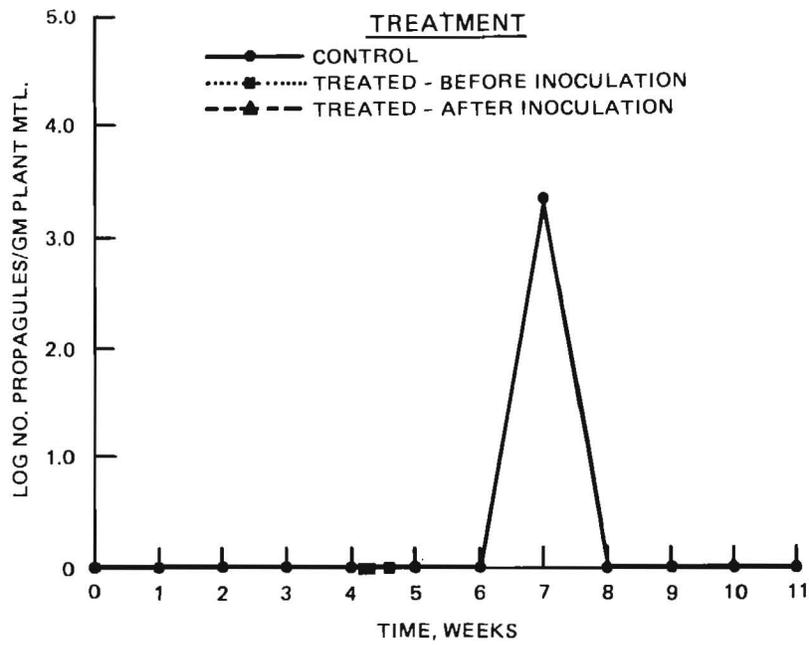


Figure 23. Populations of *M. terrestris* recovered on MA medium from control and treated *M. spicatum* tips in field experiment

Table 1  
Effect of Treatment<sup>a</sup> on Biomass of Eurasian  
Milfoil in Stockbridge Bowl, Massachusetts (1987)

Plant Part	Mean Biomass <sup>b</sup> Dry Weight Plant Material (gm/0.25 m <sup>2</sup> )		p-value <sup>c</sup>
	Control	Treated	
Stem-leaf	60.81	16.50	0.003*
Root	3.98	2.23	0.190
Combined	64.79	18.73	0.002**

<sup>a</sup> *M. terrestris* (*M.t.*) applied as a biological control.

<sup>b</sup> Average weight in grams of dried plant materials (105° C, 72 hr) from eight 0.25-m<sup>2</sup> samples/plot after 4 weeks.

<sup>c</sup> p-values obtained from ANOVA F values and t-test of 8 sets of replicates.

\*p ≤ 0.05 significant.

\*\*p ≤ 0.01 highly significant.

decline of this plant. Implicit in this technique is the derivation of the control organism from the plant ecosystem to which it is returned as an invasive and ultimately lethal agent.

It is important to note that this year's application methodology differed substantially from that employed previously. The original procedure included the inoculation of a strongly pectinolytic and growth stressing bacterium together with *M.t.* Laboratory trials, however, had indicated that *M.t.* alone could be effective in bringing about plant decline. In the interests of simplifying the application, as well as reducing fermentation costs, it was decided that this year's field trials would be conducted with *M.t.* alone.

The results indicate that though a significant level of control was achieved in the absence of the companion bacterium, there were, however, qualitative differences in the results. While in 1986 there was virtually a total disappearance of plant material in the treated quadrant, this year there appeared to be a random deletion in growth.

A number of elements which may have functioned to differentiate this year's results from previous applications include the time of application and the number of applications. As our results have indicated, the efficacy of *M.t.* is both temperature and dose conditioned with maximum results achieved at ca 25° C and with increasing dose levels. This year's application was made 1 month later than last year's at cooler ambient temperatures. Too, in distinction to last year, in which two applications of both bacterium and fungus were made, this year only one application of the fungus was provided. Nonetheless, a strong measure of control is indicated even in these circumstances. It should, however, be emphasized that maximum control potential rests with applications made at the highest summer temperatures and with the maximal dose practicable.

Consonant with our previous studies, population dynamics reflect the strong microfloral commitment to the plant host. Numbers of organisms in the water profile are invariably several orders of magnitude lower than those on plant surfaces, reflecting diminished substrate possibilities. On the plant, on the other hand, where secretions,

sloughed off tissue components, and saprophytic possibilities exist, the higher numbers reflect these substrate options. It is of interest that populations differ with the different plant zones. While the roots serve as the richest substrate provider for bacteria, fungi tend to remain at similar densities on all areas of the plant.

The introduction of *M.t.* as inoculum, on the other hand, while raising its numbers on tips and midsection, did not increase its presence on the roots. This fact may reflect the manner of *M.t.* application just below the water surface, and accordingly the longer period of time required for the fungus to reach and establish itself on the plant root in contrast to its rapid establishment on tips and stems.

In general, there was little perturbation of heterotrophic microflora in the phyllosphere, while in the rhizosphere shifts were more pronounced. Most noticeable was the change in the pectinolytic, pitting, microflora whose numbers increased significantly in the plant midsection subsequent to inoculation with *M.t.* This change would logically follow the invasive action which would make more substrate available to the pectinolytics and stimulate a flush in their growth. Viewed overall, however, the introduction of *M.t.* does not have a lasting effect on population dynamics and thus in no way stresses the ecosystem.

## CONCLUSIONS AND RECOMMENDATIONS

The results of this, our third year of field applications, again confirm both the validity of our ecosystem approach and its efficacy in the control of *M. spicatum*. A departure from previous years was the utilization of a single organism, *M. terrestris*, as a source of inoculum, in contrast to the two microorganisms previously used. *M. spicatum* control can be achieved with *M.t.* alone with appropriate regard for water temperature, inoculum density, and plant physiological status. To maximize plant kill, the time of application should also be adjusted to assure sufficient contact between plant and inoculum. Although fluctuations in microbial populations occurred subsequent to inoculation, no permanent changes were observed. On the other hand, the *M. spicatum* decline which was achieved may be seen as a harbinger of the return of a more balanced plant community.

At the application level, primary attention must be given to the development of appropriate delivery systems and to further the scaling up of *M. terrestris* production. At the research level, further studies are indicated as to the mechanism of action, whereby *M. terrestris* effects its pseudopathogenic function. Such studies, beyond their academic interest, are also necessary to establish specificity limits. Nor should the interaction between microorganisms such as BSP8 and *M. terrestris* in bringing about plant decline be overlooked. Such synergistic activities may provide insights into accelerating target plant decline as well as in extending the range of target plant species.

## ACKNOWLEDGEMENTS

We wish to thank Mr. Edwin A. Theriot, Project Manager, Aquatic Plant Control Research Program, US Army Engineer Waterways Experiment Station, for his continued cooperation and support of this project.

We also wish to thank Dr. Randall Swartz of the Tufts University Biotechnology Engineering Center for his help in the fermentation of the fungal inoculum.

We would like to express our gratitude to Dr. Richard A. Rohde, Associate Director of the Agricultural Experiment Station, College of Food and Natural Resources at the University of Massachusetts, for providing transportation for the project.

Particular appreciation is expressed to the town of Stockbridge, Massachusetts, and Dr. Don W. Deno, Chair of its Conservation Commission, for the opportunity provided by the use of Stockbridge Bowl for our field trials.

Special thanks also to Peter D. Gamble and Phasuvudh Kanechorn for their dedication and help in this year's field trials.

We would like to express our particular thanks to Mr. and Ms. John D. Bender, our hosts at the test site, for their continued enthusiasm, support, and generosity.

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# Biological Control of Hydrilla with Plant Pathogens

by  
Gary F. Joye\*

## INTRODUCTION

These studies were based on the theory that microorganisms, which exist in the natural microflora of hydrilla, have a latent ability to attack the plant when conditions favor the microbe and/or stress the plant. This research was similar to the biological control project on Eurasian watermilfoil (Gunner 1983).

Previous research has identified 20 fungal isolates that produced enzymes lytic to hydrilla tissues, six of which caused damage to hydrilla in test tube assays (Theriot and Pennington 1985). Isolate 224 (*Cladosporium cladosporioides*), which was the only isolate to produce pectinase, failed to disease hydrilla in test tube assays. However, pectinase production may be a predisposing factor for the pathogenicity of many plant pathogens. Therefore, 224 was included with the other six (Table 1). These seven isolates were evaluated in aquaria assays. From this assay, isolate 224 completely destroyed hydrilla within 2 weeks after inoculation.

Table 1  
Candidate Microorganisms Evaluated in Aquaria Assays

<i>Isolate Number</i>	<i>Scientific Name</i>
56	<i>Aspergillus awomori</i> Nakazawa
156	<i>Humicola</i> sp. with <i>Tricoderma</i> sp.
161	<i>Humicola</i> sp. with <i>Tricoderma</i> sp.
170	unidentified
224	<i>Cladosporium cladosporioides</i> Fresen. de Vries
236	<i>Fusarium moniliforme</i> Sheldon var. <i>subglutinans</i> Wr. & Reink
249	<i>Aspergillus awomori</i> Nakazawa

For a candidate organism to be an effective biocontrol agent, it must express pathogenicity, be host-specific, thrive in conditions favored by the target weed species, and compete with other microflora (Shrum 1982). Based on these criteria, the objectives of this research were to evaluate the host specificity of isolate 224, to determine its potential toxicity to fish, and to evaluate 224 in the field. Other objectives included collection and evaluation of microorganisms collected from hydrilla during the 1987 growing season.

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

## MATERIALS AND METHODS

### Host specificity of *Cladosporium cladosporioides*

A host specificity aquarium assay was conducted under controlled conditions (light, 2,000-ft candles, 14-hr light period, and a constant temperature of 25° C) in an environmental chamber measuring 2.6 × 2.3 × 2.4 m. Aquaria measured 30 × 30 × 75 cm. Each of the eight aquaria was filled with 48.5 l of nutrient solution; reverse osmosis (RO) water, CaCl<sub>2</sub> × 2 H<sub>2</sub>O, 4.456 g; MgSO<sub>4</sub> (anhydrous), 1.635 g; KHCO<sub>3</sub>, 0.747 g; and NaHCO<sub>3</sub>, 2.84 g/48.5 l. Three 13-cm apical nonflowering sprigs of hydrilla, (*Hydrilla verticillata*), Eurasian watermilfoil (*Myriophyllum spicatum*), Elodea (*Elodea densa*), and Coontail (*Ceratophyllum demersum*) were planted in one 0.24-l plastic cup four-fifths filled with sterilized Brown Lake sediment (Waterways Experiment Station, Vicksburg, Mississippi) and covered with 1 cm of washed silica sand. Each aquarium contained three cups of hydrilla and three cups each of two of the other species for a total of nine cups and three species per aquarium. The plants were allowed to grow 4 weeks before application of the fungal inoculum. There were two aquaria for each plant species combination—one for control and one for treatment. The inoculum consisted of the fungus and the culture media (whole inoculum). The formulated media was made of 200 ml of V-8 juice, 3-g CaCO<sub>2</sub>, 5-g sodium polypectate, 2-g yeast extract, and 800-ml dH<sub>2</sub>O. After 1 week in this media, the fungus had grown to a concentration of 10<sup>5</sup>cfu's/ml. For each treatment, 600 ml of whole inoculum plus 3 × 10<sup>6</sup> spores/ml was applied to four aquaria. Spores were grown by the open tray method (Walker, 1980). The test continued for 8 weeks. Data collected included total living biomass above and below ground, number of shoots from roots, number of branches off shoots, and maximum plant length. Data were subjected to T-test (Steel and Torrie 1980).

### Fish toxicity of *Cladosporium cladosporioides* on hydrilla

*Cladosporium cladosporioides* was tested for toxicity to the White Amur. The Triploid White Amur, female *Ctenopharyngodon idella* × male *Hypophthalmichthys nobilis* (Ozark Catfisheries, Springfield, Missouri), was chosen as the test fish because it feeds directly on hydrilla foliage. Therefore, it would be in direct contact with the fungus. Ten aquaria measuring 30.5 × 21.5 × 15.2 cm were filled with 8 l of RO water (Reverse osmosis). Within each aquarium, 50 g of hydrilla foliage was emersed in the water. Ten White amur were placed in each aquarium. Five randomly assigned aquaria were inoculated with 150 ml of fungal whole inoculum (10<sup>5</sup> cfu's/ml) plus a spore concentration of 3 × 10<sup>6</sup>/ml. The water was not filtered or changed after inoculation. The effects of any toxic activity may be removed if the water had been filtered. However, due to increase toxicity from an increase in the concentration of their fecal materials, it was expected that the fish would eventually die. The fish were observed daily for any toxic effect by the fungus. Data collected included pretest weight of total fish/aquarium, posttest fish weight, and number of dead fish. Changes in feeding habit were monitored and an examination of five randomly sampled fish was made of their digestive tracks and gills. The test continued for 2 weeks. Data were subjected to a paired T-test analysis comparing differences between treated fish and untreated fish (Steel and Torrie, 1980). The test was conducted twice.

### Field evaluation of isolate 224.

With the help of the Fish and Wildlife Department of Texas, Lake Nacodoches was the best site to conduct a small field test of 224. However, the test was not completed because the City Council of Nacodoches had received numerous inquiries into our plans for a similar test. The City of Nacodoches changed its mind and did not give permission to conduct the test in Lake Nacodoches.

### Test tube assays.

Two separate test tube assays were conducted on previously collected fungal isolates. The first test included isolates known to produce lytic-enzymes (Pennington 1985). The second test included fungal isolates collected from hydrilla during the summer of 1987 (Figure 1).

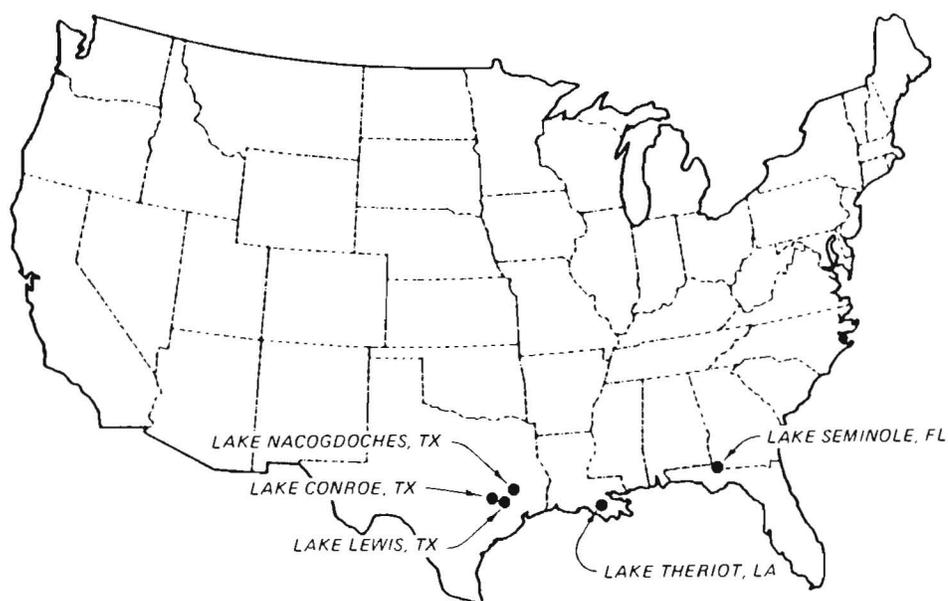


Figure 1. Map of the United States with locations indicating where microorganisms were isolates from collected hydrilla samples (1987)

In the first test, eight fungal isolates were tested for efficacy on hydrilla by the method described by Pennington (1985). These isolates included the fungal species; *Aspergillus awomori*, *Cephalosporium acremonium*, *Hemicola* sp. with *Trichoderma* sp., *Fusarium moniliforme* var. *subglutinans*, *Cladosporium cladosporioides*, and *Fusarium roseum* var. *culmorum*. *F. roseum* var. *culmorum* was received from Charudattan of the University of Florida. Test tubes (20 × 2.3 cm) were filled with 60 ml of nutrient solution (described in methods for the host specificity test) amended with 3-mg/l streptomycin. A 10-cm apical tip of hydrilla was inserted into each tube.

The fungi were grown separately in 100 ml of Potato Dextrose Broth (PDB) in 250 Erlenmyer flasks at 120 rpm on a shake table. *C. cladosporioides* was grown in a V-8 juice media as described in the host specificity methods. Two ml of inoculum of each

fungus was randomly placed in each hydrilla test tube. The hydrilla inoculated test tubes were incubated at 25° C with 12-hr light periods in a Hotpack programmed refrigerator incubator model number 352620, Philadelphia, Pennsylvania. The test continued for 6 weeks. After 6 weeks the remaining living biomass was weighed (g). During the course of the test, notes were made on the condition of the hydrilla plant for each fungal isolate. All treatments were replicated five times.

The second test tube assay was conducted in a similar manner as the first. Fifteen fungal isolates collected from hydrilla during 1987 were screened in this assay. These fungal isolates were grown on solid media which consisted of Potato Dextrose Agar (PDA) amended with 3-mg/l streptomycin and 2-g yeast extract. A 1-cm plug was placed in each test tube and forced to the bottom of the tube (Rejmankova, Blackwell, and Culley 1986). All treatments were replicated 10 times.

Data from both assays were subjected to analysis of variance procedures, and mean comparisons between isolates and controls were made using Tukey's test (Steel and Torrie 1980).

#### **Aquarium assay of fungal isolates efficacy on hydrilla.**

A study was conducted to test the effects of five fungal isolates that showed potential as biocontrol agents in test tube assays on the growth of hydrilla. Species included in this assay were *Aspergillus awomori*, *Fusarium roseum* var. *culmorum* (donated by Charudattan, University of Florida, Gainesville), *Cladosporium cladosporioides*, and two unidentified isolates. Three hydrilla stem tips (13 cm) were planted in plastic cups (0.24 l) filled with pond sediment and covered with silica sand. Nine cups were placed in each of six aquaria. One aquarium was designated as the control. Environmental conditions were maintained as previously described. After the plants had grown to the top of the water column, whole inoculum of each isolate was poured into one of the six aquaria. For each isolate, a concentration of 10<sup>6</sup> cfu's/ml was applied. The test was continued for 3 weeks. Biomass of each cup from each aquaria was weighed. Data were subjected to analysis of variance procedures, and Tukey's test was performed to compare means (Steel and Torrie 1980).

