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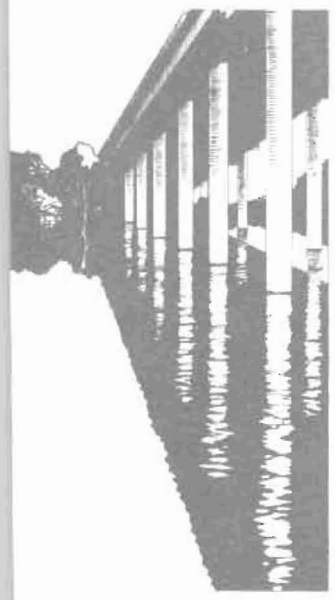

AQUATIC PLANT CONTROL RESEARCH PROGRAM

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PROCEEDINGS, 23RD ANNUAL MEETING, AQUATIC PLANT CONTROL RESEARCH PROGRAM

14-17 NOVEMBER 1988
WEST PALM BEACH, FLORIDA

June 1989

Final Report

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The 23rd Annual Meeting of the US Army Corps of Engineers Aquatic Plant Control Research Program was held in West Palm Beach, Florida, on 14-17 November 1988, 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.

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PREFACE

The 23rd Annual Meeting of the US Army Corps of Engineers Aquatic Plant Control Program was held in West Palm Beach, Florida, on 14-17 November 1988. The meeting is required by Engineer Regulation 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, MS.

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. E. Carl Brown was Technical Monitor for the Headquarters, US Army Corps of Engineers.

Ms. Billie F. Skinner, Program Manager's Officer, EL, was responsible for coordinating the necessary activities leading to publication. The report was edited by Ms. Jessica S. Ruff of the Information Technology Laboratory (ITL), WES. Ms. Betty Watson, ITL, designed and composed the layout.

Commander and Director of WES during preparation of this report was LTC Jack R. Stephens, EN. Technical Director was Dr. Robert W. Whalin.

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AGENDA

23rd Annual Meeting
US Army Corps of Engineers
AQUATIC PLANT CONTROL RESEARCH PROGRAM

West Palm Beach, Florida
14-17 November 1988

MONDAY, 14 NOVEMBER 1988

12:30 p.m. -5:00 p.m.	Registration – The Board Room (above main lobby)
6:30 p.m. -8:00 p.m.	Reception – Poolside

TUESDAY, 15 NOVEMBER 1988
General Session, Regency Room (A, B, & C)

7:30 a.m. -9:00 a.m.	Registration Continued (outside Regency Room)
8:00 a.m.	Call to Order and Announcements – J. L. Decell, Manager, Aquatic Plant Control Research Program Waterways Experiment Station (WES), Vicksburg, Mississippi
8:10 a.m.	Welcome to Jacksonville District – COL Robert L. Herndon, Commander, USAE District, Jacksonville, Florida
8:25 a.m.	Comments by Dr. Robert W. Whalin – Technical Director, WES Vicksburg, Mississippi
8:40 a.m.	US Army Engineer District, Jacksonville – G. Gren, Chief, Operations Division Jacksonville, Florida
8:55 a.m.	Presentation by Dr. Ernest L. Corley – Area Director, South Atlantic Area Agricultural Research Service, Athens, Georgia
9:10 a.m.	TVA Aquatic Plant Research and Cooperative Efforts with the Aquatic Plant Control Research Program – A. L. Bates, Tennessee Valley Authority Muscle Shoals, Alabama
NOTE:	Computer model demonstrations - Board Room (above lobby) 9:00 a.m. - 4:00 p.m., 15 Nov 9:00 a.m. - Noon, 16 Nov

9:25 a.m.	Hydrilla Program in California, Current Status – N. Dechoretz, California Department of Food and Agriculture, Sacramento, California
9:40 a.m.	Plan of Study for Determining Economic Values of Aquatic Plant Management – J. Henderson, WES
9:55 a.m.	BREAK

Biological Control of Aquatic Plants
– A. Cofrancesco, WES, Presiding

10:20 a.m.	An Overview of the Biocontrol Program – A. Cofrancesco, WES
10:30 a.m.	Foreign Exploration - Insect Biocontrol for Hydrilla – J. Balciunas, University of Florida, Gainesville
10:45 a.m.	Quarantine Work - Insect Biocontrol for Hydrilla – G. Buckingham, USDA, Gainesville, Florida
11:00 a.m.	Release and Establishment of Insect Biocontrol on Hydrilla – T. Center, USDA, Fort Lauderdale, Florida
11:15 a.m.	Pathogen Study on Hydrilla – G. Joye, WES
11:30 a.m.	Status of Eurasian Watermilfoil Pathogen – H. B. Gunner, University of Massachusetts, Amherst
11:45 a.m.	Pathogen Study on Eurasian Watermilfoil – L. Winfield, WES
12:00 Noon	LUNCH
1:00 p.m.	Status of Insect Biocontrol Agents on Waterlettuce – D. Habeck, University of Florida, Gainesville
1:15 p.m	Genetic Engineering: Host Specificity – S. Kees, WES
1:30 p.m.	Impact of Chemicals on Waterhyacinth Insects – M. Grodowitz, WES
1:45 p.m.	Expert System Development – H. Lemmon, USDA-ARS, Albany, California
2:00 p.m.	USAE Division/District Working Session (Polo Room E & F)
2:00 p.m.	Federal Aquatic Plant Management Working Group (Polo Room D)

WEDNESDAY, 16 NOVEMBER 1988
General Session, Regency Room (A, B, & C)

Chemical Control Technology Development
– H. Westerdahl, WES, Presiding

8:00 a.m.	Overview - Chemical Control Technology – H. Westerdahl, WES
8:15 a.m.	Herbicide Application Technique Development for Flowing Water – K. Getsinger, WES
8:30 a.m.	Dye Dispersion and Relation to Herbicide Contact Time with Plants – A. Fox, Center for Aquatic Plants, University of Florida, Gainesville
8:45 a.m.	Herbicide Concentration/Exposure Time Relationship - Endothall/Hydrilla and Eurasian Watermilfoil – R. Green, WES
9:00 a.m.	Carbohydrate Partitioning in Hydrilla and Waterhyacinth – K. Luu, WES
9:15 a.m.	Bioassay Development for Assessing Plant Growth Regulator Effects on Submersed Aquatic Plants – C. Lembi, Purdue University, West Lafayette, Indiana
9:30 a.m.	BREAK

Special Session - Lake Okeechobee

9:45 a.m.	A History of Lake Okeechobee – L. Mitchum, Okeechobee, Florida
10:15 a.m.	Roland Martin, Clewiston, Florida
10:45 a.m.	Algae of Lake Okeechobee: The Good, The Bad and The Ugly – E. Phlips, University of Florida, Gainesville
11:00 a.m.	A GIS Approach to Ecological Studies of Wetlands of Lake Okeechobee – W. Kitchens, University of Florida, Gainesville
11:15 a.m.	Environmental Impacts of Fluridone Application in Lake Okeechobee, Florida – K. Langeland, University of Florida, Gainesville
11:30 a.m.	LUNCH
12:30 p.m.	Field Trip to Lake Okeechobee
5:00 p.m.	Dinner Cruise
9:00 p.m.	Arrive at Palm Hotel

THURSDAY, 17 NOVEMBER 1988
General Session, Regency Room (A, B, & C)

Ecology of Submersed Aquatic Plant Species
—J. Barko, WES, Presiding

8:00 a.m.	Interactive Effects of Environmental Variables on the Growth of Submersed Aquatic Macrophytes —J. Barko, WES
8:15 a.m.	Competitive Interactions of Submersed Aquatic Macrophytes in Relation to Water Chemistry and Other Environmental Conditions —R. M. Smart, WES
8:30 a.m.	Effects of Environment on Growth and Tuber Formation in Hydrilla —D. McFarland, WES
8:45 a.m.	Effects of Benthic Barriers on Substratum Conditions: An Initial Report —D. Gunnison, WES
9:00 a.m.	Phenolics in Aquatic Macrophytes: Implications for Studies of Community Interactions —W. C. Kerfoot, University of Michigan, Ann Arbor
9:15 a.m.	Habitat Value of Submersed Aquatic Macrophytes: Invertebrates —A. C. Miller, WES
9:30 a.m.	Habitat Value of Submersed Aquatic Macrophytes: Fish —K. J. Killgore, WES
9:45 a.m.	BREAK
10:00 a.m.	Effects of Submersed Aquatic Macrophytes on Water Quality in the Potomac River: An Update —V. Carter, US Geological Survey, Reston, Virginia
10:15 a.m.	Effects of Submersed Aquatic Macrophytes on Sedimentation in the Potomac River —H. L. Eakin, WES
10:30 a.m.	Effects of Submersed Aquatic Plants on Sedimentation in Eau Galle Reservoir: An Update —W. F. James, WES

Computer-Aided Simulation Procedures for Aquatic Plant Management
—R. M. Stewart, WES, Presiding

10:45 a.m.	Introduction and Overview —R. M. Stewart, WES
11:00 a.m.	Applications of INSECT, A Computer Model of Waterhyacinths and *Neochetina* —F. G. Howell, University of Southern Mississippi, Hattiesburg
11:15 a.m.	Validation Studies in Texas for INSECT —M. J. Grodowitz, WES
11:30 a.m.	Laboratory and Field Validation of 2,4-D Fate Algorithms Included in HERBICIDE (Version 1.0) —J. H. Rodgers, Jr., University of North Texas, Denton
11:45 a.m.	An Improved Hydrilla Growth Model - Description and Validation with Florida Field Data —J. W. Wooten, University of Southern Mississippi, Hattiesburg
12:00 Noon	A Prototype Environmental Data Base for Aquatic Plant Management —M. R. Kress, WES
12:15 p.m.	LUNCH
1:30 p.m.	1988 Activities, Aquatic Plant Control Operations Support Center and Report of Tuesday's Division/District Working Group Sessions and General Forum —W. Zattau, USAE District, Jacksonville
3:00 p.m.	Adjourn 23rd Annual Meeting
3:30 p.m.	FY90 Civil Works R&D Program Review, Directorate of R&D, HQUSACE (Polo Room D & F - Corps of Engineers representatives only)