## **RESULTS AND DISCUSSION**

### **Host specificity.**

None of the test species were diseased by *C. cladosporioides* including hydrilla. This fact may be explained through evidence that long-term storage of pathogenic organisms may lose their virulence even though viability is maintained (Hawksworth 1984). This change is usually due to a genetic mutation, and the gene(s) for virulence may be lost. Occasionally, virulence may be regained through modification of the growth media, but such has not been the case with *C. cladosporioides*.

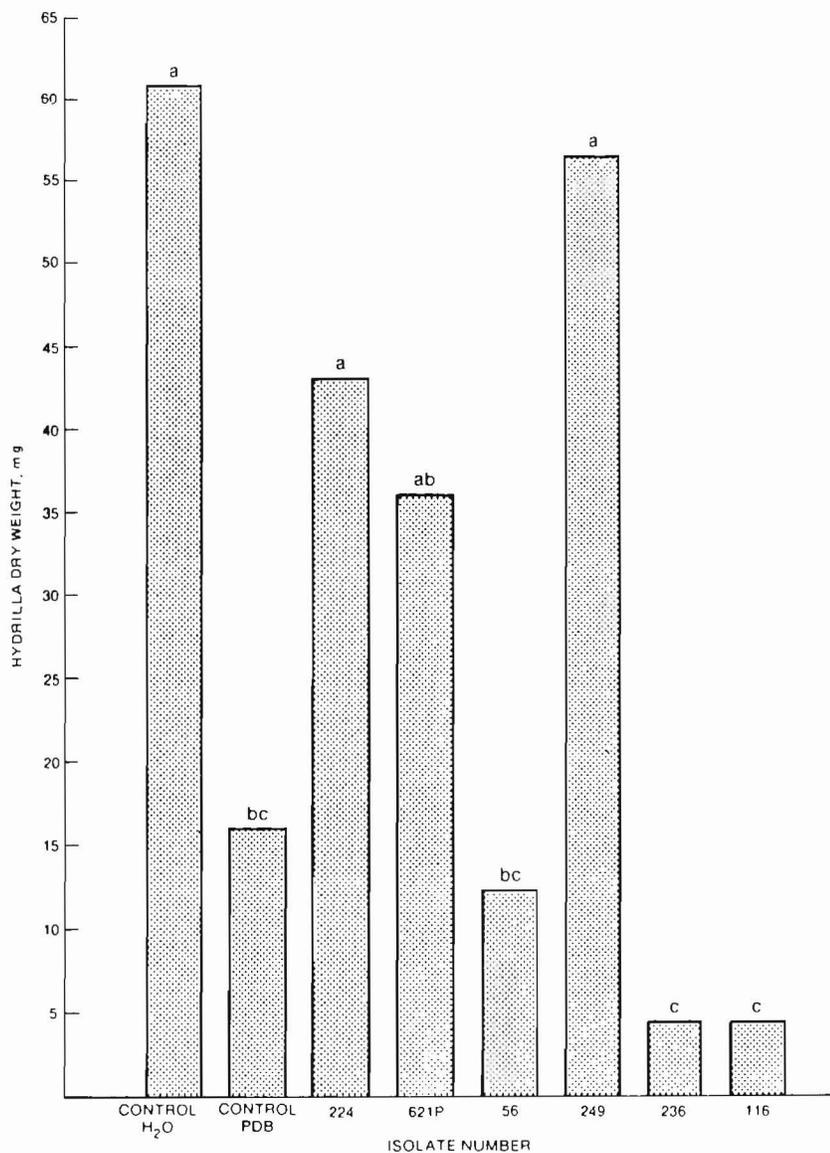
### **Fish toxicity.**

The white amur were not affected by the presence of *C. cladosporioides*. Ninety percent or more of the hydrilla was consumed within 10 days. Fish in the treated aquaria were observed ingesting the fungus as well the hydrilla foliage. Also, their feces were of a

much darker color than the untreated fish. Both fungal hyphae and hydrilla tissue could be removed from their digestive tracts. No fungal material was removed from the gills. There was no significant difference between treatments for mortality or weight loss. Results of this test suggest that any exudates from this fungus would not pose a threat to fish behavior or mortality. The fact that this organism is known to exist in endogenous waters and soils (De Vries 1967) would indicate cohabitation with a variety of fish species with no ill effects.

### Test tube assays

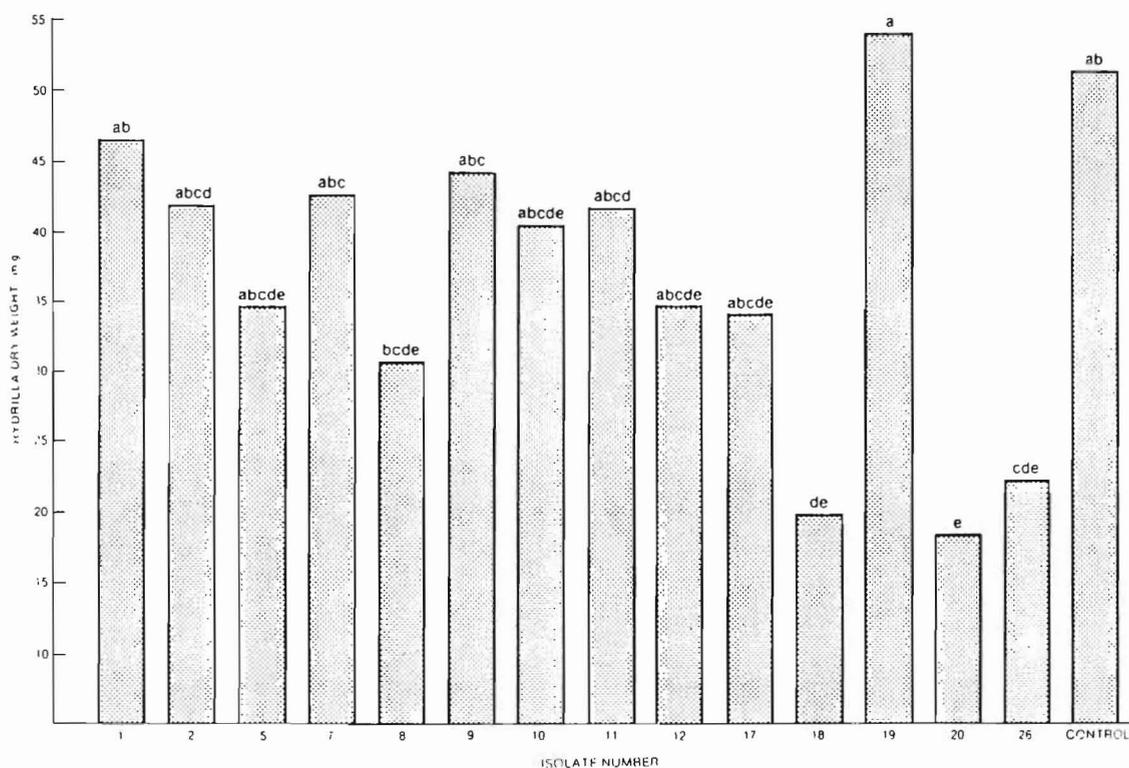
**Assay 1.** The analysis of variance procedures showed a significant difference in the efficacy between the treatments ( $r^2=0.66$ ,  $Pr > F = 0.0001$ ). Fungal isolates 56, 116, and



**Figure 2.** Effects of previously collected fungal isolates on hydrilla (Table 1). Tukey's Test ( $P < .05$ ). Bars with the same letter are not significantly different

236 were significantly more effective than isolates 224 or 621P in reducing hydrilla biomass. However, the more effective isolates were not significantly better than the control with PDB (Figure 2). The effects of the PDB on hydrilla were not expected. From this surprise phenomenon, it was speculated that either PDB was contaminated or the PDB concentration was too high for hydrilla to survive.

**Assay 2.** The analysis of variance procedure showed a significant difference in the effectiveness of the fungal isolates ( $r^2=0.16$ ,  $Pr > F =0.04$ ). There was an expected lower coefficient of determination in this test than the first test. Unlike the isolates of the previously discussed assay, these fungal isolates were not evaluated for any particular mode of pathogenicity such as the degradation of host tissue from the production of lytic-enzymes or any other predisposing virulence factor. Thus, any isolate could express a wide range of efficacy to hydrilla. However, several isolates were significantly more effective than others (Figure 3).



**Figure 3. Effects of fungal isolate collected from hydrilla during 1987. Tukey's Test ( $P < 0.05$ ). Bars with the same letter are not significantly different**

Those isolates which showed potential were tested in greenhouse aquaria studies. They will also be taxinomically characterized.

#### **Isolate efficacy aquarium assay.**

None of the test isolates impacted hydrilla plants. There was no significant difference between the control and treatments. Even isolate 224 which had severely impacted hydrilla in a previous experiment did not disease hydrilla. Since these organisms were

not impacting hydrilla initially in the field, they may have been effective only in the test tube assay because of a limited nutrient source or some other unknown factor. However, in the case of 224, pathogenicity of organisms may be lost through repeated transfer of cultures. Given the lack of facility of the past, improper storage conditions may have rendered the isolate nonpathogenic. As described above, changes in pathogenicity of organisms such as *C. cladosporioides* are usually genetic. Thus, the isolates of 224 in the culture collection have been recultured to the point that they have become laboratory artifacts. The recent acquisition of a cryofreezer (Revco) should reduce the chance of such changes in stock cultures from occurring. Ultra-freezing of cultures reduces genetic mutations, and many fragments may be taken from a single isolate (Dhingra and Sinclair 1985).

## CONCLUSIONS

It was concluded that:

- a. Isolate 224 did not cause disease of hydrilla in any assay and was thus considered a laboratory artifact because of extensive reculturing of the organism and the improper storage facility.
- b. Isolate 224 did not have any toxic effects on the triploid white amur. From this study, isolate 224 was considered nontoxic to fish.
- c. Several isolates collected from hydrilla showed potential as biocontrol agents in test tube assays.
- d. None of the isolates tested in aquarium assays diseased hydrilla.

## FUTURE RESEARCH

From these studies, it has become evident that the pursuit of a plant pathogen to control hydrilla is a monumental task. Thus far, no isolate has been considered outstanding as a biocontrol agent. Several isolates now in culture will be tested in the greenhouse, and titer tests will be conducted to determine the minimum cfu counts for effective control. These isolates will be taxonomically characterized. A survey will continue in search of plant pathogens of hydrilla. An effort will also be made to reisolate 224 from hydrilla in the field.

The future of this project will be dependent on the persistence of all persons involved to conduct extensive surveys of hydrilla mats in both endogenous and exotic waters to collect potential pathogens. The basic principles outlined in the introduction should be the guide for determining whether or not an organism should be considered for biological control of hydrilla.

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# Aquatic Macrophyte Changes In Devils Lake, Oregon, and Keevies Lake, Washington, Following Stocking Of Triploid Grass Carp (*Ctenopharyngodon idella*)\*

by

Gilbert B. Pauley,\*\* G. L. Thomas,\*\* Scott A. Bonar,\*\* Steven L. Thiesfeld,\*\* and Jonathan Frodge\*\*

## INTRODUCTION

Aquatic weeds are a major management problem in many waters of the Pacific Northwest. One method of controlling aquatic weeds is with the use of grass carp (*Ctenopharyngodon idella*). Prior to the advent of induced triploidy, there was considerable concern by management agencies regarding the use of this fish (Pauley et al. 1985). Even though reliable methods of separating diploid and triploid fish exist (Bonar et al. 1985) and triploid fish are used by managers in many areas (Allen and Wattendorf 1987), the triploid fish did not reduce the controversy in some areas (Pauley et al. 1987). Although grass carp are not a panacea for aquatic macrophyte control, they appear to have their place in certain instances depending upon a variety of variables. They have been used with success to eradicate certain aquatic plants such as *Hydrilla verticillata* (Stocker and Hagstrom 1985). However, actual control of aquatic plants has met with varied success because of the individual management goals of different agencies and the varied local environmental conditions of widely separated geographic regions (Bonar, Thomas, and Pauley 1987). This fact has led to wide variations in the numbers of grass carp reported to effect macrophyte control (Bonar, Thomas, and Pauley 1987).

The purpose of this paper is to report the efficacy of triploid grass carp in controlling aquatic macrophytes in Devils Lake, Oregon, and Keevies Lake, Washington, in the first growing season for the plants following stocking of the fish.

## MATERIALS AND METHODS

The basic design of the studies in Devils Lake, Oregon, and Keevies Lake, Washington, have been previously described (Pauley et al. 1985, 1987). The method of estimating grass

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\*Funding for this research project was accomplished through the Washington Cooperative Fishery Research Unit by the Washington State Department of Ecology, the Washington State Department of Wildlife, the US Fish and Wildlife Service, the US Army Corps of Engineers, the US Environmental Protection Agency, the Devils Lake Water Improvement District, and the University of Washington.

\*\*The Washington Cooperative Fishery Research Unit, US Fish and Wildlife Service, School of Fisheries, University of Washington, Seattle, Washington. (The Unit is sponsored jointly by the US Fish and Wildlife Service, the Washington State Department of Fisheries, the Washington State Department of Wildlife, and the University of Washington.)

carp stocking rates has been noted by Bonar, Thomas, and Pauley (1987), and those stocking rates used in Devils Lake and Keevies Lake are listed in Table 1.

Table 1  
Grass Carp Stocking Rates in Devils Lake, Oregon, and Keevies Lake,  
Washington

<i>Test Site</i>	<i>Number of Fish</i>	<i>Stocking Rate (kg/ha)</i>	<i>Stocking Rate (kg/metric ton)</i>
Devils Lake	27,090	19.4	1.4
Keevies Lake (Area 1)	1,722	84.6	1.4
Keevies Lake (Area 2)	1,595	104.5	2.9

Macrophytes were sampled three times in each lake during 1986 and 1987 at bimonthly intervals. Macrophytes were sampled by a 0.25-m<sup>2</sup> quadrat sampler patterned after one designed by Pukerson and Davis (1975). The sampler was a plexiglass box with a hinged door and an opening on one side where a mesh onion sack is attached. The sampler was placed on the lake floor and the hinged door was opened. A scuba diver then used a knife or his hands to remove all plant material, including the roots, from inside the quadrat sampler. Each sample of plants was retained in a separate onion sack. The lakes were sampled by either the simple random or the stratified random method, depending on the correlation of biomass to depth within each lake and the ease of the sampling. On Devils Lake there were four control enclosure areas, measuring 25 by 25 ft. Shallow area sampling at depths shallower than 2.5 m was done on Devils Lake to give a closer comparison with the control enclosure areas than the lake as a whole would give to them.

A grid was superimposed over a map of each lake, and sampling sites within the grid were assigned using a random numbers table to determine the different grid squares within each area and strata. Pilot surveys were conducted at the time of maximum biomass in the first year of the study to determine the variance of plant biomass in each lake. This variance was then used to calculate a sample size to estimate the mean biomass per meter squared, with a confidence of 15-30 percent, depending on the variability of the plant biomass within each lake. These confidence values are accepted standards for estimating aquatic macrophyte biomass (Nichols 1984, Osborne 1984). Sample sizes for each lake have been determined from standard simple random and stratified random sample size equations presented in Cochran (1977). The sample sizes for each lake and the degree of estimation precision for mean wet biomass per square meter is presented in Table 2.

Once samples were collected they were stored in airtight bags and transported back to the laboratory where the bags were filled with water and put into a cold room at 6.0° C to preserve them. Samples were sorted by species, and wet weights were determined for each species. After wet weight determination was completed, samples were spun in a washing machine to a constant weight and then weighed to the more accurate fresh spun weight. After recording the weight, macrophyte samples were pressed between wire screens and dried for approximately 7 days on a rack with fans. Plants were then

Table 2  
 Sample Sizes Used to Estimate Aquatic Macrophyte Biomass in Test Sites Within a Certain Percentage (r) of the Population Mean with 95 Percent Confidence

<i>Test Site</i>	<i>Sample Size</i>	<i>r (Estimated)</i>	<i>r (Obtained)</i>
Devils Lake	90	0.15	0.17
Keevies Lake (Control Area)		0.20	0.26
Strata 1	10		
Strata 2	5		
Keevies Lake (Area 1)		0.20	0.27
Strata 1	10		
Strata 2	5		
Keevies Lake (Area 2)		0.25	0.42
Strata 1	10		
Strata 2	5		

weighed on an analytical balance to obtain the dry weight. All macrophytes were then placed in paper sacks and tagged for additional chemical analysis.

## RESULTS

The macrophyte levels in both Devils Lake, Oregon, and Keevies Lake, Washington, changed between 1986 and 1987, following the stocking of triploid grass carp in both lakes. Peak biomass in both lakes occurred between July and September. The overall biomass in both lakes increased with a shift in the dominant plants in both lakes.

In Devils Lake, both the biomass and percent of Brazilian waterweed (*Elodea densa*) increased dramatically (Figures 1 and 2). Although the percent of coontail (*Ceratophyllum demersum*) was increased between the 2 years only in September (Figure 1), the biomass of this plant showed an overall increase (Figure 2). Watermilfoil (*Myriophyllum spicatum*) and Canadian waterweed (*Elodea canadensis*) showed percentage decreases (Figure 1) and biomass decreases (Figure 2). The shallow regions of less than 2.5 m in Devils Lake (Figures 3 and 4) and the enclosure control areas (Figures 5 and 6) in the lake both exhibited macrophyte relationships similar to those found in the lake as a whole in 1987. However, the control enclosure areas did not have the precise relationship of plants exhibited by the shallow areas and the entire lake

The control area of Keevies Lake had very similar relationships in 1986 and 1987 for floating-leafed pondweed (*Potamogeton natans*), watershield (*Brasenia schreberi*), and bladderwort (*Utricularia vulgaris*) for both percentage of plants (Figure 7) and plant biomass (Figure 8). Treatment area 2 (highest stocking rate) in Keevies Lake had a definite increase in *B. schreberi* in 1987 for both percentage (Figure 9) and biomass (Figure 10). *P. natans* in this treatment area exhibited some decrease in both percentage (Figure 9) and biomass (Figure 10). Overall biomass in treatment area 2 remained about the same, but the peak occurred later in the year in 1987 (Figure 10). Treatment area 1 (lowest stocking rate) in Keevies Lake showed a pronounced increase in both the percentage (Figure 11) and the biomass (Figure 12) of *B. schreberi* as well as an increase in the total biomass (Figure 12). *P. natans* exhibited a noticeable decline between years in both percentage (Figure 11) and biomass (Figure 12).