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23rd Annual Meeting
US Army Corps of Engineers
AQUATIC PLANT CONTROL RESEARCH PROGRAM

West Palm Beach, Florida
14-17 November 1988

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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
cubic yards	0.7645549	cubic metres
Fahrenheit degrees	5/9	Celsius degrees or kelvins*
feet	0.3048	metres
gallons per acre	0.00093	cubic decimetres per square metre
inches	2.54	centimetres
miles (US statute)	1.609347	kilometres
ounces (US fluid)	0.02957353	cubic decimetres
pounds (mass)	0.4535924	kilograms
pounds (mass) per acre	0.000112	kilograms per square metre
pounds (mass) per gallon	0.12	kilograms per cubic decimetre
quarts (US liquid)	0.9463529	cubic decimetres
square feet	0.09290304	square metres
square miles	2.589998	square kilometres
tons (mass) per acre	0.22	kilograms per square metre

*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: $K = (5/9)(F - 32) + 273.15$.

23rd 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 to 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 Headquarters, US Army Corps of Engineers; the Program Manager, APCRP; and representatives of the operations elements of various CE Division and District 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, 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, Engineer Circulars, and Engineer Manuals. 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 23rd Annual Meeting held in West Palm Beach, Florida, 14-17 November 1988.

TVA Aquatic Plant Research and Cooperative Efforts with the Aquatic Plant Control Research Program

by
A. Leon Bates*

The Tennessee Valley Authority (TVA) and various Divisions of the US Army Corps of Engineers (USACE) have, for several years, initiated and implemented cooperative projects because of their mutual interests in water resources management. Aquatic plant management efforts accomplished cooperatively by the Aquatic Plant Control Research Program (APCRP) and other agencies, such as TVA, have been coordinated through the Interagency Research Coordinating Conference. Since 1972, the Federal Aquatic Plant Management Working Group (FAPMWG), formed under the umbrella of the interagency coordinating conference, has helped to coordinate aquatic plant research among the Federal agencies.

Although TVA, USACE, US Bureau of Reclamation, and Bonneville Power Administration are the primary participants as specified by the coordination mandate, the FAPMWG has involved other Federal agencies, such as the US Department of Agriculture, Agricultural Research Service, and the US Environmental Protection Agency (USEPA), as well as State agencies and university participants, to further facilitate research coordination. The overall objective is to ensure that aquatic plant control research is coordinated among the public agencies and not duplicated while expediting transfer of control technology to aquatic plant management specialists. The APCRP has been instrumental in the comprehensive development of aquatic plant control and subsequent technology transfer, and the FAPMWG members and participants have benefited significantly from the extensive data base.

Several applied research studies have been conducted jointly by TVA and the APCRP. An example includes the herbicide concentration/exposure time relationships (Getsinger 1988) conducted in the flume channels at TVA's Aquatic Research Laboratory facility located at Browns Ferry Nuclear Plant. Laboratory analyses of herbicide residues for this study were completed by TVA's Laboratory Branch, Chattanooga, Tennessee.

A joint field study between TVA and the APCRP was initiated in 1987 and involved the use of popnets to evaluate the density and standing crop of fish in colonies of Eurasian watermilfoil in Guntersville Reservoir (Killgore et al. 1988). These studies serve to provide data applicable to the management of aquatic vegetation which will result in the enhancement of the fisheries resource. Both APCRP and TVA biologists were involved with this field study, as well as fisheries

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biologists from the Alabama Game and Fish Commission. This study serves as a typical example of cooperative Federal and State efforts dealing with aquatic vegetation management studies which have implications for enhancing technology transfer to numerous public reservoir projects.

Previous joint operational control programs have been implemented by TVA and USACE both in the assessment phase and weed control operations. The TVA Aviation Unit has conducted aerial photography overflights used for aquatic vegetation assessment within Mobile Bay in the Mobile District, USACE, and Lake Barkley in the Nashville District, USACE. Under the provisions of existing interagency agreements, TVA's Aviation Unit applied granular fluridone herbicide by helicopter to an infestation of hydrilla in the Aliceville Pool, Tennessee-Tombigbee Waterway, soon after its discovery in late summer 1988.

Historically, staff from WES and the Aquatic Plant Control Operations Support Center have cooperated with TVA in several releases of flea beetles (*Agasicles hygrophila*) and thrips (*Amynothrips andersonii*) for alligatorweed control in the Tennessee Valley. Unfortunately, the insects did not successfully overwinter because of climatic conditions, and releases have been discontinued.

Additional cooperative studies and technology transfer projects have included:

a. Previous cooperative efforts to expand the TVA 2,4-D aquatic label for Eurasian watermilfoil control to include USACE and Bureau of Reclamation projects.

b. Efficacy testing of aquatic herbicides under the provisions of Experimental Use Permits conducted to secure USEPA-approved aquatic herbicide labels.

c. Transfer of HARVEST and STOCK models from WES to TVA for computer simulation of these plant control techniques in TVA reservoirs.

d. Field surveys of Eurasian watermilfoil populations in the Tennessee Valley to screen for presence of pathogens and insects that show promise as a biological control agent.

In October 1988, a Congressional delegation visited Guntersville Reservoir in northeast Alabama, one of the most heavily weed-infested reservoirs in the TVA system, to view the problems caused by dense aquatic vegetation. Technical staffs from the USACE and TVA were subsequently charged to pool their technical expertise in aquatic weed control and to develop a joint proposal for a large-scale test demonstration weed control project on Guntersville Reservoir. The technical staffs from TVA, WES, and the Nashville District, USACE, developed a draft joint proposal for agency review. The status of the joint proposal will be determined in early calendar year 1989.

In summary, the research coordination and technology transfer with the USACE have allowed TVA to more effectively and economically manage aquatic weeds in the Tennessee Valley. Likewise, some of the weed control technologies developed

by TVA and the specialized research facilities available within TVA have contributed to enhancing water resource management capabilities throughout the Nation.

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Hydrilla Program in California: Current Status

by
Nathan Dechoretz*

INTRODUCTION

In 1976, hydrilla (*Hydrilla verticillata* Royle) was first found in California infesting Lake Ellis in Marysville (Yuba County). This 35-acre lake was drained, hydrosoil removed, and the lake bottom treated with herbicides prior to refilling. The procedure was a success, and hydrilla was declared eradicated in 1984 following 3 years of intensive survey to confirm the absence of hydrilla. Subsequent to the initial discovery, hydrilla has been found infesting various aquatic sites in 13 California counties. The following paragraphs discuss the current status of the hydrilla eradication programs in three northern California counties (Sonoma, Shasta, and Calaveras).

SONOMA COUNTY

In 1984, hydrilla was found in Spring Lake, a 72-acre flood control reservoir for the City of Santa Rosa. Complete knockdown of hydrilla and other submersed aquatic plants including Eurasian watermilfoil (*Myriophyllum spicatum* L.) and sago pondweed (*Potamogeton pectinatus* L.) was achieved 2 weeks after a subsurface application of KOMEEN (ethylenediamine complex of copper) at a rate of 16 gal/acre. The lake was then drained and, after a brief drying out period, a massive excavation project was initiated. Over a period of 5 months, approximately 300,000 cu yd of soil was removed from the lake, transferred to large borrow pits, and covered with clean soil.

After the hydrosoil removal component of the eradication project was complete, selected areas of the lake bottom were treated with metham at a rate of 1 qt per 100 sq ft. After completing the metham treatment, the entire lake bottom was treated with diuron at 48 lb active ingredient (ai)/acre and refilled after the herbicide had been fixed in the lake bottom.

The entire lake was surveyed by scuba divers annually from 1986 through 1988. No hydrilla was detected during any of the surveys, and eradication was declared in November 1988. Cost of the eradication project was approximately $1.2 million.

*California Department of Food and Agriculture, Sacramento, California.

SHASTA COUNTY

In 1985, hydrilla was found in seven ponds adjacent to the Sacramento River near Redding, California (see Table 1). Three of the ponds were treated with

Table 1
Shasta County Ponds Infested with Hydrilla

Pond	*Date of Find*	*Surface Area acres*	*Average Depth ft*
Anderson River Park*	9/13/85	0.2	ND**
North Market Street I*	9/21/85	1.5	ND
North Market Street II*	9/23/85	0.5	ND
Shea Sand and Gravel I*	9/24/85	3.0	ND
Shea Sand and Gravel II	9/24/85	6.0	10
Fish and Game	8/27/86	10	8
Tenny	9/25/85	16	5
Raley's I	7/03/86	7.5	8
Raley's II	7/30/86	1.0	4
City Pond	7/24/86	6	12

*Ponds drained and filled in with clean soil.
**Not determined.

KOMEEN to remove established plants and were treated 1 month later with dichlobenil at 10 lb ai/acre to prevent regrowth. The remaining four ponds were treated with KOMEEN, drained, treated with dichlobenil or diuron, and then filled in with clean soil.

In 1986, an extensive survey of the area around the infested ponds resulted in the discovery of four new infestations. Since established plants were present in five ponds, KOMEEN was applied, followed by an application of dichlobenil 2 weeks later. The remaining two ponds were treated with dichlobenil only. Good control was obtained in most of the ponds. However, significant regrowth did occur during the fall, and retreatment with KOMEEN was required.