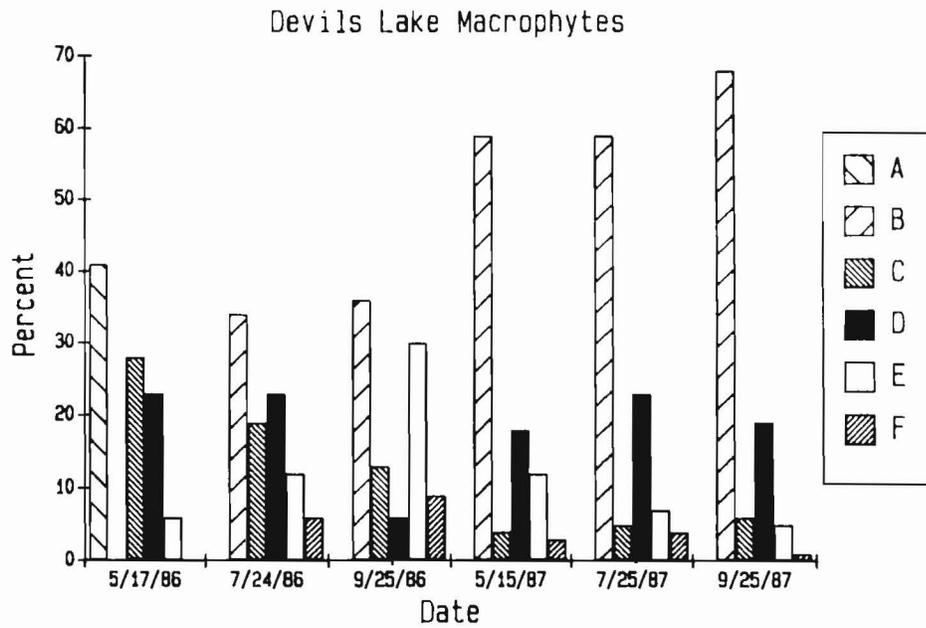


Figure 1. Devils Lake macrophyte percent in the entire lake in 1986 and 1987: A = *Elodea* spp., B = *E. densa*, C = *M. spicatum*, D = *C. demersum*, E = miscellaneous plants, F = *E. canadensis*

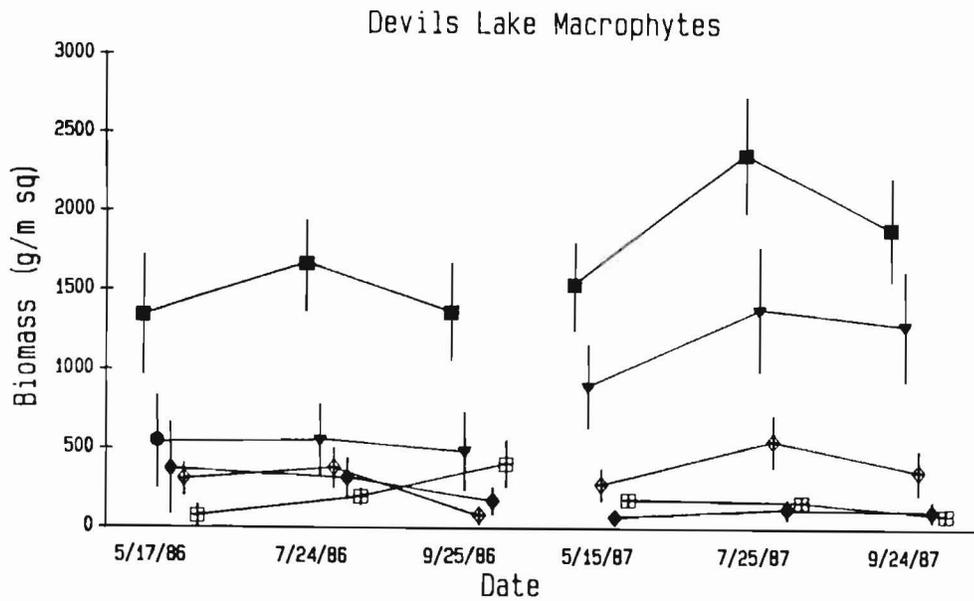


Figure 2. Devils Lake macrophyte biomass in the entire lake in 1986 and 1987: ■ = total biomass, ▼ = *E. densa*, ◆ = *Elodea* spp., ◇ = *C. demersum*, ◊ = *M. spicatum*, ◻ = miscellaneous plants

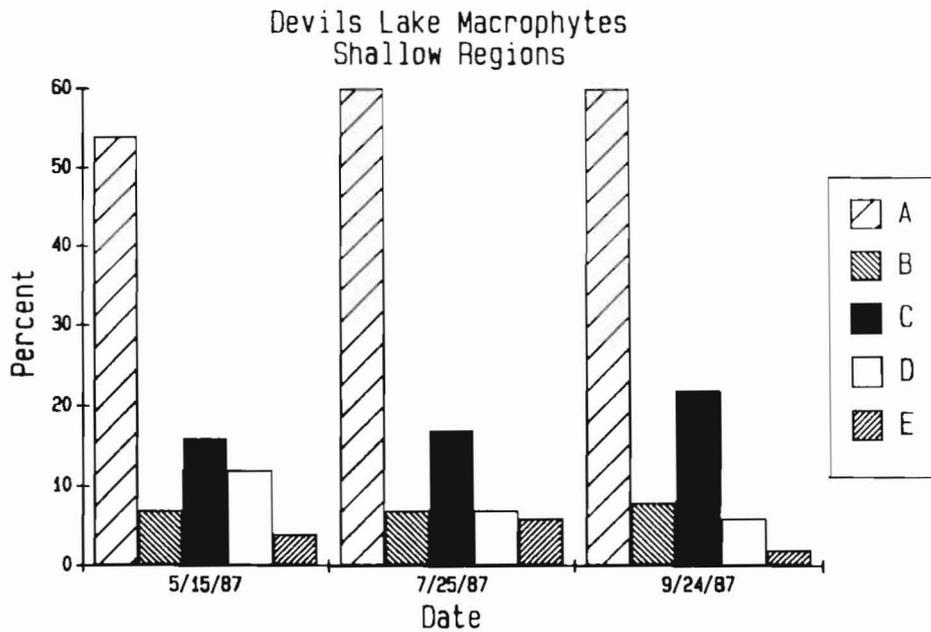


Figure 3. Devils Lake macrophyte percent in the shallow regions of the lake that are less than 2.5 m in 1987: A = *E. densa*, B = *M. spicatum*, C = *C. demersum*, D = miscellaneous plants, E = *E. canadensis*

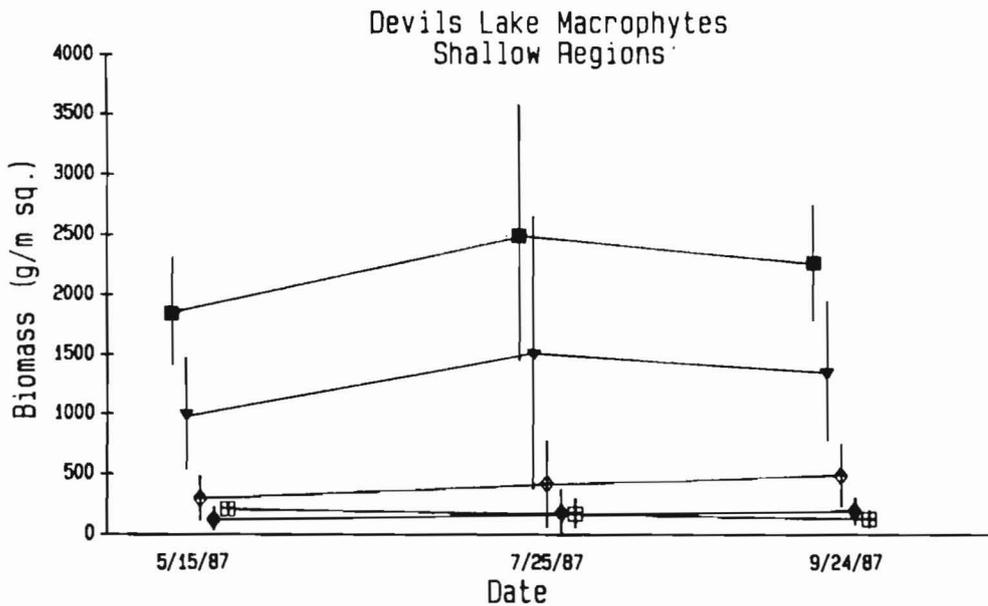


Figure 4. Devils Lake macrophyte biomass in the shallow regions of the lake that are less than 2.5 m in 1987: ■ = total biomass, ▼ = *E. densa*, ◆ = *C. demersum*, ◆ = *M. spicatum*, ⊕ = miscellaneous plants

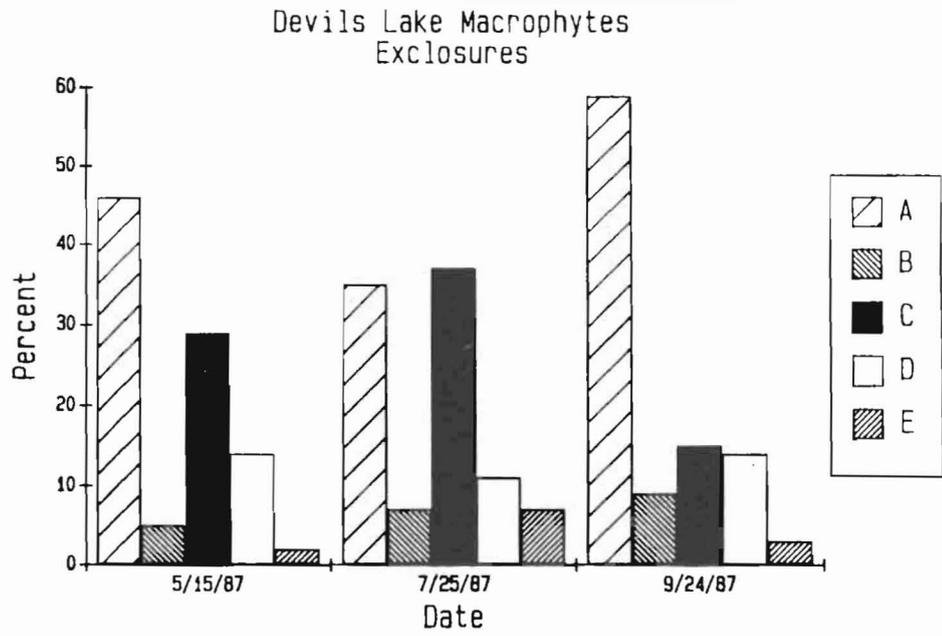


Figure 5. Devils Lake percent macrophytes in control exclosure areas in 1987: A = *E. densa*, B = *M. spicatum*, C = *C. demersum*, D = miscellaneous plants, E = *E. canadensis*

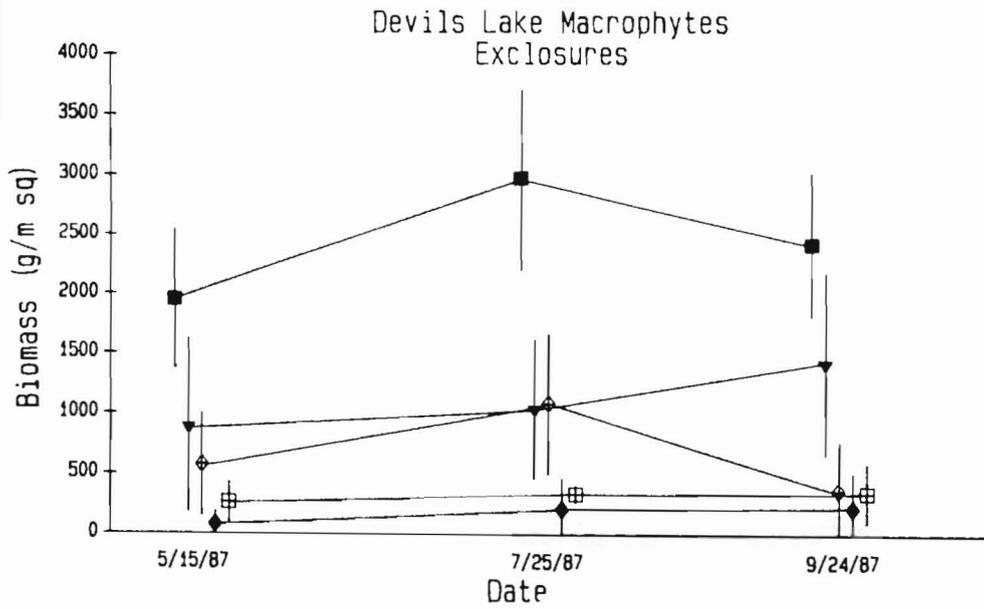


Figure 6. Devils Lake macrophyte biomass in control exclosure areas in 1987: ■ = total biomass, ▼ = *E. densa*, ◆ = *C. demersum*, ◆ = *M. spicatum*, □ = miscellaneous plants

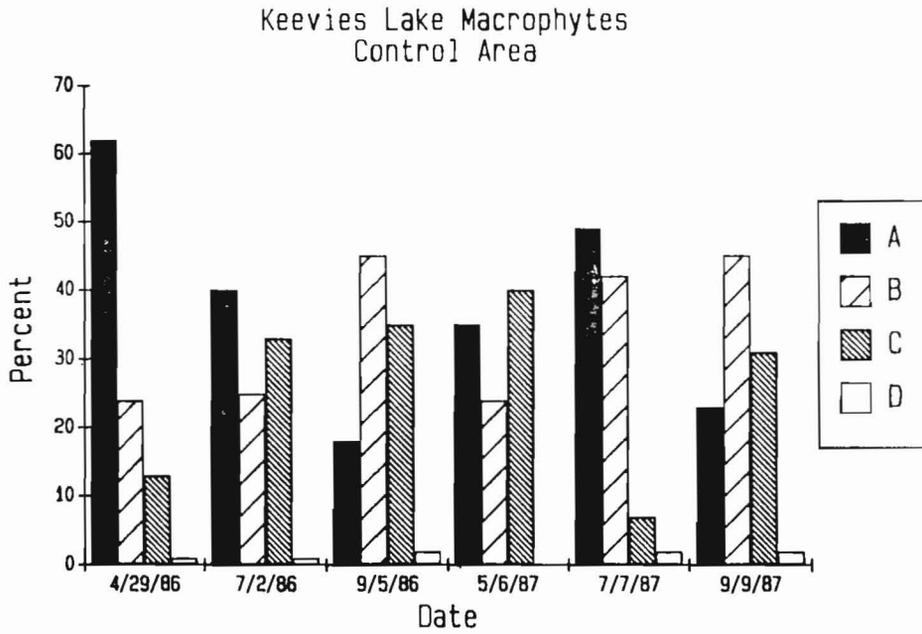


Figure 7. Keevies Lake macrophyte percent in control area in 1986 and 1987: A = miscellaneous plants, B = *P. natans*, C = *B. schreberi*, D = *U. vulgaris*

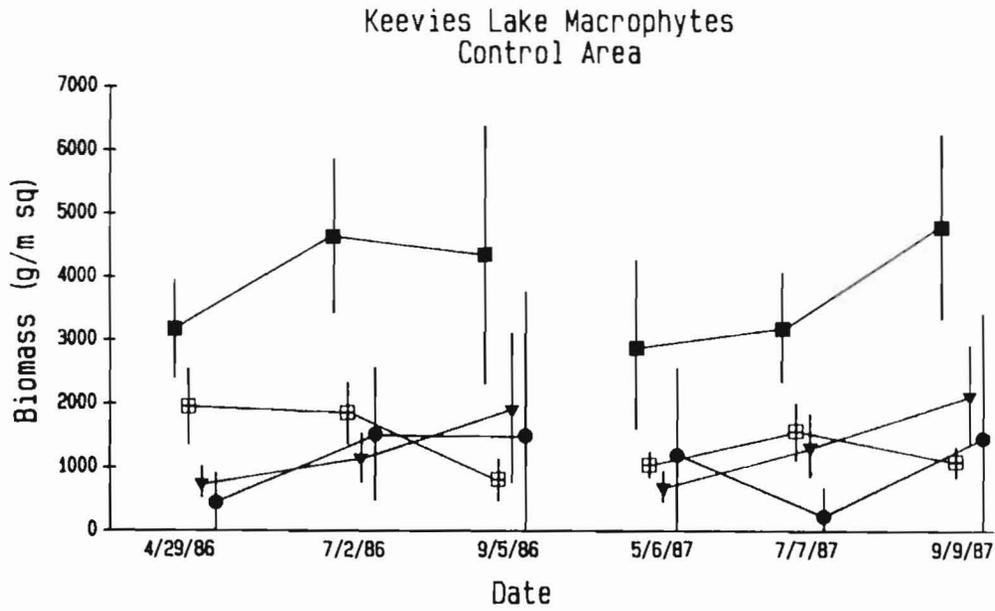


Figure 8. Keevies Lake macrophyte biomass in control area in 1986 and 1987: ■ = total biomass, ▼ = *P. natans*, ● = *B. schreberi*, ◻ = miscellaneous plants

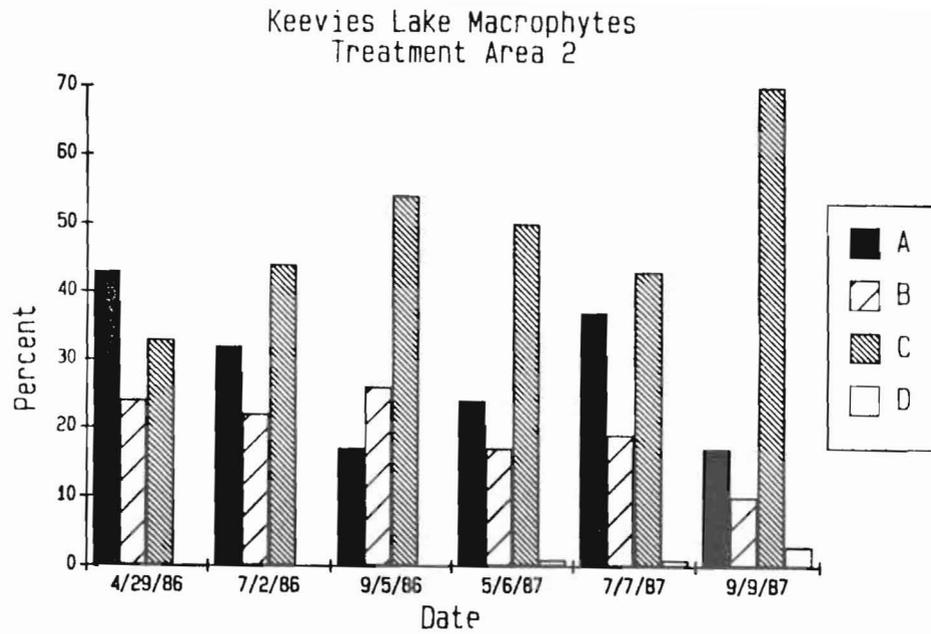


Figure 9. Keevies Lake macrophyte percent in the low stocking treatment area 2 in 1986 and 1987: A = miscellaneous plants, B = *P. natans*, C = *B. schreberi*, D = *U. vulgaris*