Based on a recommendation from a Science Advisory Panel, fluridone (5P formulation) was applied in 1987 at 4 lb ai/acre to six of the infested ponds. The 100-acre pond was treated with dichlobenil at 15 lb ai/acre. Excellent season-long control of hydrilla was obtained in ponds treated with fluridone. Plants collected at the end of the growing season were stunted and chlorotic, indicating that herbicide activity was still present at least 5 months after treatment.

In contrast to the control obtained in the fluridone-treated ponds, the efficacy of the dichlobenil treatment was not satisfactory. Surveillance activity in this pond at the end of the growing season produced viable plants that did not exhibit symptoms of herbicidal activity. KOMEEN was applied in the fall to the dichlobenil-treated pond to remove the existing vegetation and inhibit tuberization.

In conjunction with the fluridone applications, a monitoring study was conducted to determine the dissipation of fluridone in the treated ponds. Duplicate 1-ℓ samples were collected pretreatment and 1, 7, 14, 21, 30, 60, 90, 120, 150, and 180 days after treatment from each fluridone-treated pond. The samples were collected at middepth, frozen, and transported to the California Department of Food and Agriculture (CDFA) laboratory for analysis.

The results of the analysis are presented in Table 2. Maximum concentration of fluridone was obtained 7 days after treatment and remained relatively constant for 90 days. The excellent hydrilla control obtained with fluridone is probably attributed to the persistence of fluridone in the pond water.

Table 2
Concentration of Fluridone in Water of Hydrilla-Infested Ponds After Application of Sonar 5P

Days After Treatment	*Fluridone Concentration** *ppbw*
1	20.7 (±4.3)
7	47.0 (±16.2)
14	42.5 (±13.6)
21	34.5 (±9.5)
30	30.7 (±6.8)
60	21.2 (±5.6)
90	19.5 (±6.4)
120	13.0 (±4.0)
150	7.6 (±3.4)
180	7.2 (±3.1)

*Value represents mean ± standard error; n = 6.

Since dichlobenil did not provide satisfactory control of hydrilla in the 100-acre pond, fluridone was applied in May 1988 to 30 percent of the pond at a rate of 4 lb ai/acre. The other six ponds were retreated in their entirety with fluridone at the same time. Excellent inhibition of hydrilla growth was obtained in all the treated ponds. Although slight regrowth of hydrilla was found in two of the treated ponds, the plants were chlorotic and stunted. As a precautionary measure, these two ponds were treated with KOMEEN to remove the plants.

The herbicide monitoring program was expanded in 1988. Samples were collected to evaluate the fate of fluridone in water and soil. Furthermore, water samples collected from the fluridone-treated ponds will be analyzed for the possible presence of N-methylformamide, a potential photolysis metabolite of fluridone.

CALAVERAS COUNTY

Hydrilla was first detected in Calaveras County when a landowner submitted an aquatic weed sample to the CDFA on May 26, 1988. As a result of this discovery, an intensive delimiting survey resulted in hydrilla being found in six more ponds

(Table 3). The ponds are located on the Bear Creek drainage basin. Bear Creek starts in northwestern Calaveras County, flows west through San Joaquin County, and eventually empties into the Sacramento-San Joaquin delta at Disappointment Slough. Scattered hydrilla plants were found in four areas within Bear Creek in Calaveras County. However, no plants were found in San Joaquin County or in Disappointment Slough. How hydrilla was introduced in the Bear Creek area is not known at this time.

Table 3
Calaveras County Ponds Infested with Hydrilla

Pond	*Surface Area acres*	*Average Depth ft*	*Degree of Infestation*	*Other Weeds*
Baker	17.0	6	Moderate	*Ceratophyllum*
Perock	0.5	4	Moderate-dense	*Ceratophyllum* *Najas*
Kesterson	3.0	3	Light	*Potamogeton* *Najas* *Myriophyllum* *Ceratophyllum*
Kraft	0.6	4	Dense	None
Whalen	0.4	2	Dense	None
Ghiradelli	0.2	2	Dense	*Ceratophyllum* *Najas*
Holmes	0.1	1	Moderate-dense	*Potamogeton* *Najas*
Kerstan I	0.25	5	Dense	*Potamogeton* *Ceratophyllum*
Kerstan II	0.1	4	Light	None

In addition to the infestations within the Bear Creek area, hydrilla was detected on August 30, 1988, in two ponds approximately 20 miles east of the seven infested ponds associated with Bear Creek. In contrast to the Bear Creek infestation, the source of hydrilla in these two ponds is known. Approximately 10 to 15 years ago, the landowner purchased some ornamental waterlilies and placed them in the pond (Kerstan II). As has occurred in other areas in California, hydrilla was probably associated with the waterlilies as packing material around the roots and was introduced into the pond simultaneously. Once established in Kerstan II, hydrilla easily infested Kerstan I, which is approximately 25 ft downstream.

As described previously, KOMEEN and fluridone were applied to the ponds to kill the established plants and prevent regrowth, respectively. Since eight of the ponds were relatively small, fluridone was applied at 2.0 lb ai/acre. Baker Pond

was treated at the 4.0 lb ai/acre rate. Fluridone was applied 2 weeks after the KOMEEN applications. Control for the remainder of the growing season was attained, and additional herbicide applications were not required.

A herbicide monitoring program was conducted for the Calaveras County hydrilla eradication project. Protocols for sampling were similar to those established for the Shasta County project. Results of these analyses, in conjunction with those from Shasta County, will be presented at a later date.

Due to unseasonably dry conditions during 1988, the total area within the Bear Creek drainage basin infested with hydrilla could not be determined. Approximately 75 percent of the area normally containing surface water was dry as early as June. By the end of October, this condition had increased to over 95 percent. At least half of the infested ponds had almost completely dried up. Significant areas within Bear Creek and in adjacent ponds may have had hydrilla prior to the drought. If normal rainfall occurs in 1989 and viable nondormant hydrilla tubers are present, the infested area may be substantially larger than the area infested in 1988. Extensive survey and detection activities will be initiated in March and continued through the summer to determine the extent of the infestation. Herbicides and various mechanical control methods will be used to eradicate hydrilla from infested sites.

Plan of Study for Determining Economic Values of Aquatic Plant Management

by
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INTRODUCTION

Webster says that economics is "a social science concerned chiefly with description and analysis of the production, distribution, and consumption of goods and services." Economic analysis leads to investigation of human choices to determine the most desirable, efficient, and effective way to accomplish personal or societal objectives. Applied to public programs, economic analysis is used to ensure that public programs are implemented to make the best use of public revenues and achieve the goals/objectives of the programs. Because there are always more program needs than there is funding or tax base to meet the goals, economic analysis is used to compare the merits or benefits of funding one project over another. The whole area of Benefit Cost Analysis derived from this need to compare benefits versus costs.

Cost and benefit analysis has proved challenging in evaluating the merits of competing natural resource programs. There is uncertainty regarding application and the long-term effects of the control technologies. The research and documentation of field applications reduces this uncertainty, to the extent that it can be controlled. Another uncertainty is the costs of implementing the control programs, such as the availability of organisms and control substances. The uncertainty of the costs is also being addressed by research and field applications. In addressing a natural resource challenge such as aquatic plant control, a great deal of research, time, and experience is required to get to the point where these costs and benefits can be reliably estimated.

VALUATION OF AQUATIC PLANT MANAGEMENT

This paper sets out a Plan of Study for establishing the value of aquatic plant management. Through the use of market and nonmarket valuation methods and public perception information, decisions on aquatic plant management can be improved. Valuation requires consideration of the range of impacts of control efforts and the economic benefits and costs associated with those impacts.

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Benefits and costs of aquatic plant control

Resources have economic value to the extent that they (a) provide consumer satisfaction or enjoyment, i.e., provide a desirable service, and (b) are scarce. Economic value includes several components--both tangible and intangible--that when combined are the total value of a resource, good, or service (Loomis and Peterson 1984). For aquatic plant control, recreation and commercial values are user or direct benefits resulting from increased use of a waterway. Option, existence, and bequest values are nonuser benefits obtained by nonusers of the waterway, or society in general. These nonuser values refer to the availability for potential future use and to the knowledge that the free-flowing waterway exists, or is available for use (Loomis and Peterson 1984).

From an agency standpoint, decisions have to be made on the basis of the overall benefits and costs to society. This requires that an understanding of societal benefits and costs be used. The benefits of an open waterway in terms of recreation, aesthetics, and habitat are economic benefits to be considered and included along with the project purpose benefits of commercial navigation, flood control, and water supply. Additionally, public response to the cleared waterway will include secondary effects such as changes in land use, recreation use patterns, and improvement of aesthetic qualities.

The benefit of aquatic plant control is the willingness to pay for increases in recreation, navigation, water supply, and other services. These direct benefits, associated with providing the services, are often readily measurable by economists. The offsite or nonuser benefits, which are the intangible option, existence, and bequest values, are not usually evaluated in Corps analyses. These benefits are valued by the maximum willingness to pay to avoid their loss.

The direct costs of aquatic plant control include equipment, time, and the costs of other resources used for aquatic plant control that cannot be put to other purposes. Just as there are secondary and nonuser benefits in addition to navigation and other project benefits, there are also costs which are not accounted in looking strictly at the labor, equipment, and supply costs. These costs are the opportunity costs of using public monies for purposes other than plant control. Because decisions are made not to fund plant control projects, there are costs in terms of such things as displacement of recreation use, loss of access to lake areas, fish and wildlife habitat losses, loss of flood control benefits, and diminished quality of recreation experience.

Decisionmaking

While these other-than-project benefits and opportunity costs are more difficult to quantify and place a dollar value on, it is these benefits and costs which likely have the greatest impact on the public at large. An accounting of these benefits and costs must be made in decisions on the level of required control or the control strategy to be used, and decisions of using one control technology over

another. The point is that better, more informed decisions can be made if all the impacts and effects of aquatic plant control are identified. That is, if the costs (of both opportunity and implementation) and the benefits from cleared plants and from the secondary impacts, e.g., improved recreation, are identified, then better, more informed decisions will be made.