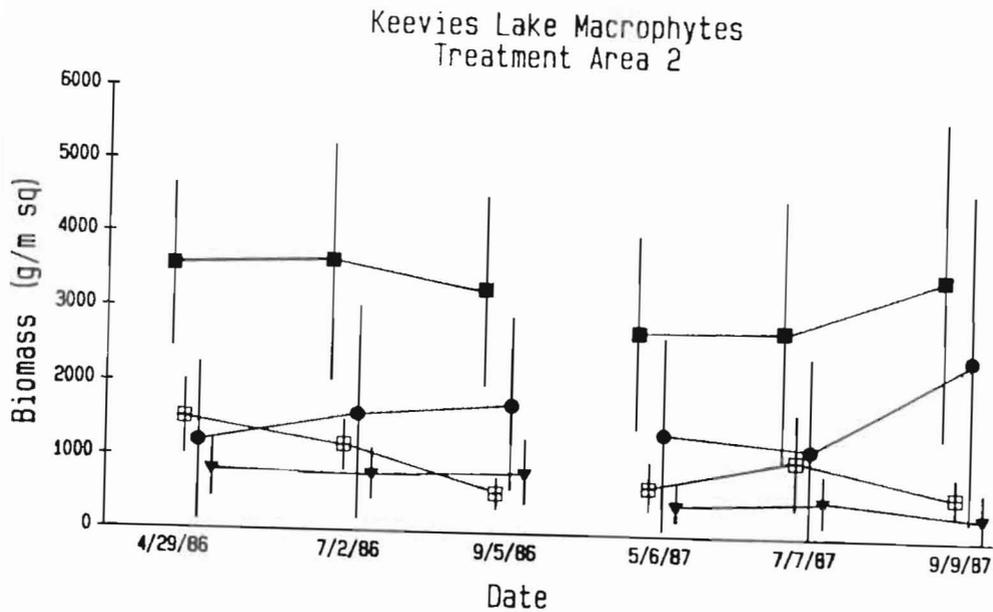


Figure 10. Keevies Lake macrophyte biomass in the low stocking treatment area in 1986 and 1987: ■ = total biomass, ▼ = *P. natans*, ◆ = *B. schreberi*, ⊠ = miscellaneous plants

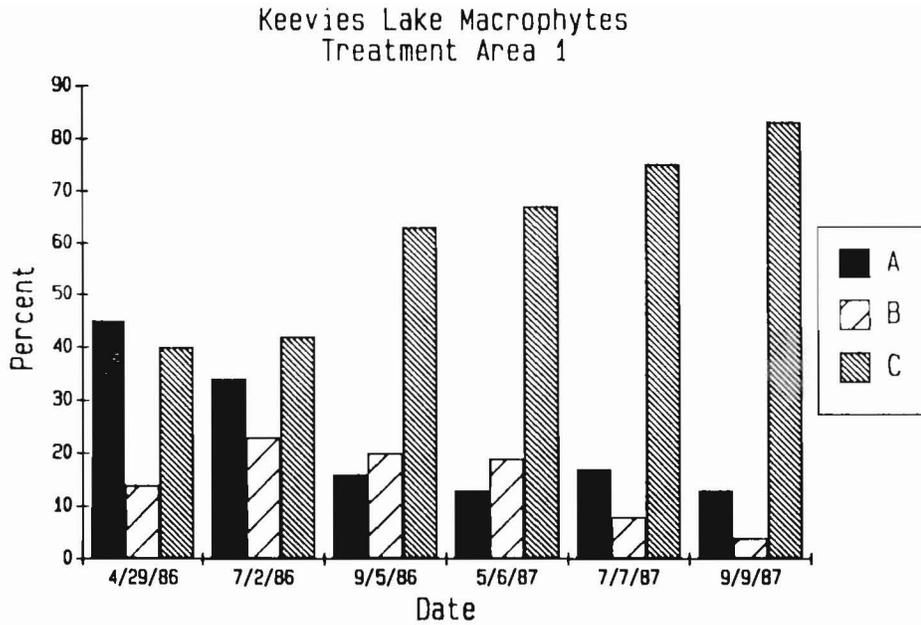


Figure 11. Keevies Lake macrophyte percent in the high stocking treatment area 1 in 1986 and 1987: A = miscellaneous plants, B = *P. natans*, C = *B. schreberi*

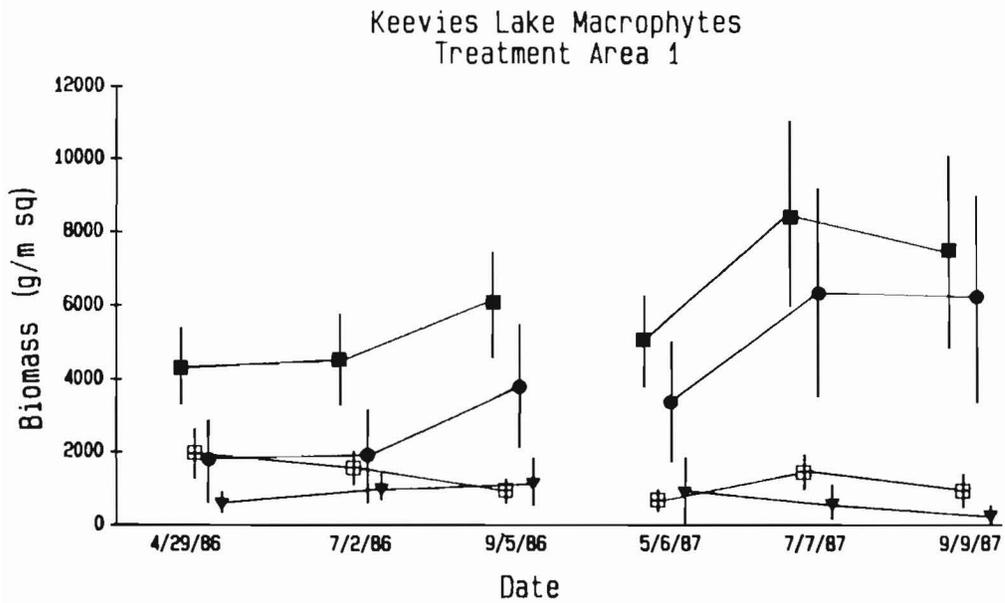


Figure 12. Keevies Lake macrophyte biomass in the high stocking treatment area in 1986 and 1987: ■ = total biomass, ▼ = *P. natans*, ◆ = *B. schreberi*, □ = miscellaneous plants

## DISCUSSION

Initial examination of the changes in aquatic macrophytes between 1986 and 1987 in Devils Lake indicated that these plants have been altered in a fashion that is predicted by the feeding preference studies performed by Bowers, Pauley and Thomas (1987), which indicated that the nonpreferred *E. densa* should flourish if other more preferred plants are eaten by the grass carp. This change has occurred in Devils Lake. The highly preferred *E. canadensis* should be reduced to levels lower than normal, which has occurred in that lake. The feeding preference work also predicts that the closely preferred *M. spicatum* and *C. demersum* should decrease and have the *C. demersum* increase or stay the same, which has occurred in Devils Lake. It is unfortunate that the control exclosures were not in place in 1986 and therefore could not be sampled for comparison to 1987. The shallow region test areas were designed to be more comparable to the controls than was the entire lake. The shallow regions resembled very closely the lake as a whole, while the control exclosures differed from both of them only somewhat subtly at the July and September samplings. As a result, it is possible that the changes in aquatic macrophytes seen in Devils Lake may have been just natural yearly fluctuations and variations, without any relationship to the grass carp. Another possibility exists in that both preferential feedings occurred on the plants to some degree and that feeding was observed in combination with natural yearly plant fluctuations. The latter hypothesis appears most probable to us, but sampling in subsequent years is needed to be certain. Stocking rates in Devils Lake were on the conservative side (Bonar, Thomas, and Pauley 1987), which also supports the hypothesis that the effects were the result of both grass carp impact and a yearly fluctuation in the plants, rather than an effect caused entirely by the grass carp.

The control area in Keevies Lake indicates that little change has taken place in that section of the lake. However, there was a progressive change that occurred in both the lower stocking rate treatment in area 1 and in the higher stocking rate treatment in area 2. The changes observed in areas 1 and 2 were most likely due to the feeding of the grass carp planted in those two sections. The changes are similar, but they were more accentuated in area 1 than in area 2. The changes seen were in keeping with what is expected from the preferential feeding work of Bowers, Pauley, and Thomas (1987). In both areas 1 and 2, the preferred *P. natans* was consumed by the grass carp and shows up in reduced amounts in 1987. The less preferred *B. schreberi* remained unconsumed or was eaten in minuscule quantities and thereby was allowed to increase in both percent and biomass. The preferential plant changes observed in our experiments commonly have been observed in many situations where grass carp have been stocked in lakes (Mitzner 1978; Mitchell 1980; Van Dyke, Leslie, and Nall 1984; Fowler 1985). The results in Keeview Lake demonstrate that both the grass carp stocking rate and the grass carp feeding preference exert an effect upon the subsequent plant composition of a body of water.

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# Influence of Herbicides on Weevils Used as Biocontrol of Waterhyacinth

by  
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## INTRODUCTION

Even though some problems with aquatic plants existed in previous centuries, the main invasion and spread of nuisance aquatic plants in lakes and navigation systems worldwide have occurred this century. Waterhyacinth, *Eichhornia crassipes* (Martius) Solms-Laubach, one of the world's leading nuisance aquatic plants, is native to Brazil (Godfrey and Wooten 1979, Penfound and Earle 1948). A member of the Pontederiaceae family, this free-floating, mat-forming perennial now infests nearly all tropical and sub-tropical regions of the world (Holm et al. 1977, Little 1965, Penfound and Earle 1948). This aquatic plant has caused extensive problems in the United States.

Its introduction into the United States is linked to the Cotton Centennial Exposition in New Orleans in 1884, although Penfound and Earle (1948) mentioned evidence of waterhyacinth cultivation as a greenhouse and landscape exotic following the Civil War. By 1897, waterhyacinth infestations in the United States created serious problems in navigable waters of the south (Webber 1897, Zeiger 1962). Today it is found in the southern United States from South Carolina to Texas and in California (Sanders, Theriot, and Perfetti 1985).

The many detrimental effects of waterhyacinth on aquatic systems along with its estimated maximum relative growth rate of 5-6 percent per day (Center and Spencer 1981) suggests need for effective control. The US Army Corps of Engineers has been involved in waterhyacinth control in navigable waters since before 1897 (Zeiger 1962). The first attempt at control was through the use of log barriers to prevent downstream movement (Zeiger 1962) and a crusher boat to physically remove the mats (Sanders, Theriot, and Perfetti 1985; Wunderlich 1962). Unfortunately, the lack of knowledge of waterhyacinth growth patterns and optimal harvesting times together with the slow progress of the boat resulted in little impact to the infestation (Wunderlich 1962).

The predominant method for waterhyacinth control since 1950 has been the use of chemical herbicides, especially 2, 4-D (2, 4-dichlorophenoxyacetic acid) (Sanders, Theriot, and Perfetti 1985). The seasonal reapplication requirements of herbicides make them expensive to use and effective only as short-term management agents. Herbicides employed today mainly include 2, 4-D, diquat, and glyphosate (Haag 1986b).

These herbicides are believed to be relatively safe in the concentrations used for waterhyacinth control, although the safety factor remains questionable. Among possible side effects are oxygen depletions and resulting fish kills caused by rapid decomposition

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to massive amount of plant material (Patton and Starnes 1970) and possible pollution of ground-water supplies (Haag 1986a).

The use of biological control offered a possible long-term, low-cost remedy to the waterhyacinth management problem (Perkins 1973a). In the 1960's, the US Department of Agriculture (USDA) began investigating South American insects that feed on waterhyacinth as potential biocontrol agents (Bennett and Zwolfer 1968, DeLoach and Cordo 1976a, Perkins 1974). This research, funded by the Corps of Engineers, led to the release of three exotic insects including two species of waterhyacinth weevils, *Neochetina eichhorniae* (Warner 1970) and *N. bruchi* (Hustache 1928), Coleoptera: Curculionidae and the waterhyacinth moth, *Sameodes albiguttalis* (Warren), Lepidoptera: Pyralidae (Center 1982; Center and Durden 1981; Center, Durden, and Corman 1984; Perkins 1973b; Perkins and Maddox 1976; Sanders, Theriot, and Perfetti 1985).

These waterhyacinth weevils are semiaquatic, holometabolous members of the tribe Bagoini which undergo development in and feed on various parts of the waterhyacinth plant almost exclusively (DeLoach 1975, 1976; DeLoach and Cordo 1976a, b; Perkins and Maddox 1976). Weevil damage to individual plants and the effects of the damage on waterhyacinth populations have been well documented (Cofrancesco, Stewart, and Sanders 1985; DeLoach and Cordo 1983; Forno 1981; Goyer and Stark 1984; Perkins 1974; Sanders, Theriot, and Perfetti 1985).

Typically, biocontrol of aquatic weeds results in a gradual decline of the target species, which frequently is not rapid enough for heavily used waterways (Center, Steward, and Bruner 1982). In high-access areas rapid reduction of waterhyacinth infestations is achieved through the use of herbicide application; however, the weevil population is reduced because of the abrupt loss of immature and nonmigrating individuals, habitat (Haag 1986a), and food source (Center, Steward, and Bruner 1982). Effective biological control results from a dynamic equilibrium between target weed and biocontrol agent (Wright and Center 1984). Population declines of the target weed and its biocontrol agent caused by repeated chemical application precludes reestablishment of the dynamic equilibrium because the population regrowth of the target weed grossly exceeds that of its agent (Center, Steward, and Bruner 1982).

Effective, low-cost, long-term, safe waterhyacinth control is still the management ideal for many state and federal agencies. In an attempt to achieve these goals, research is being directed toward the integrated use of herbicides and biocontrol agents.

In addition to the aforementioned impact of herbicides on weevil populations, there may exist other less conspicuous repercussions. In a series of integrated pest management studies, Haag (1986a, b) found that the waterhyacinth weevils do not die as a direct result of exposure to 2,4-D, diquat, glyphosate, or their surfactants and that adult weevils migrate away from declining, sprayed plants toward healthy waterhyacinth plants. Although direct exposure to these herbicides is not lethal to adult weevils, the effects on the reproductive abilities of adults ingesting treated plant material and the population size of subsequent generations have not been thoroughly examined.

Buckingham and Passoa (1984) and Haag (1986b) found evidence that the use of herbicides on waterhyacinth may directly or indirectly affect flight muscle development in *Neochetina*. Haag proposed that an environmental cue like herbicide exposure or a

decline in plant quality (as seen following herbicide application) may trigger flight muscle generation.

Research on waterhyacinth control indicates that more information is needed on the interactions that occur between chemical and biological control techniques. Understanding these interactions will allow more efficient utilization of resources and a reduction in cost.

## PURPOSE AND OBJECTIVES

The overall purpose of this study is to develop management strategies that will utilize chemical and biological agents to gain rapid control of waterhyacinth with long-term effects. The objectives are to:

- Reaffirm that the weevils migrate over short distances from herbicide-treated plants to untreated plants.
- Examine the toxic effects of the herbicides and compare the reproductive capabilities of insects which have been subjected to herbicide applications with control populations.

## APPROACH

### Study I

Since migration of weevils over short distances was observed both in the field and in aquaria (Haag 1986b), it is necessary to substantiate whether similar behavioral responses are attainable under the conditions of this study. Specific points of this study are to determine the following:

- a. Whether the waterhyacinth weevils (*Neochetina eichhorniae*) feed on plants treated with 2,4-D or diquat when offered healthy, unsprayed plants.
- b. Whether the waterhyacinth weevils will migrate to clean plants.
- c. The amount of time required for the weevils to migrate and begin feeding on clean (unsprayed, weevil-free) plants.
- d. The number of weevils that successfully migrate to the clean plants.

### Study II

Study II will examine the direct impact of herbicides on the weevils and their reproductive capabilities. Specific points of this study are to:

- a. Determine if there is a lethal impact to the weevils from the herbicide.
- b. Determine if adult *Neochetina eichhorniae* exposed to herbicides produce the same number of eggs as unexposed adults.
- c. Determine if the progeny of exposed and unexposed conspecific adults are equally capable of eclosion.

## MATERIALS AND METHODS

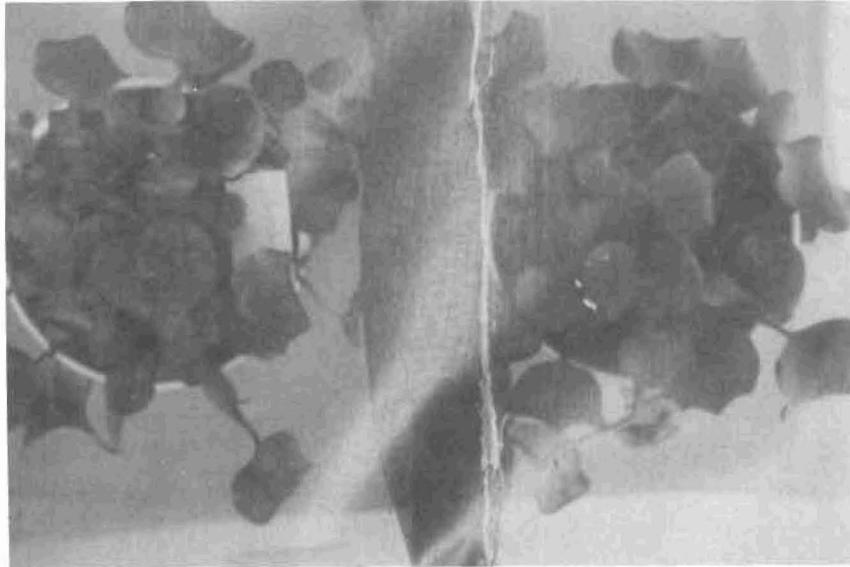
### Study I

Twenty-four large nalagene tanks were utilized in the insect movement studies (Figure 1), twelve as treatments and twelve as controls. Inside each large nalagene tank two smaller containers of plants were placed. Prior to placement into the large nalagene tank, one of the smaller containers received 10 male and 10 female weevils. The plant container receiving the insects were then subjected to herbicide spray (2,4-D) or distilled water spray (control). The second container of plants to be introduced into the large nalagene tank was healthy, untreated waterhyacinth plants that the insects could move towards. Destructive sampling was conducted on days 1, 3, 5, and 7 after treatment. Six large nalagene tanks were selected, three treated and three controls on each of the 4 sampling days, and the movement of insects was recorded. This test was replicated three times.