In recent years, the development of methods for valuation of natural resource benefits has lessened the argument that these costs and benefits cannot be quantified. These natural resource economic methods determine the value of natural resource amenities and services, e.g., aesthetics or recreation, through procedures that determine the public's willingness to pay for such things as aesthetics, bag or catch for fish and wildlife, or increased real estate values due to more desirable resources. This last example is a natural resource service (i.e., residential or commercial location) being sold in a market, whereas recreation experience and aesthetics require nonmarket valuation techniques.

An economic analysis of aquatic plant programs can provide decisionmakers with better information. Decisions can be made with an understanding of public perceptions of different technologies and willingness to pay for varying levels of control or management. Information on all the benefits and costs associated with aquatic plant control will lead to better allocation of scarce resources. This benefit and cost information and willingness to pay information can be used to determine the:

a. Value of the program, from a local, District, state, or national perspective.

b. Benefits and costs associated with aquatic plant control, including secondary or indirect benefits and costs.

c. Economic trade-offs of different technologies.

PLAN OF STUDY

Phase I - Literature review

Development of appropriate procedures for determining economic values of aquatic plant management will be accomplished in four phases. Phase I entails a literature review to identify the critical attributes to consider in aquatic plant control valuation. Only a few studies have addressed valuation of aquatic plant control efforts. In addition to the valuation studies, there is a need to examine the literature or documentation on aquatic plants to identify the decision criteria, e.g., water demand, used to formulate the implementation plans for aquatic plant control. This Phase I literature review is basically an in-the-office effort.

Phase II - Determination of impacts and public perceptions

Phase II involves determining the benefits and costs of aquatic plant control and determining public perceptions. This phase requires data collection and is to be performed in conjunction with District personnel and the Waterways Experiment Station (WES), with requisite public input. Phase II involves the identification of all effects, impacts, and changes attendant to aquatic plant problems and management and the public perceptions of plant problems and management technologies. This information will be used to develop the valuation framework, which is similar to an environmental assessment framework. The framework identifies the human and natural resources affected by the aquatic plant problems and control technologies, and determines the limits of valuation.

As indicated above, there has never been an effort to identify and evaluate all of the changes, impacts, and effects that result from aquatic plant problems and management plans. For instance, the impacts to recreation caused by aquatic plants result in persons being displaced to other recreation areas, not recreating at all, or not participating in some recreation activities. The aesthetics enjoyed by nearby residents are likewise affected by aquatic plants.

Recreation and aesthetics have been valued for other resources through use of nonmarket valuation methods, such as the Contingent Valuation Method (CVM), that determine the willingness to pay to preserve or maintain certain levels of resource conditions (Bergstrom, Dillman, and Stoll 1985; Titre et al. 1988).

Public perceptions. Critical to understanding the public value of aquatic plant control is to identify the public perceptions of problem and nonproblem plant levels. Although high levels of control are possible, willingness to pay will be minimal for levels of control beyond what the public perceives as necessary or desirable. Another important public consideration is perceptions of and preferences for different management technologies. Public perceptions of the management technologies available to control an aquatic plant problem will affect the willingness to pay for the different management efforts.

As important as the willingness to pay is for calculating the benefits, it is also critical in formulating management plans to be able to determine what management efforts will and will not be supported by the public. Information about public support for different types of management technologies can assist in formulating plans that will be supported by state and local cost-sharers. In a recent water quality study on two lakes in New York, recreation users (swimmers, boaters) were asked to agree or disagree with the statement "Algae or aquatic plants should be cleared out with chemicals." Of the respondents, 85.3 percent either disagreed or strongly disagreed (Henderson 1988). When the question was asked in the negative way ("Chemicals should not be used in the lake for plant control"), a slightly lower percentage (77.9 percent) agreed or strongly agreed. These particular lakes are in watersheds made up primarily of agricultural lands,

and receive heavy inflow from cropland and dairy farm runoff. The use of land use controls to limit the inflow of nutrients to the reservoirs received high support, with 92.8 percent either agreeing or strongly agreeing with "Zoning and land use controls should be used to help keep the streams and lakes clean."

Valuation framework. The Phase II information is brought together in the form of a valuation framework. By identifying the primary and secondary effects and impacts, the benefits and costs of aquatic plant control are also identified. It is recognized that while identification of some secondary effects may be possible, the quantification and valuation of these effects may not be possible. However, it is important to be able to identify all benefits and costs, even if quantification is not readily possible.