**Figure 1. Large nalagene tank that were used for insect movement study**

The above movement study was modified in order to allow the experiment to continue for a longer duration and evaluate two herbicides. The major difference was that destructive sampling was not conducted. The two small containers placed inside the large nalagene tank were separated by a screen (Figure 2). This screen prevented the insects from moving to the new food source. Instead a healthy trap plant was placed on the side of the tank having the insects. This plant was replaced each day, and the adults present were recorded. The experiment was allowed to run for 18 days and blocked three times. One tank of diquat with the X-77 surfactant, one tank of 2,4-D with the sidekick surfactant, and one control were evaluated each of the three times.



**Figure 2. A screen was utilized to separate the treated and the untreated plants in the large nalagene tanks used in the movement study**

## **Study II**

Insect mortality studies were conducted to evaluate the impact of herbicides and their surfactants on weevils. The herbicides utilized were diquat with the surfactant X-77 and 2,4-D with the surfactant sidekick. The herbicide was mixed according to label instructions, and 20 male and 20 female weevils were treated with 50 ml of herbicide. A similar number of controls were treated with distilled water. All weevils were maintained at 24°C for 48 hr, and insect mortality was monitored at 24 and 48 hr.

The impact herbicides have on weevil reproduction was examined in a preliminary study exposing pairs of male and female weevils to normal herbicide concentrations and maintaining them for 5 days on herbicide treated plants. The number of eggs and larvae were compared to controls. All containers having the insects were placed in an environmental chamber at 24° C.

## **RESULTS**

### **Study I**

Generally, female weevils moved first in all replicates. Insect movement in the initial study is depicted in Figure 3. On day 5, there was a significantly higher movement of insects from the 2,4-D treated plants than from the control plants. However, after day 7, 35 percent of the weevils were still present on the 2,4-D treated plants.

Results of modified insect movement study are depicted in Figure 4. The insects exposed to the diquat treated plants moved first. The movement trends for insect exposed to the 2,4-D treated plants are similar to the movements observed in the initial test; at 4-5 days there is significantly higher movement of these insects than in the controls. Low movement levels were recorded for the insects exposed to plants treated with distilled

**NEOCHETINA EICHHORNIAE  
MOVEMENT IN RESPONSE TO 2,4-D**

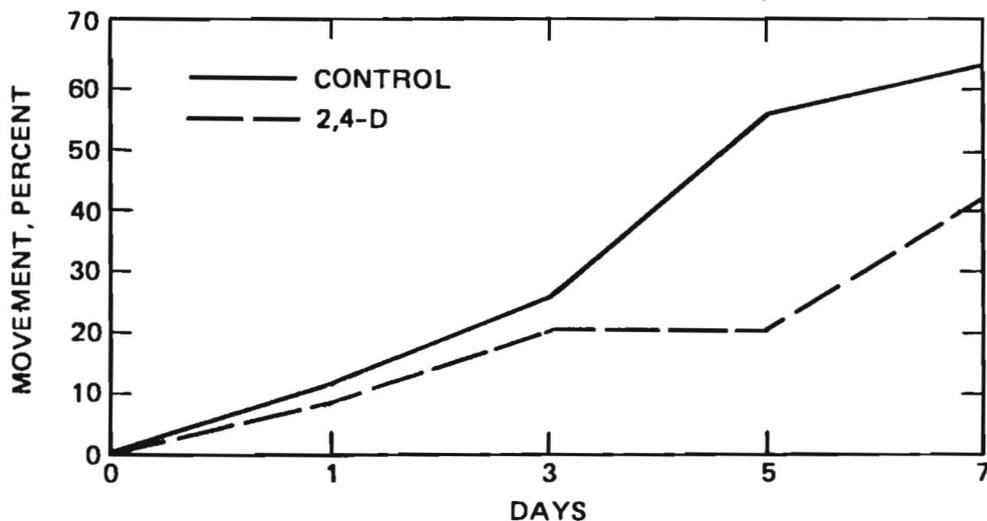


Figure 3. The movement of insects exposed to 2,4-D

**NEOCHETINA EICHHORNIAE MOVEMENT**

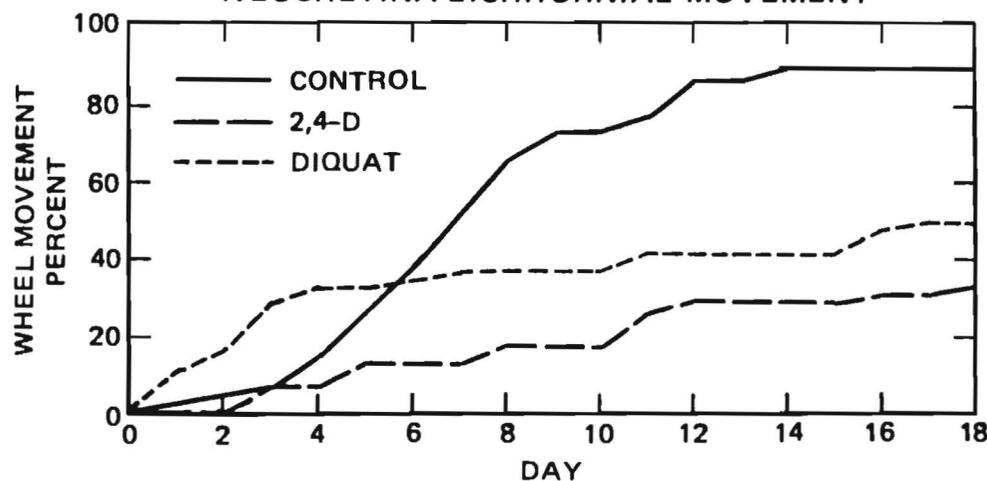


Figure 4. The movement of insects exposed to 2,4-D and Diquat

water (controls). The diquat exposed insects never exceeded a 50 percent level of movement. The physical condition of the plants was the most apparent change. After just 3 days, the diquat treated plants were brown and dry (Figure 5a), the 2,4-D treated plants were showing signs of geniculation (Figure 5b), while the controls were healthy and green (Figure 5c). At the completion of day 18, the large nalagene tanks and their contents were broken down and examined. During the examination, it was found that there was almost a 45 percent mortality in the insects exposed to diquat. The insects exposed to 2,4-D and the controls had low mortality.



**Figure 5. (a) Plant condition three days after exposure to diquat.  
(b) Plant condition three days after exposure to 2,4-D.  
(c) Plant condition three days after exposure to a spray of water  
(control)**

## Study II

Insect mortality studies were conducted to evaluate the impact of diquat with the surfactant X-77 and 2,4-D with the surfactant sidekick. Insect mortality was significantly higher than the controls for diquat at 24 and 48 hr (Figures 6 and 7). At 48 hr, the insects treated with diquat had a 85 percent mortality as compared to 1.7 percent for the controls and 6.7 percent for the 2,4-D treated weevils.

The tests on reproduction were restricted to the herbicide 2,4-D because of the high mortality with diquat. The number of eggs varied significantly within controls and treated-weevils replicates. Weevils that were treated with 2,4-D did lay eggs, and the eggs hatched and larvae were produced.

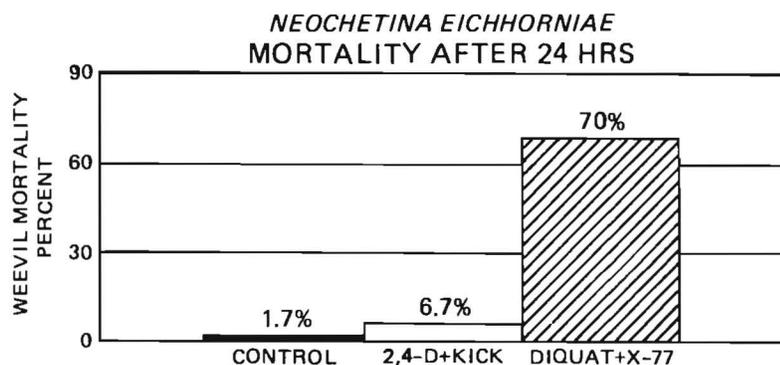


Figure 6. *Neochetina eichhorniae* mortality after 24 hr

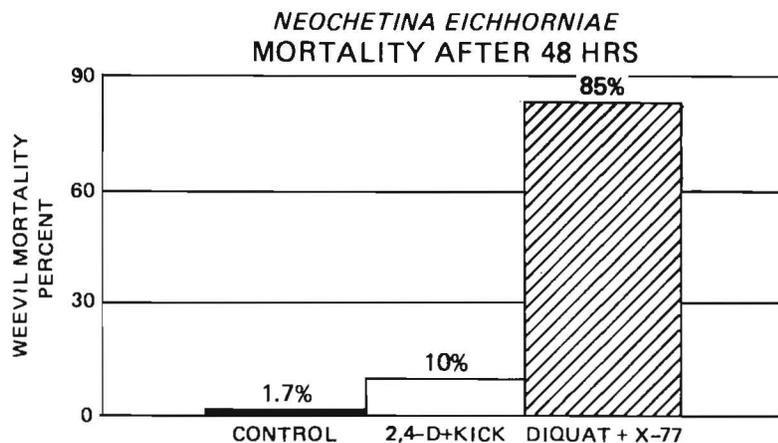


Figure 7. *Neochetina eichhorniae* mortality after 48 hr

## DISCUSSION

The movement of insects away from the plants treated with herbicides is pronounced. Plant condition varied greatly between the herbicides even for the same exposure time (Figures 5a, b, c). The movement, however, appears to be linked to a food quality in the 2,4-D treated plants rather than the presence of the herbicides directly. The ability of the weevils to move off plants treated with the herbicide 2,4-D and still produce first instar larvae indicates that integrated control procedures are possible. In addition, the high mortality exhibited by the weevils exposed to diquat and the surfactant X-77 indicates that further studies are necessary to determine if the toxicity was caused by the herbicide or the surfactant and if other herbicides and their surfactants have the same impact.

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# Field Study for the Verification of the Model, INSECT

by  
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## INTRODUCTION

A model is a representation of some portion of "reality" and is typically portrayed by mathematical algorithms. Models consist of various subcomponents called submodules which represent important aspects of the system being modeled. For example, in biological models submodules may be developmental rates, reproduction, mortality, etc. Submodules are connected to each other by pathways that represent actual relationships between the submodules in the system being modeled. These submodules and their associated interconnections actually allow the model to function. Since models represent some aspect of "reality," they must adequately simulate that portion of the world being modeled (Streifer 1974). Hence, it is important to the development of a model that it be compared with data collected under actual field conditions. Biological models are frequently compared to actual data to determine if the model fits the real world situation (Whisler et al. 1986; Bartlett and Murray 1986; Witz et al. 1985; Oddson and Aggarwal 1985; Fye, Reddy, and Baker 1984).

Verification becomes especially important when one considers that the majority of the algorithms used in the submodules comprising biological models are developed from laboratory experiments where environmental conditions are held constant and the experiment is repeated under several different environmental regimes. This procedure is in contrast to what is found in the field where environmental conditions typically fluctuate widely. Even more important is the consideration that biological models typically perform differently in various geographical regions. Such changes in behavior can be caused by variations in weather patterns, differences in plant and animal strains, and/or a combination of several factors.

The present paper describes how field data are being collected that will eventually be used for verification of the model, INSECT, under southern Texas conditions. Since this research is currently being conducted, I will not discuss the final comparison to model output but will leave that for next year's proceedings. Similar verification and actual comparisons to model output have been accomplished under both north and south Florida conditions\*\* and are discussed in the article by Howell, Akbay and Stewart in these proceedings.

Presently, INSECT is a first generation biological model that describes the interaction between waterhyacinth, *Eichhornia crassipes* (Mart.) Solms., and two species of

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\*\*Personal Communication September 1987, Fred Howell, University of Southern Mississippi, Hattiesburg, Mississippi.

*Neochetina* sp. (i.e., *N. eichhorniae* Warner and *N. bruchi* Hustache). INSECT simulates plant growth, insect development, and plant/insect interactions, specifically the insect's impact to the plant. Planned uses of this model include development of biological concepts concerning insect/plant interactions and as an evaluation tool for biological control (Akbay, Wooten, and Howell 1988).

## STUDY SITE

The study site used for the collection of verification data is located in the US Army Engineer facility at Wallisville, Texas. Wallisville is located ca. 40 miles east of Houston, Texas, directly south of Interstate 10. Plant and insect sampling is being accomplished in an ca. 5-acre borrow pit within the Wallisville facility.

This particular study site was chosen for several reasons. Firstly, this general area was an original release site for *N. bruchi*. Releases were made in October 1980 (50 adults), April 1981 (50 adults), and August 1981 (150 adults). Coinciding with the releases was a quarterly insect sampling of the release site beginning in June 1980 and continuing through June 1983. Hence, information is available that describes general insect population changes which can be used as baseline data for our present sampling effort. This study also represents continued assistance to the Galveston District. In addition, no complete insect/plant population data are available in this region of Texas that is coupled with accurate onsite weather data.

## CURRENT STATUS

The project began in June 1987 with the installation of remote weather data collection equipment and initial plant and insect sampling. Biological sampling is currently scheduled to continue monthly through the end of FY 1988.

Weather data are being collected via a Campbell Scientific, model CR-21X data logger system. Data are collected once per minute, averaged hourly, and downloaded to cassette tape. The tape is later retrieved, and the data uploaded onto a IBM-AT computer at the Waterways Experiment Station facility. Weather data currently being collected include relative humidity, solar radiation, wind speed and direction, precipitation, and air temperature. In addition, three different water temperatures are being monitored including surface, 1 ft from the bottom, and the bottom water temperature.

The biological information includes both plant and insect data. All samples are taken from two 1/4-m<sup>2</sup> samples taken adjacent to one another. One of the 1/4-m<sup>2</sup> samples is used for plant measurements while the other is for insect counts. Differences in plant height within the mat prompted the stratification of the sampling effort to reduce typically high variation normally associated with biological sampling. Toward this goal three distinct areas were delineated within the mat based primarily on plant height; i.e., area 1 containing very small, stunted plants, area 2 having medium height plants, and area 3 containing the tallest plants. These differences are presumably due to variations in plant nutrient status and/or insect population differences. Sampling within the mat is done by randomly throwing the sampling square onto the mat and removing only those plants that are >50 percent within the sampling square. A similar sample is taken adjacent to

the first sampling. Three such sampling pairs are made for each study area giving a total of nine sample pairs for the entire site.

Plant measurements deal almost exclusively with biomass determinations partitioning the total biomass between living and dead material. For example, total wet weight of the 1/4-m<sup>2</sup> plant material is recorded. Number of living and dead leaves are quantified for each plant and subsequently totaled for the entire sample. Determination of living leaves is a subjective determination based primarily on the amount of green material remaining. If the leaf has > 50 percent green material remaining, it is classified as living. The living material above the water, below the water, and all dead material is then weighed separately. This measurement gives total biomass, living biomass above the waterline, living biomass below the waterline, and total dead biomass. In addition to plant biomass data, numbers of plants per 1/4-m<sup>2</sup>, or density, are quantified. This information is subsequently converted to dry weight (multiplication by 0.05) on a per meter basis for model input.

Insect data consist of numbers of individuals for each life stage (i.e., larvae, pupae, and adults). Numbers of each life stage are found by carefully examining each plant in the sample. The numbers are then summed over the entire sample and subsequently converted to a per meter basis. Species determinations of larval and pupal stages cannot be done with any accuracy and hence are not made. However, adults are fixed in alcohol and brought back to the laboratory where accurate species ID's can be ascertained.

## PLANNED EXPANSION

Currently, there are certain areas of this study that will be added or expanded such as the determination of physiological age of female weevils based primarily on ovarian condition. Such age-grading can greatly help in determining a more accurate portrait of the population's age structure as well as the quantification of the proportion of individuals that have laid eggs previously. This information can aid in the initialization procedures of the model, check for accuracy, and may aid plant managers in the timing of chemical applications relative to the reproductive status of the insects. In addition, water quality samples are being collected and analyzed for such parameters as pH, dissolved oxygen, redox potential, conductivity, nitrates, nitrites, ammonia, as well as essential minerals such as calcium, potassium, and phosphorus. Nutritional quality of the plant tissues may eventually be quantified as well.

Longer-term expansion projects may include using the site for validation of various submodules within the model including insect development, reproductive rates, effects of nutritional changes in the plants in relation to reproductive condition, etc. Such expansion projects will aid in delineating concepts concerning the insect's biology and plant/insect interactions as well as the derivation of more accurate parameter estimates currently used in INSECT. Lastly, modification of ambient air temperatures by the plant canopy, plant tissues, and water may be characterized under field conditions using data collection units. With such data sets ambient or water temperatures can be modified using regression techniques so that more accurate temperatures can be used to drive the model.

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# Quarantine Research Program—Hydrilla Insects

by  
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## INTRODUCTION

The hydrilla insect quarantine program is conducted at the Biological Control Laboratory, Division of Plant Industry, Florida Department of Agriculture and Consumer Services, Gainesville, Florida. Since the Division of Plant Industry is responsible for regulating the introduction of insects into the state and for protecting agriculture, they provide the quarantine facility and promote biological control. Hydrilla research, however, is an Agricultural Research Service/US Department of Agriculture (USDA) program conducted with personnel support through a Research Support Agreement with the University of Florida. To enter the quarantine facility, one must pass through three doors and two small anterooms. White labcoats are donned in the inner anteroom. The coats make it easier to see insects that hitchhike on the researcher. Negative air pressure in quarantine ensures that air will rush inwards when the doors are opened, thus preventing insects that are near an opened door from being swept outside. Access to the locked quarantine is limited to those conducting research inside or to occasional escorted visitors. We conduct hydrilla research in quarantine in lieu of or in addition to foreign research because we must determine early in the program that the insect will attack our US hydrilla strains but not our native plants, because we often do not have a laboratory or researcher in the foreign country to conduct tests there, and because we wish to utilize the expertise and manpower provided by the quarantine team.

## ARRIVAL OF A SHIPMENT

When a package of insects arrives from overseas, it is carried into quarantine and into a locked maximum security room where it is opened inside a cage fitted with two cloth sleeves. The sleeves allow the researcher's arms to be inserted into the cage but prevent insects from escaping. Packages are sent to us via air freight with the usual transit time about 4-10 days. Major delays are usually encountered at ports-of-entry in the United States—for example, Miami or New York—not at overseas locations. Although flight times have been reduced through the years, bureaucratic red tape and snafus still occur causing death of the insects. Fortunately, US dispatch agents now handle our shipments at the airports and have contributed greatly to the success of the hydrilla program. Packaging varies with the type of insect. Fly larvae in hydrilla leaves were sent in water-filled plastic bags and cylinders. Adult weevils were sent in plastic containers with moist blotter paper, wood excelsior, and hydrilla sprigs. New insects are held in maximum security until the identity is confirmed. Diseased individuals, parasitic

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insects, and contaminants are removed before the insects are moved to the research areas of quarantine. The period in maximum security may be a few hours, a few days, or a few months, and several generations.

## **IDENTIFICATION OF BIOCONTROL AGENTS**

Most insects can be readily identified to genus by those of us in quarantine or by cooperating taxonomists. Identification to species, however, is a different matter. The correct identity of an Indian tuber-feeding weevil was still unknown 3 years after we initiated studies with it. A visit to the British Museum of Natural History in London by the weevil taxonomist Dr. C. W. O'Brien, Florida A. and M. University, Tallahassee, finally led to discovery of its identity. A companion Indian weevil that was collected and studied at the same time was a new species that is only now being described by Dr. O'Brien. An Indian leaf-mining fly that we studied was easier to identify because it had been described as a result of an earlier hydrilla project in Pakistan. Even that identification, however, took several weeks for confirmation by a specialist in Washington, DC. The Australian stem-mining weevil that we are currently studying was misidentified by an Australian specialist as a known species but is actually an undescribed species. This fact could not be confirmed by Dr. O'Brien until specimens were sent from London for comparison. These examples have been presented to illustrate both the importance of taxonomists to our programs and the difficulties we have to obtain correct names for our insects.

Entomologists usually understand why insects are difficult to identify, but others often do not. "Numbers" are the principal reason. There are just too many insects. It has been estimated that there are over 1 million insects and related arthropods already described. Estimates range from 1-7 million for the number of still undescribed and undiscovered species. What do these numbers mean? Based upon current estimates, insects and their relatives comprise about 53.2 percent of the world's organisms. About 38.8 percent of the organisms are in the Plant Kingdom, 5.6 percent are lower animals, and only 2.4 percent are vertebrates, including all fish, reptiles, amphibians, mammals, and birds. Insect taxonomists are abundant, but not if one considers the number of botanists, pathologists, and zoologists in the world. Actually, it is amazing that insect taxonomists do so well when providing us with identifications.

## **ARE THE BIOCONTROL AGENTS SAFE?**

The theme of our research in quarantine, which is the question that I am asked most often is "Are they safe?" "Will they eat my cabbage?" The public's perception of our program is often that we are playing with fire. New gypsy moths, Japanese beetles, or fruit flies are awaiting release. Is this concern valid? I do not believe so, although caution and careful research is warranted. Relative specialization of insects on plant species or plant groups is the rule in nature, not the exception. Most gardeners are familiar with cabbageworms on their cole crops. They know that they do not attack corn, or beans, or tomatoes. The tomatoes in turn are attacked by hornworms, which attack only the solanaceous plants. There are indeed polyphagous garden pests that attack many crop

plants, but they are few. It has been estimated that no more than 1 to 5 percent of the known insect species are pests, and few of those are of major importance. During a presentation at a recent meeting in Florida, a chemical company representative stated that only 546 pests, including pathogens, nematodes, weeds, and others, in addition to insects, were targets worldwide for pesticides. Only those pests were important enough economically to be targeted. Although the risk of careful researchers releasing a polyphagous insect is almost nil, the cabbageworm-hornworm feeding habits do demonstrate that plants closely related to the target plant might be at risk, and this risk must be evaluated. Temporary or minor feeding on nontarget plants has been reported for a few of the 192 organisms introduced around the world for biological control of weeds from 1832 to 1979, but none of the organisms has become a major pest.

## HYDRILLA INSECT FAUNA

The herbivorous insect fauna that is associated with hydrilla is not large compared to those of many terrestrial plants. However, it does offer a variety of insect types and life styles. These types include weevils, or snout beetles, that feed in submersed stems and in both stems and tubers out of water, ephydrid flies that mine leaves and stems, midges that feed in the tips of stems and inside stems, caddisflies that feed on the leaves, caterpillars that feed on the leaves and stems, and possibly leaf beetle larvae that feed on roots. Our first choices for intensive quarantine study have been an Indian weevil that attacks tubers during drawdowns, an Indian fly that mines leaves on submersed stems, and an Australian weevil whose larvae initially feed in submersed stems, but complete their development when stems excised by adult feeding float to shore. The Australian weevil is discussed in another paper in these proceedings and will not be discussed here.

Both Indian insects were released in Florida by Waterways Experiment Station, COE, personnel during 1987. The weevil, *Bagous affinnis* Hustache, was released in April and the fly, *Hydrellia pakistanae* Deonier, in October. Although these species were new to the United States, they were not unusual organisms for our country. In fact, approximately 50 percent of the known species of *Hydrellia* flies are native to the United States (55 of 113 total) and approximately 25 percent of the known *Bagous* weevils (33 of 138 total) are native. No host plants are recorded for the majority of the native species in these two genera.

## THE INDIAN HYDRILLA FLY

The Indian fly, whose larvae mine inside hydrilla leaves, was reared on hydrilla in gallon jars held in greenhouses and temperature cabinets. Adults fed on pollen and honeydew, not on hydrilla. Because larvae are confined to water, only aquatic plants and most submersed species were included in the host-range tests. Most tests were conducted by placing 5 eggs in each of 10 test tubes containing sprigs of test plants. Fifty plant species were tested during a 2-year period. Species that larvae attacked in the initial test or that we believed were at risk of attack were tested several times throughout the testing period. Small numbers of adults emerged from six plant species: four pondweeds, naiad, and elodea. The largest number, averaging only 8 percent, emerged from a major

introduced weed, curly-leaf pondweed, *Potamogeton crispus* L. This weed is native with hydrilla in Pakistan and India, but the fly does not attack it there. The results of our intensive 2-year testing program demonstrated that the fly is not a threat to our native aquatic plants. Larvae might occasionally develop in native plants, but damage would be negligible and overshadowed by that from native flies.

## THE INDIAN TUBER WEEVIL

The Indian weevil, whose larvae eat tubers during drought or drawdown periods, was more difficult to rear and test than the fly. Females laid eggs in small pieces of moist wood which we then buried with tubers in moist sand in terracotta vases. A layer of hydrilla stems was placed on the sand to retard evaporation and allow the sand to dry slowly. This rearing technique, which was adopted after a visit to India, was developed to approximate natural conditions. Since newly hatched weevil larvae crawl through the soil in search of tubers, larval tests included roots of aquatic plants that would survive drawdowns and many vegetables. Many species of aquatic plant were excluded because larvae cannot live in water and present no risk to them. Unlike adult flies, weevil adults feed and had to be tested against both aquatic plants and vegetables. Most larval tests were conducted by confining one or two newly hatched larvae in plastic cups (1 oz) with test plant material and vermiculite. Thousands of cups were set up during this laborious project, and approximately 110,000 tubers were field collected by hand, usually 1,500-2,500 per day. A water pump and a screen were used to wash the soil from the tubers. A total of 52 plant species were tested. Minor larval development occurred only on watercelery and two pondweeds, which survive drawdowns principally as seeds.

## TEST PLANT SELECTION

The two most important criteria for choosing test plants are relationship of the test plant to the target plant and status as a host plant of insects related to the biocontrol agent. True plant relationship is unknown, of course, and many schemes have been proposed. Generally, the closest relatives of the hydrilla-elodea-watercelery family are thought to be the arrowhead family and the pondweed-naiad family which we emphasize when choosing test plants. Families closely related to waterlilies (dicots) are considered more primitive than hydrilla, and other aquatic monocots are considered more advanced. Test plants are chosen from these groups as well as from other dicot groups. Interestingly, the known host plants of many *Hydrellia* flies are also found in those families closest to hydrilla. The host plants of *Bagous* weevils, however, do not fit a pattern and thus provide little direction for choosing test plants.

## APPROVAL FOR INSECT RELEASES

Permission to release hydrilla insects and even to import them into quarantine must be obtained first from the federal interagency Technical Advisory Group (TAG) for biological control of weeds, then from the Florida Department of Agriculture and Consumer Services, and ultimately from the Animal and Plant Health Inspection Service (APHIS), USDA. The request to the TAG is in the form of a detailed report

documenting what is known about the insect and the test results. If the request is approved by all groups, APHIS issues a permit and labels which must be affixed to the shipping boxes. The TAG has four members from the USDA, four from the US Department of Interior, one from the Corps of Engineers, one from the Environmental Protection Agency, one from the Weed Science Society of America, and one from the National Plant Board. These members represent a broad selection of interests and disciplines. Release of the biocontrol agents in another state must be approved by that Department of Agriculture and by APHIS.

## **POTENTIAL FOR CONTROL OF HYDRILLA**

Now that hydrilla insects have been released from quarantine will pre-hydrilla conditions return to our waterways? This question is difficult to answer. Definitely, waterways will always have hydrilla because insects will not eliminate it. Even when biological control is successful, a residual population of the target plant remains. Hopefully, however, the residual populations will be low. I believe that the potentially most damaging species of the present hydrilla insects is the fly because all immature stages develop underwater. Although stems were not eaten, they often rotted in our rearing jars after heavy attack. It is not possible to predict if degradation will happen to vigorously growing stems in the field, but at least the plant growth rate should be reduced by extensive leaf destruction. Calculations made from our laboratory data suggest that one female could produce 6.7 million females after five generations under optimum conditions. Obviously, field populations will not increase at this optimum rate, but we expect 4-6 generations in much of Florida. One larva eating 9 to 12 leaves could not be very effective, but millions of larvae each eating that number of leaves might be.

The tuber weevil is well adapted to the distinct wet-dry seasons found in India and thus may have difficulty establishing under most conditions in the United States. Numbers sufficient to quickly overwhelm the tuber population would be needed for release during short-term drawdowns. Weevils might be able to establish, however, during longer drawdowns and in waterways with natural summer low-water periods. Unfortunately, data on natural infestations are lacking except from one site in Pakistan. That site was a small pond at which 46 percent of the tubers were attacked in April, 90 percent in May, and almost all in June. The rains began in August, but hydrilla had not returned by December, even though it was present at other sites. That data and data from our laboratory studies indicate that under the proper conditions the weevil can greatly reduce tuber populations.

# Australian Insects to Control Hydrilla

by  
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## OBJECTIVES

The goal of this project is to locate, test, and evaluate Australian insects which, if imported into the United States, would have potential serving as biocontrol agents of hydrilla (*Hydrilla verticillata*). During 1987, our objectives in Australia were : (a) to maintain our colonies of the hydrilla stem-boring weevil, *Bagous n. sp. Z.*; (b) to obtain necessary Australian export permits for *Bagous n. sp. Z.* and ship it to quarantine facilities in Gainesville, Florida; (c) to continue our field studies of this weevil's host range, behavior, ecology, and distribution; (d) to begin laboratory and field studies of the leaf-mining Ephydrid fly, *Hydrellia n. sp. A.*; (e) obtain export permits necessary to ship this fly to quarantine facilities in Gainesville; and (f) to begin laboratory and field studies of the stream dwelling moth, *Nymphula prob. eromenalis*.

## INTRODUCTION

Hydrilla *Hydrilla verticillata* (L. fil.) Royle was introduced into the United States in the early 1960's. It was apparently imported by the aquarium trade, then released into various natural water bodies. The earliest herbarium specimens from the United States were collected near Orlando, Florida, during 1962. Since that time hydrilla has spread throughout the country and is now considered one of our worst aquatic weed problems. It now occurs extensively throughout the southern United States to California and in scattered locations north to Delaware. Although the problem is already serious, the number of water bodies infested continues to multiply.

Because it represents such a serious problem, and because control is both expensive and difficult, hydrilla has become a top priority for biological control. For example, the US Department of Agriculture-Agriculture Research Service (USDA-ARS) Research Planning Conference on Biological Control listed it in a 1984 report as the top priority aquatic weed. Further, the 1982 report of the USDA-ARS Research Planning Conference on Aquatic Weed Control placed biological control of hydrilla near the top of a list of national aquatic weed control research priorities. That conference also identified foreign exploration for potential biological control agents of hydrilla to be a national priority and the top priority of the Fort Lauderdale Aquatic Plant Management Laboratory. Hydrilla control is also a top priority of the US Corps of Engineers Aquatic Plant Control Research Program which has financially supported the USDA-ARS effort to find and develop biological controls for hydrilla.

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Many species of plants become serious weeds after being introduced from other parts of the world to new areas which provide favorable growing conditions. At the time of introduction, these plants are usually free of natural enemies and can grow without interference from host-specific herbivores. In so doing, they often grow vigorously and become more abundant in their adventive range than they were in their native range. The classical biological control approach strives to find, within the plant's native range, host-specific herbivores that are capable of slowing this rampant plant growth, then introduce them into areas where the plant has become a weed. Foreign exploration then is an essential, integral part of a classical biological control effort. Faunal inventories of associated herbivores from regions where the target weed is native are necessary to select potentially useful biological control agents. However, in the case of hydrilla, a precise area of origin is unknown. It occurs as a native plant on at least three continents (Africa, Asia, and Australia) as well as on the many island countries of the Indo-Pacific region. India, Africa, and Australia have variously been identified as the areas of origin of hydrilla.

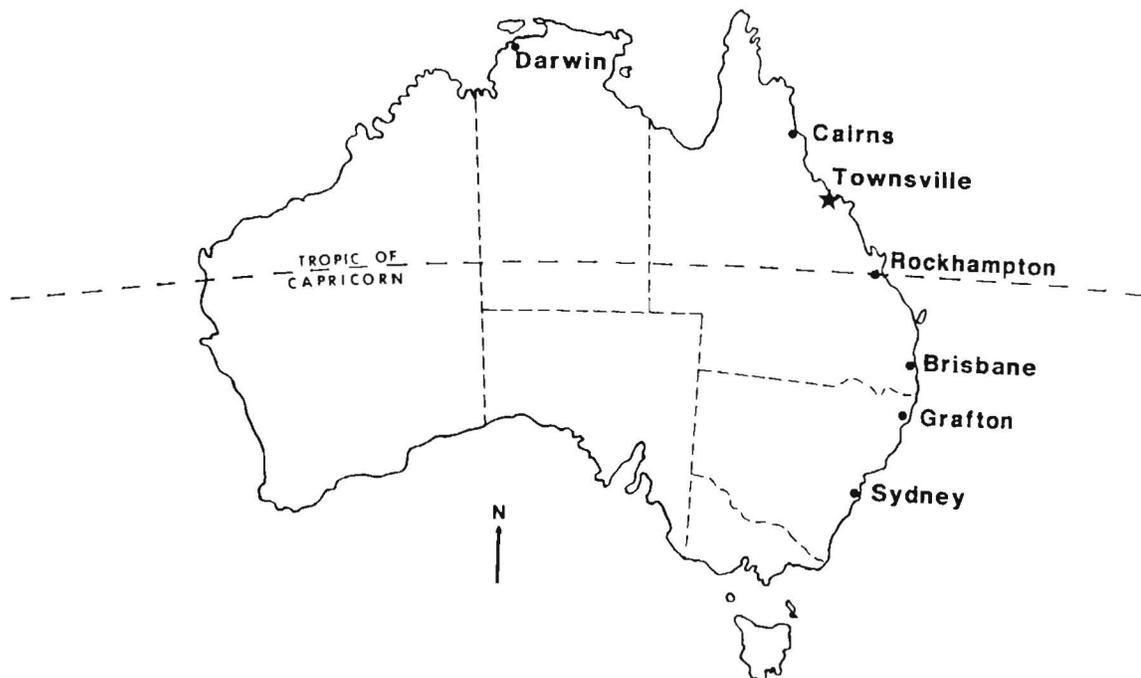
Determining the area of endemism of a weed is the first step in a biological control project, but this area could not be ascertained with certainty for hydrilla. Global surveys were therefore undertaken to compile lists of the natural enemies of hydrilla throughout its native range. Rather than thoroughly survey in just one or a few areas, instead it was decided to survey briefly in as many areas as possible. This decision was based upon the knowledge that most of the previously successful biological control agents were usually found early in the survey phase of their respective projects. Thus, in the case of a species such as hydrilla which occupies a large, disjunct range, more potential biological agents would likely be found by cursory sampling from a large portion of its range rather than by sampling thoroughly from within a small portion. Economics were also a consideration since air fares with many stops on a circumglobal route were generally equivalent to single destination fares for distant localities. Thus, many countries could be visited as cheaply as a few. Therefore, during a 3-year period (1981-1983), I spent a total of about 15 months traveling throughout India, Indonesia, the Philippines, Southeast Asia, and Australia searching for hydrilla and its natural enemies. This survey was made possible through a cooperative agreement between the USDA and the University of Florida.

In addition, the Commonwealth Institute of Biological Control Kenya Station, under a cooperative agreement with the USDA searched for natural enemies of hydrilla in Africa. The end result was that many phytophagous insects were found which fed on hydrilla. Most of these were new, undescribed species. The importation and use as biological agents has not begun. Australian and Indian insects are being evaluated first. This report describes the progress of our research on hydrilla insects in Australia.

## APPROACH

This research program was initially based on the northeast coast of Australia at the Commonwealth Scientific and Industrial Research Organization's (CSIRO) Davies Laboratory in Townsville, Queensland (ca. 19° S latitude). However, my space at CSIRO was no longer available this year, so in April I shifted my laboratory and office to the campus of James Cook University, only several miles away. Supplemental research

funding and a favorable exchange rate of US currency permitted the continued employment of a technician, Matthew Purcell, at CSIRO's Longpocket Laboratory in Brisbane 1,000 miles south of Townsville (Figure 1). The geographical range over which collections could be obtained was increased with the employment. During 1987, Matthew made monthly collecting trips to Grafton, New South Wales. This field work delimited the range of hydrilla herbivores, increased the number of potential host-plant species examined, and provided additional sources of weevils and host plants of Matthew Purcell.



**Figure 1. Map of Australia showing the location of our lab in Townsville, Queensland. Most of the collecting there is done between Townsville and Cairns, with one of the technicians collecting in the Brisbane vicinity, with monthly trips to Grafton**

To determine the seasonal variation in the abundance of the weevil and other hydrilla insects, hydrilla from each of a dozen sites in the Townsville-Cairns region and a similar number in the Brisbane vicinity were sampled on a regular schedule.

Samples of hydrilla and other aquatic plant species were collected in the field, then transported to the laboratory for processing. A portion of each plant sample was weighed, then placed in a berlese funnel to slowly dry (over a period of 4-5 days) from the heat of a light bulb mounted inside the top cover of the funnel. The animals from the drying material escaped into a container at the bottom of the funnel and were collected daily. This method proved to be an effective means for collecting stem-boring and leaf-mining insects, such as weevil larvae and *Hydrellia* fly larvae. Immature herbivores were reared to the adult stage to facilitate species determinations, but other animals

were identified only to family or generic level, then counted and preserved in 75 percent ethanol.

Our emphasis during 1987 was on shipping hydrilla stem-boring weevil, *Baqous n. sp. Z.*, to the quarantine facilities in Gainesville, Florida, and on collecting and providing field and lab data about this weevil which would speed up its quarantine testing and eventual release. During the latter part of 1987, we also began colonizing the leaf-mining Ephydrid fly, *Hydrellia n. sp. A* (Figure 2). We also began the testing and colonization of three species of aquatic moths, whose larvae feed on hydrilla growing in fast-flowing streams.

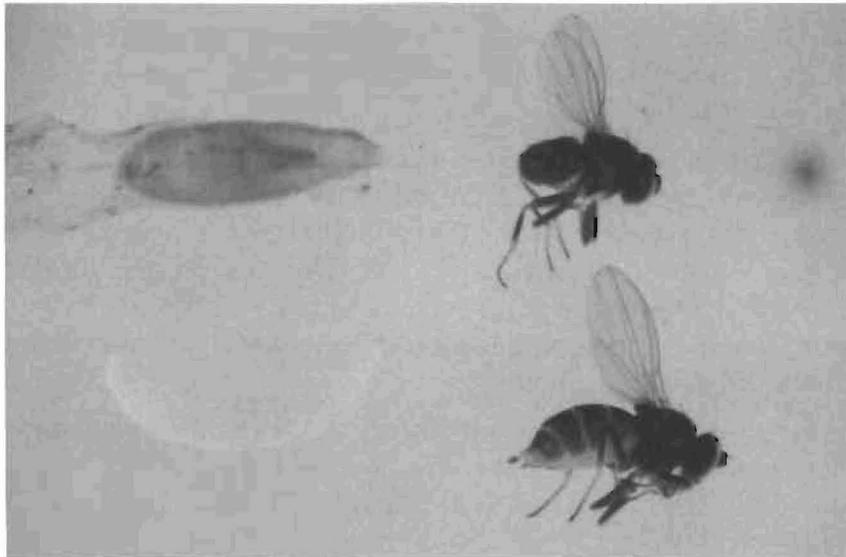


Figure 2. Life stages of the hydrilla leaf-mining fly, *Hydrellia n. sp. A.*, which will be imported into Gainesville, Florida, quarantine facilities in early 1988

## RESULTS AND DISCUSSION

Drought conditions persisted throughout many of our collecting sites in Queensland. In Townsville, it has now been 6 years since the last “good” rainy season. Very few of our sites near Townsville now contain water. We now have to rely on our sites near Cairns, several hundred miles to the north, for not only our insect collections but also for the aquatic plants used in our tests and maintaining our insect colonies. Sporadic heavy rainfalls near Brisbane have replenished some reservoirs, but many remain nearly empty.

Despite these continued adverse weather conditions, our field collecting was very productive. We made an additional 123 collections of hydrilla during 1987. Since the project began in 1985, we have made 464 collections of hydrilla. During 1987, we also made 263 collections from aquatic plants other than hydrilla, raising to 781 the total number of nonhydrilla collections made in Australia. Most of the nonhydrilla collections were focused on plants closely related to hydrilla, especially other Hydrocharitaceae: *Vallisneria gracilis*—122 collections, *Egeria densa*—76 collections, *Blyxa octandra*—49 collections, and other Hydrocharitaceae—42 collections.

We continued to intensively collect herbivores on aquatic plants in the field, since these collections form the basis for our decisions on the host specificity. In the artificial conditions of a laboratory, feeding tests of these same herbivores usually indicate host specificity that is broader than what we actually encounter in the field. With more than 1,200 collections, we can now fairly confidently predict what species of aquatic plants will be attacked *in the field* by a particular herbivore species.

## WEEVILS

Dr. Charlie O'Brien, the world expert on *Bagous* weevils, after examining our specimens and comparing them to the type specimen from London, has informed us that the weevil we have been calling *Bagous australasiae* was a new, undescribed species of *Bagous* and not *B. australasiae*. Until he describes this species, we shall refer to this hydrilla stem weevil as *Bagous n. sp. Z*.

We were hoping to ship this weevil to Gainesville quarantine facilities at the beginning of 1987. However, we did not receive all the necessary permits until the end of March. We dispatched our first shipment on 10 April 1987, and a second shipment was sent 29 May. At the end of July, we received word that the weevils were well established in Gainesville quarantine and that immediate supplemental shipments would not be needed. We therefore terminated our remaining laboratory colonies of this weevil and shifted our laboratory research to new candidates.

We are, however, completing some life history studies of this weevil under controlled environmental conditions in Brisbane. We hope to submit these results for formal publication by early 1988. We are also continuing to monitor population levels of this weevil in the field.

This weevil is the most damaging insect on hydrilla that I have seen in my 10 years of research on hydrilla insects. If this weevil receives permission for release and becomes established in the United States, I feel that it will quickly begin to play a key role in reducing hydrilla infestations there.

## HYDRELLIA FLIES

In early June, the Australian specialist on Ephydrid flies who graciously has been identifying the thousands of Ephydrid flies which we have collected, notified me that all the *Hydrellia* flies collected from aquatic plants other than hydrilla were *Hydrellia* new species near *unigena*—the same species as those collected from hydrilla—rather than *Hydrellia unigena* as he had earlier informed me. Thus, the new species of *Hydrellia* (hereafter referred to as *Hydrellia n. sp. A.*), instead of being restricted only to hydrilla, also occurred occasionally on some other aquatic macrophytes. After reexamining all of our field records for *Hydrellia* from the past 3 years, I found that 97 percent of our specimens of *Hydrellia n. sp. A.* were collected from hydrilla. Only 440 specimens of *Hydrellia n. sp. A.* have been collected from plants other than hydrilla, while our hydrilla collections have produced more than 12,000 individuals of this species. Over 800 specimens are sometimes found in a single hydrilla collection, while nonhydrilla collections usually yield less than 5 specimens (max.—52 from a *Najas* collection) of this

fly. Thus, it is evident from our field data that hydrilla is the preferred—and perhaps the only—host for this fly. The relatively few specimens from plants other than hydrilla may just represent incidental occurrences, rather than breeding populations.

Therefore, laboratory testing of this promising fly appears to be more than justified. Accordingly, Matthew Purcell, after terminating his weevil colonies in Brisbane, began to collect and colonize *Hydrellia n. sp. A*. We have now received the necessary permits to import *Hydrellia n. sp. A* into the United States and to export it from Australia. We hope to make the first shipment early in 1988.

## STREAM-DWELLING MOTHS

After terminating our weevil colonies in Townsville in late April, we began to focus our field and laboratory research on the stream-dwelling moth, *Nymphula eromenalis* (Figure 3). After a few weeks of intensive study, it became apparent that the larvae of at least two other moth species were being collected and confused with *N. eromenalis*. These additional moth species have at last been identified as *Strepsinoma repititalis* (Figure 4) (in previous reports, referred to as Type 1) and *Aulacodes siennata* (Figures 5 and 6) (referred to as Type 3). Our Type 2 species has been confirmed to be *Nymphula eromenalis*.

All three of these species appear to be restricted to streams with permanent flows and have been collected almost exclusively from three species of Hydrocharitaceae: *Hydrilla verticillata*, *Blyxa octandra*, and *Vallisneria gracilis*. In our preliminary tests, when given a choice of three macrophytes, all three species of moth larvae preferred hydrilla, with *N. eromenalis* and *A. siennata* producing the heaviest damage. While the other two species feed primarily on the leaves of hydrilla, *A. siennata*, the species with the largest larvae, also devours hydrilla stems.

Rearing and colonizing these stream-dwelling moths has proven to be especially difficult and time-consuming. As yet, we have been unable to establish colonies large enough to supply individuals for our laboratory tests.

## PLANS FOR 1988

Emphasis, both in Townsville and Brisbane, will remain on field research, especially determining the host range and geographic distribution of the insects being considered as potential control agents. Matthew will continue his monthly sampling in New South Wales.

In Brisbane, Matthew will colonize and test the leaf-mining fly, *Hydrellia n. sp. A*. Since Gary Buckingham has already requested the importation of this fly into United States quarantine, we hope to send the first shipment in January 1988. Once this fly is well-established in the Gainesville quarantine, we will terminate our laboratory colonies and studies—hopefully by July 1988.

In Townsville, our laboratory research will focus on one of the three stream-dwelling moth species, most likely *Aulacodes siennata* since it appears to cause the most damage.

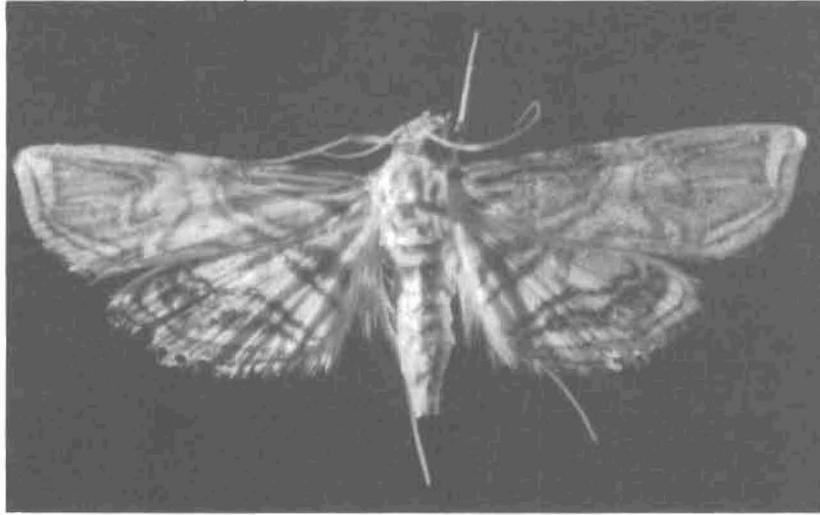


Figure 3. Adult of the moth *Nymphula eromenalis*. Larvae of this moth feed on hydrilla growing in fast-flowing streams

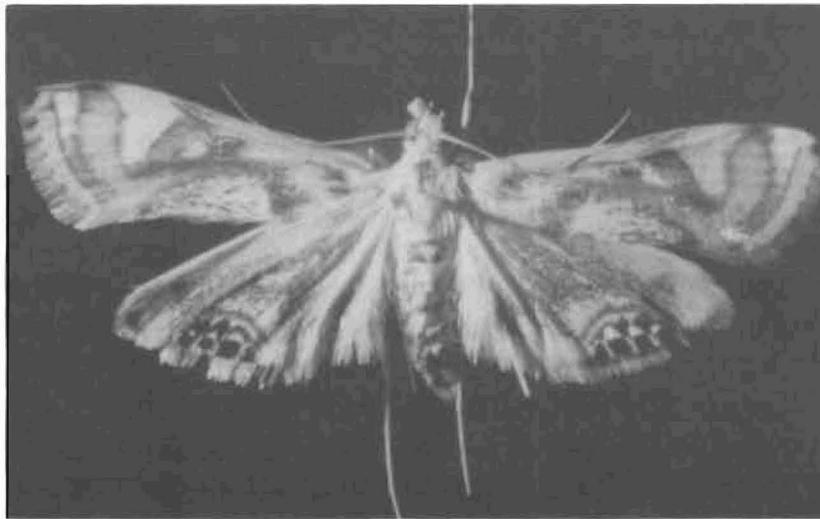


Figure 4. Adult of the moth *Strepsinoma repitalis*. Larvae of this moth also feed on hydrilla growing in fast-flowing streams

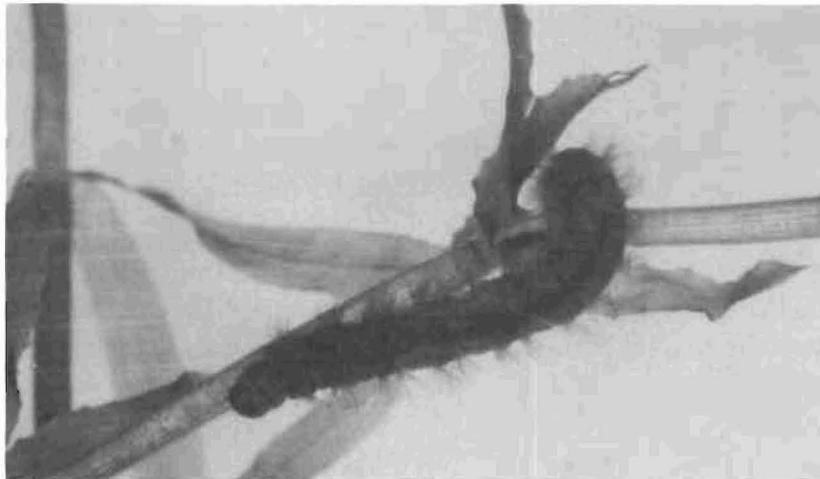


Figure 5. Larva of the moth *Aulacodes siennata*. The large larvae of this moth species will be the focus of our studies in Australia during 1988

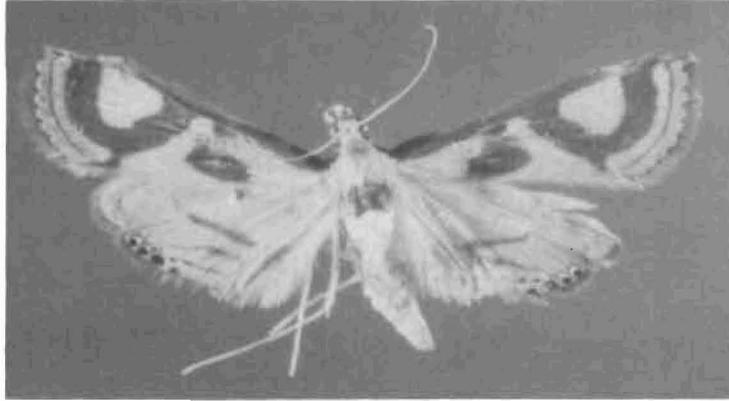


Figure 6. Adult of the moth *Aulacodes siennata*

If we are able to colonize this species, we should be able to complete our host testing in early 1989.

Alternatively, if sufficient additional funds (approximately \$25,000) are provided so we can hire someone to take care of our insects and plant cultures, we would probably be able to simultaneously test a second moth species, probably *Nymphula eromenalis*.

# Biological Control of Waterlettuce

by

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Ted D. Center,† and Joe K. Balciunas\*\*

## INTRODUCTION

The decrease in populations of waterhyacinth *Eichhornia crassipes* (Mart.) Solms, due both to maintenance herbicide treatments and the pressure exerted by three insects introduced for biological control, has allowed waterlettuce *Pistia stratiotes* L. to increase in many waterways in Florida. In view of this increase and the successful use of a weevil *Neohydronomus pulchellus* Hustache by CSIRO in Australia to control waterlettuce, a program was initiated in Florida to study the weevil. The weevil has been highly host-specific in studies in South America (DeLoach, DeLoach, and Cordo 1976), its natural home, and Australia. Subsequent releases in Australia (Harley et al. 1984) and South Africa (Cilliers 1987) were encouraging. Host-specificity tests in Florida were equally promising (Thompson and Habeck 1989, in press), and permission for field release was obtained in mid-November 1986.

The moth *Namangana pecticornis* Hampson is a common insect on waterlettuce throughout much of tropical Asia. There is some confusion on the generic placement of this noctuid moth. It has been called *Namangana* in Thailand (Suasa-ard 1976) and India (George 1963). In Indonesia, it was called *Proxenus hennae*, but this name is synonymous with *N. pectinicornis* according to Dr. J. Holloway, an English authority on the macrolepidoptera of southeast Asia.†† Eventually, this species may be placed in the genus *Athetis* or even *Spodoptera*. George (1963) estimated that 100 *N. pectinicornis* caterpillars hatching from one average-sized egg mass could completely destroy one square metre of waterlettuce during their development. He also reported that caterpillars fed only on waterlettuce in ponds with mixed vegetation and would starve rather than feed on other plant species. In Indonesia, Mangoendihardjo and Nasroh (1976) tested 26 plants and found that newly hatched larvae did not feed on any of them.

Later Mangoendihardjo (1983) reported that *Proxenus hennae* was tested on 44 plant species in 20 families and that larvae developed normally only on waterlettuce. In Thailand, Suasa-ard and Napompeth (1976) tested 74 plant species from 34 families, and waterlettuce was the only plant on which third instar larvae could survive. *Namangana pectinicornis* was mass reared and released at several sites in Thailand. Complete control of two infestations of 4.5 and 10 km<sup>2</sup> was obtained within 6-10 weeks (Napompeth 1982).

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††Personal Communication, 1986, Dr. J. Holloway, British Museum of Natural History.

## METHODS AND MATERIALS

Weevils were maintained in a cage in a nonquarantine greenhouse to build up populations for field release. New plants were supplied weekly, and previously exposed plants were kept in shallow greenhouse pools while larvae developed. As adults emerged, they were placed in the cage with the other adults.

The moth *Namangana pectinicornis* Hampson has been in quarantine in Gainesville since late September 1986. This insect was supplied by Dr. Banpot Napompeth, Director of the National Biological Control Research Center in Bangkok, Thailand. Sixty plant species in 29 families (Table 1) were exposed to newly hatched larvae. Tests were conducted in 1-oz plastic cups. Each test consisted of 3 replicates of 10 caterpillars. Caterpillars were checked after 48 hr to determine initial survival and daily thereafter until death.

## RESULTS AND DISCUSSION

The weevils were first released at Torrey Island in Lake Okeechobee in south Florida on April 29, 1987. Subsequently weevils were released at three other south Florida sites, Kramer Island (also in Lake Okeechobee), Port St. Lucie, and Plantation. The weevil has become established and is increasing at all four sites. The populations of both weevils and waterlettuce are being monitored monthly. As yet, no changes in waterlettuce populations have been observed. Several north Florida sites have been tentatively selected for field release when more weevils are available.

The mature caterpillar of *Namangana pectinicornis* is up to 25 mm long. It tunnels in the leaves during the early stages and does extensive damage to the plant. Pupation occurs in a cavity eaten by the caterpillar near the base of the leaf. Development from egg to adult requires about 28 days.

In Florida, 60 species of plants in 29 families were exposed to newly hatched larvae (Table 1). Each test consisted of 3 replicates of 10 caterpillars. Except for one caterpillar which lived 6 days, none lived more than 72 hr and none molted to second-stage caterpillars. The plants tested included 24 food plants, 10 landscape or indoor plants, and 17 aquatic and semiaquatic plants including some weeds. Tests of first- and third-stage caterpillars in Florida and Thailand with 116 plant species in 48 families have shown this insect to be highly host-specific and a very promising candidate for release against waterlettuce.

## ACKNOWLEDGMENTS

The authors especially express their appreciation to Dr. Banpot Napompeth for supplying the pupae of *Namangana pectinicornis*. The assistance of numerous personnel, especially Margaret Glenn, Center for Aquatic Weeds, University of Florida, Judy Gillmore, John Watts, and Debbie Matthews, Department of Entomology and Nematology, University of Florida, and Dr. Gary Buckingham and Chris Bennett, USDA Biological Control Group, Gainesville, is gratefully acknowledged. The financial support of the US Army Corps of Engineers (Jacksonville District and Waterways Experiment Station) made this research possible.

**Table 1**  
**Host-Specificity Tests of First Instar Larvae of *Namangana pectinicornis* (Hampson)**

PLANTS TESTED		
<i>Family</i>	<i>Genus and Species</i>	<i>Common Name</i>
Alismataceae	<i>Sagittaria montevidensis</i> Cham & Schlecht.	California arrowhead
Amaranthaceae	<i>Alernanthera philoxeroides</i> (Mart.) Griseb. <i>Amaranthus retroflexus</i> L.	Alligatorweed Pigweed
Anacardiaceae	<i>Mangifera indica</i> L.	Mango
Apiaceae	<i>Hydrocotyle umbellata</i> L. <i>Daucus carota</i> L. var. <i>sativa</i> DC.	Water pennywort Carrot
Araceae	<i>Aglaonema</i> sp. <i>Anthurium</i> sp. <i>Arisaema dracontium</i> (L.) Schott <i>Arisaema triphyllum</i> (L.) Schott & Endl. <i>Dieffenbachia</i> sp. <i>Orontium aquaticum</i> L. <i>Peltandra virginica</i> (L.) Kunth. <i>Pistia stratiotes</i> L. <i>Spathiphyllum</i> sp.	Aglaonema Anthurium Green dragon Jack-in-the-pulpit Dumb cane Goldenclub Green arum Waterlettuce Spathe flower
Asteraceae	<i>Bidens mitis</i> (Michx) Sherff. <i>Cirsium horridulum</i> Michx. <i>Lactuca sativa</i> var. <i>crispa</i> L. <i>Gnaphalium purpureum</i> L.	Begger tick Yellow thistle Lettuce Purple cudweed
Balsaminaceae	<i>Impatiens sultanii</i> Hook f.	Impatiens
Brassicaceae	<i>Brassica campestris</i> var. <i>napobrassica</i> (L.) DC <i>Brassica deraceae</i> var. <i>viridis</i> L.	Rutabaga Kale
Cannaceae	<i>Canna flaccida</i> Salisb.	Golden canna
Chenodiaceae	<i>Beta vulgaris</i> L.	Beet
Commelinaceae	<i>Tradescantia crassifolia</i> Cav.	Spiderwort
Convulvulaceae	<i>Ipomoea batatas</i> (L.) Lam.	Sweetpotato
Crassulaceae	<i>Crassula argentea</i> Thunb.	Jade
Curbitaceae	<i>Curcubita pepo</i> var. <i>melopepo</i> (L.) Alef. <i>Cucumis sativus</i> L.	Summer squash Cucumber
Ericaceae	<i>Rhododendron indicum</i> (L.) Sweet	Azalea
Fabaceae	<i>Phaseolus vulgaris</i> L. <i>Lathyrus odoratus</i> L. <i>Pisum sativum</i> L.	Bean Snow pea Sugar snap pea
Haloragaceae	<i>Myriophyllum aquaticum</i> (Vell.) Verdc.	Parrotfeather
Hydrocharitaceae	<i>Limnobium spongia</i> (Bosc.) Steud.	American frogbit
Lemnaceae	<i>Lemna minor</i> L. <i>Spirodela punctata</i> (Meyer) Thomps.	Common duckweed Giant duckweed
Liliaceae	<i>Asparagus officinalis</i> L. <i>Allium cepa</i> L.	Asparagus Onion
Malvaceae	<i>Gossypium hirsutum</i> L. <i>Hibiscus</i> sp.	Cotton Hibiscus
Poaceae	<i>Oryza sativae</i> L. <i>Saccharum officinarum</i> L. <i>Zea mays</i> var. <i>saccharata</i> (Sturtev.) Bailey <i>Triticum aestivum</i> L.	Rice Sugarcane Corn Wheat
Polygonaceae	<i>Polygonum densiflorum</i> Meisn. <i>Rumex</i> sp.	Smartweed Dock

(continued)

Table 1 (Concluded)

PLANTS TESTED		
<i>Family</i>	<i>Genus and Species</i>	<i>Common Name</i>
Pontederiaceae	<i>Eichhornia crassipes</i> (Mart.) Solms <i>Pontederia cordata</i> L.	Waterhyacinth Pickerel weed
Rosaceae	<i>Fragaria chiloensis</i> Duchesne var. <i>ananassa</i> Bailey	Strawberry
Rubiaceae	<i>Gardenia jasminoides</i> Ellis	Gardenia
Rutaceae	<i>Citrus limon</i> (L.) Burm.	Rough lemon
Salviniaceae	<i>Azolla caroliniana</i> Willd. <i>Salvinia minima</i> Baker	Carolina mosquitofern Water fern
Solanaceae	<i>Solanum melongena</i> L. <i>Solanum tuberosum</i> L. <i>Lycopersicon esculentum</i> Mill. <i>Physalis</i> sp.	Egg plant Red potato Tomato Ground cherry
Theaceae	<i>Camellia japonica</i> L.	Camellia
Typhaceae	<i>Typha latifolia</i> L.	Common cattail

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# Tennessee-Tombigbee Waterway

by  
Joe Kight\*

## INTRODUCTION

The Tenn-Tom, as the waterway is called, is a 234-mile-long navigation system that connects the Tennessee River to the Tombigbee River which ultimately winds up as the Mobile River at Mobile, Alabama, on the Gulf of Mexico. There are 10 locks and dams in the system, 11 counting the one at Demopolis, which hold 43,483 surface acres of water. The project was completed in December 1984.

An aquatic plant survey was conducted by project personnel in the summer of 1986. Eurasian watermilfoil was not found. Another survey was conducted in the summer of 1987 in which milfoil was found. I made a visit to the Tenn-Tom in September 1987 and recorded the following:

Problem Plants  
Tennessee-Tombigbee Waterway  
September 1987

Location	Surface Acres	Acres Infested	Plant
"E" Pool		200	<i>Myriophyllum spicatum</i>
		Trace	<i>Hydrilla verticillata</i>
Aberdeen	4,121	20	<i>Myriophyllum spicatum</i>
Aliceville	8,300	30	<i>Cabomba caroliniana</i>
		25	<i>Nelumbo lutea</i>
		15	<i>Myriophyllum spicatum</i>
		5	<i>Ludwigia peploides</i>
		1	<i>Eichhornia crassipes</i>
		1	<i>Alternanthera philoxeroides</i>
		1	<i>Zizaniopsis miliaceae</i>
		1	<i>Limnobium spongia</i>
Gainesville	6,400	17	<i>Myriophyllum spicatum</i>

The "trace" of hydrilla was a terminal section, approximately 4 in. long, found floating approximately 50 ft offshore from the boat ramp at Saucer Creek Recreation Area. No rooted plants were found, but small colonies could easily have been overlooked. It is possible that this particular sprig had been brought in on a boat trailer. However, the fact that it was there is reason enough for concern.

The 252 acres of milfoil were recorded from "E" Pool south to Gainesville Lake. Bay Springs Lake and the divide cut were not surveyed. Coontail (*Ceratophyllum demersum*) and alligatorweed are widely dispersed and could cause problems in the future.

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

## AREAS TREATED

The hydrilla was physically removed from the waterway. Two areas of milfoil were treated with Aquathal K at a rate of 4 parts per million (ppm). One area in "E" Pool consisted of 6 acres of milfoil located north of the Saucer Creek Recreation Area. This plot is approximately midway in some 200 acres of milfoil. The other area treated was the 17-acre infested area in the slough at Sumter Recreation Area, located on the Gainesville Lake.

Both treated areas were visited by project personnel in mid-October who stated that control was very good. Written recommendations were made to treat the entire 316 acres of problem plants. Using Aquathal K on the milfoil, 2,4-DMA on the hyacinths, and Rodeo on the other emerged plants, the estimated cost of herbicides (September 1987 prices) is approximately \$78,000. Application will add another \$5,000 to \$10,000, bringing the ante up to somewhere in the neighborhood of \$85,000 to \$90,000.

## WALTER F. GEORGE RESERVOIR

Project personnel reported *Elodea (Egeria densa)* floating in the Chattahoochee River at Columbus, Georgia, which is at the upper end of W. F. George Reservoir. I made a trip to the project, primarily to do a survey for giant cutgrass, and found healthy plants floating in the river current approximately 20 miles downstream from Columbus. It was later confirmed that the source of these plants was Goat Rock Reservoir, owned by Georgia Power Company, located a few miles above Columbus.

An onsite meeting with Georgia Power Company personnel was held. They were concerned about the plants and suggested that they would contribute some herbicides toward the control of the plants. Appropriate Corps and State offices were contacted for the purpose of conducting this control work under the Aquatic Plant Control Program. Action is pending.

## SONAR AND N-METHYLFORMAMIDE (NMF)

Fluridone, 1-Methyl-3-Phenyl-5-[3-(Trifluoromethyl)Phenyl]-4 (1H)-Pyridinone, is the active ingredient in the aquatic herbicide Sonar. In April of 1986, Sonar was approved and registered by the US Environmental Protection Agency (EPA) at the completion of a 4-year review process. EPA's conclusion was that neither Sonar nor its degradation products pose an environmental or a human health risk. The agency was aware of the metabolite, NMF, at the time of registration and considered the risk from exposure to be well within the margin of safety.

Recently, the safety of Sonar has been questioned. In response to allegations, the EPA undertook a Margin Of Safety (MOS) review for NMF. The "no effect" level describes at what concentration a compound is considered safe. The findings were that the lowest reported no observed effect level for teratogenic effects due to NMF exposure is 10.0 mg of NMF/kg of body weight.

Barlow and Sullivan (1982) did not show a "no effect" level for NMF as a teratogen or embryotoxin. This fact could indicate that no amount of NMF would be considered safe

and probably is the root of the controversy. But the point is that they did not address the “no effect” level.

In another review, Kennedy (1986) cites a separate study which reported a “no effect” level of 10 mg of NMF/kg of body weight in rabbits for maternal toxicity, fetal toxicity, and teratogenicity, i.e., the lowest “no effect” level for teratogenic effects due to NMF exposure. Using this “no effect” level and a worst case scenario that could occur in drinking water (0.15 ppm), the margin of safety for a 60-kg pregnant woman drinking 2 ℓ per day would be 30,000—or an exposure of 30,000 times less than the “no effect” level on rabbits.

The molecular weight of fluridone is 329, and NMF has a molecular weight of 59. Only one mole of NMF can be formed during the degradation of a mole of fluridone, resulting in 18 percent of the original fluridone mole (59 divided by 329 = 17.93 or 18 percent). Thus, the complete photolysis of 0.15 ppm of fluridone to NMF would produce 0.027 ppm of NMF. This calculation is a theoretical 100 percent conversion.

Saunders and Mosier (1983) found that the maximum conversion rate from fluridone to NMF observed in lake water in a glass container that was exposed to sunlight was 36 percent rather than 100 percent. This result would indicate that the actual concentration of NMF would approximate 0.010 ppm rather than 0.027 ppm (0.15 ppm × 0.18 × 0.36 = 0.010 ppm of NMF).

An artificial pond study was conducted by Lilly Research Laboratories to examine the rate and route of dissipation of C<sup>14</sup> fluridone. Fluridone was shown to dissipate rapidly in water exposed to natural environmental conditions. Photolysis was presumed to be the primary route of degradation, and no significant quantities of compounds similar to those detected in the aqueous photolysis study were observed to accumulate in outdoor ponds. Fractionation and characterization of the C<sup>14</sup> activity in water samples collected at 28, 59, and 142 days after treatment showed that radioactivity in the unextracted fraction was equivalent to 0.004-ppm fluridone. NMF, if formed, would be present in this unextracted fraction. Given that 100 percent of the unextracted fraction was NMF, the concentration of NMF would then be 0.0007 ppm (0.004 ppm × 8 percent = 0.0007 ppm).

Using Lilly’s research data of a “realistic case” of 0.0007 ppm, then the safety factor would be 434,782. EPA usually requires a safety margin of 100 for exposure to pesticides. It should also be pointed out that NMF has not been detected in the field.

$$\frac{(0.0007\text{-mg NMF}/\ell \text{ of water})(2 \ell \text{ of water})}{60\text{-kg body weight}} = 0.000023\text{-mg NMF/kg}$$
$$\frac{10\text{-mg NMF/kg}}{0.000023} = 434,782$$

## LARGE-SCALE HERBICIDE APPLICATION—LAKE SEMINOLE

Treatment of aquatic plants using Sonar herbicide was made on May 4, 6, and 7, 1987. A total of 44,000 lb of herbicide was applied, consisting of 37,680 lb of SRP (slow release pellets), 4,320 lb of 5P (normal release rate) pellets, and 2,000 lb of a dustless formulation of the SRP pellets. Each of these formulations contained 2-lb active ingredient (a.i.) per 40 lb of pellets.

Twenty-eight plots, varying in size from 16 to 100 surface acres, were treated. Twenty plots received 40 lb of pellets per surface acre. The remaining plots received either more or less herbicide per surface acre depending on water depth. In shallow water areas, the rate was reduced; in deepwater areas the rate was increased. See Table 1 for these variances. Table 2 shows a rating of the results of the May application.

Table 1  
Sonar Application, May 1987

Area	Application Date	Acres	Pounds	Pails Applied		
				SRP	5P	Dustless
1. Desser Slough	4	50	2,000	50		
2. Parramore Slough	4	36	1,400	35		
3. Pear Orchard Run	4	23	920		23	
4. Apalachee G.M.A.	4	20	800	20		
5. Howells Ramp	4	20	800	20		
6. Ranger Station	7	40	1,800	45		
7. 4-Foot Cut	7	40	1,800	45		
8. Cummings Landing	7	25	1,080	27		
9. Dr. Starling	7	25	1,080	27		
10. 253 to Sem. Sp. Lge.	6	55	2,200	55		
11. Sem. St. Pk.	6	50	2,000	50		
12. Paradise Acres	6	25	1,000	25		
13. Lewis Pond	6	33	1,320	33		
14. Rays Lake Ramp	6	25	1,000			25
15. Rays Lake Upper	6	25	1,000	25		
16. Cypress Pond Ramp	7	25	1,000	25		
17. Sealy Landing	4	25	1,000	25		
18. Reynolds Landing	7	45	1,760	44		
19. Wid-Kin Ramp	6	45	1,800	45		
20. Carls Pass North	6	25	1,000	25		
21. Carls Pass	6	25	1,000	25		
22. Ga. Refuge Slough	6	25	1,000	25		
23. Carls Pass East	6	25	1,000			25
24. 310 to 10 Mile	6	75	3,000	75		
25. 10-Mile Still	6	16	640	16		
26. Hutchenson Ferry	6	25	1,000	25		
27. River Junction	4	100	6,200	155		
28. Chattahoochee Park	4	50	3,400		85	
Totals		998	44,000	942	108	50

Application was made by Mr. Roy Morris of Morris Helicopters, Elmore, Alabama, using a Bell 47 helicopter equipped with a model #1610 simplex spreader.

Target plants, all exotic species, in descending order of importance were hydrilla, *Hydrilla verticillata*, Eurasian watermilfoil, *Myriophyllum spicatum*, and elodea, *Egeria densa*. Some native plants that will be adversely effected include *Cabomba* spp., coontail, *Ceratophyllum demersum*, and giant cutgrass, *Zizaniopsis miliacea*.

The costs based on 40-lb pellets (2-lb a.i.) per acre are tabulated below:

Sonar pellets: 44,000 lb @ \$9.54 per lb	\$419,760.00
Application: \$0.40 per lb × 44,000 lb	17,600.00
Total herbicide and application cost	\$437,360.00

**Table 2**  
**Rating\* of Results of May 4, 6 and 7, 1987, Applications**

<i>Area</i>	<i>June 9 Rating</i>	<i>October Rating</i>
1. Desser Slough	9	6
2. Parramore Slough	9	9
3. Pear Orchard Run	9	8
4. Apalachee G.M.A.	8	7
5. Howells Ramp	8	9
6. Ranger Station	8	10
7. 4-Foot Cut	8	9
8. Cummings Landing	7	8
9. Dr. Starling	7	10
10. 253 to Sem. Sp. Lge.	9	10
11. Sem. St. Pk.	10	10
12. Paradise Acres	9	10
13. Lewis Pond	9	10
14. Rays Lake Ramp	8	10
15. Rays Lake Upper	7	10
16. Cypress Pond Ramp	6	9
17. Sealy Landing	3	5
18. Reynolds Landing	8	10
19. Wid-Kin Ramp	9	8
20. Carls Pass North	6	9
21. Carls Pass	8	9
22. Ga. Refuge Slough	7	9
23. Carls Pass East	8	9
24. 310 to 10 Mile	9	6
25. 10-Mile Still	9	9
26. Hutchenson Ferry	8	9
27. River Junction	5	2
28. Chattahoochee Park	5	2

\*A subjective rating is shown of the results of each treated plot with a numeric score or rating between 1 and 10. A rating of 1 is equal to no obvious results, and a rating of 10 equals optimum control of the target plant hydrilla, based on the results observed on that date.

Herbicide and application cost per acre	\$397.60
Effective length of control—2 years	
Cost of control per acre per year	\$198.80

Based on effective control only on treated areas, this tabulation does not take into account any additional control from dispersion of the herbicide. Assuming an overall control from dispersion of 4×, then the cost per acre would be approximately \$40.00 (treated acre + 4 additional acres = 5 acres divided into \$198.80 = \$39.76).

## GIANT CUTGRASS TREATMENT

A contract was let to treat 500 acres of giant cutgrass, *Zizaniopsis miliacea*, with 7 pints per acre of Rodeo herbicide. Due to the untimely death of the contractor, the treatment was not completed as planned.

A Corps airboat was then used, with a different resource Ranger each day, to treat 50 acres of cutgrass in the most critical areas, e.g., blind curves on small-boat channels, around Corps maintained ramps and piers, public use areas, marinas, etc.

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