Phase III - Valuation methods

Description. Phase III of this work is the identification of market and nonmarket valuation methods appropriate for aquatic plant control. Many of the impacts of aquatic plant control efforts are intangible, e.g., recreation or aesthetics. For these intangible benefits, there are a number of nonmarket valuation methods. Nonmarket valuation techniques attempt to estimate the net economic value for resources for which market prices are inadequate or unavailable. Recreation benefits, for instance, are measured by willingness to pay for the recreation services (Headquarters, Department of the Army 1983). To date, the only study to identify valuation of recreation benefits related to aquatic plant control is the study of fishing conducted at Orange and Lochloosa Lake, Florida (Milon, Yingling, and Reynolds 1986).

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 decisionmaking. However, because the public expressed an interest in preservation services through protection or conservation legislation for specific resources, efforts are being directed toward estimating willingness to pay for these services.

In recent years, a number of studies have determined willingness to pay for use or preservation of natural resources, and these efforts have applicability in considering the intangible values of aquatic plant control. Titre et al. (1988) valued wetland recreation in south Louisiana through the use of a CVM questionnaire. Bergstrom, Dillman, and Stoll (1985) determined the values for preservation of prime farmland in South Carolina; Bowker and Stoll (in press) examined such values for whooping cranes; and Walsh, Loomis, and Gillman (1984) have examined preservation values for wilderness areas. These studies identified the attributes of the resource that are important, quantified the attributes, and then elicited willingness to pay values for changes to the resources.

Market valuation. The market valuation of natural resources is usually limited to increases in benefits of navigation, water supply, and flood control. The impact of natural resource conditions on the market value of such things as real estate is

often evident. Differing market values and controversies over wetland development and other resource conflicts make it clear that the quantity, quality, and type of natural resources give rise to market values. In the case of wetland development, it is obvious that the presence of natural wetland characteristics (e.g., water frontage, direct access to water) contributes to determining the sale price of wetland development sites.

A market-based method called the Hedonic Price Technique has been used to determine the value of natural resource characteristics. This approach uses actual land transfer and sale information (such as lot size, water frontage, and access to water) to identify the willingness to pay for these characteristics. This type of analysis enables valuation of the natural resources characteristics based on market data. This Hedonic Price Technique was used to develop a regression analysis of a subdivision developed in a wetland (Batie and Mabbs-Zeno 1985). Using the characteristics of the different lots and the sale price of the lots, a regression equation was developed to show the influence or importance of the different characteristics to the sale price. This analysis determined, for instance, that consumers were willing to pay $4,180 for a lot on a canal but $7,410 for a lot on open water. Lots adjacent to wetlands are valued at $1,120 less than lots not adjacent to wetlands. Other market analysis methods such as econometric land market analysis (Luken 1976) have been used to place a value on residential development in wetlands.

These market-based valuation methods will be examined for applicability to aquatic plant control. The use of natural resource valuation methods for different resources must be used with methodological care and understanding of the differences in resources. For instance, in use of the Hedonic Price Technique for wetland development, the valuation is of naturally occurring characteristics, e.g., water access. Use of this approach in aquatic plant control requires care because that valuation is of a man-induced rather than a natural condition, i.e., exotic plants.

Examination of market and nonmarket methods for use in aquatic plant control valuation will consider the suitability of the methods in terms of (a) appropriateness for use in District decisionmaking and planning and (b) appropriateness of methods for the water and land resources and the impacts resulting from aquatic plant problems and management. This evaluation of methods will be made by field representatives, resource economists, and personnel experienced in the valuation of natural resources and management programs. If the evaluation of methods shows that the market and nonmarket methods for valuation of natural resources require adaptation or that new methods are required, methods will be adapted or developed to meet the needs for valuation of aquatic plant services. Method development or adaptation will be a joint effort by natural resource economists, WES, and District personnel. The involvement of District personnel is critical in such an effort to ensure that the methods are implementable within

District resources and that the valuation results can be used in District decisionmaking.

Phase IV - Field testing and development of field guidance

Phase IV will be field testing and use by the Districts of the methods identified or developed in Phase III. The field tests will be coordinated so that, after completion, the evaluation of the field tests will form the basis for developing guidance for valuation of aquatic plant control. The emphasis in the guidance document will be on use of the valuation methods at all levels of aquatic plant management. The guidance will show how to use valuation information to improve decisions on level of control and determine trade-offs of different management strategies, based on public willingness to pay and perceptions of aquatic plant problems and control technologies.

SUMMARY

This paper sets out a Plan of Study for determining economic values of aquatic plant management through identification or development of valuation methods for aquatic plant valuation, field testing of the methods, and development of guidance for use of the methods. Aquatic plant control efforts produce a number of services valued by the public. A cleared waterway, with aquatic plants managed at nonproblem levels, is an important resource to the public, valued for recreation, aesthetic, and preservation values, in addition to the navigation, water supply, and flood control benefits. Better decisions can be made at all levels of aquatic plant management by considering the costs and benefits of all the services, both tangible and intangible, provided directly and indirectly by aquatic plant control efforts.

Market and nonmarket valuation methods have been developed for the economic services provided by natural resource management programs. These valuation methods will be evaluated for use in aquatic plant management. Existing valuation methods will be adapted or new methods developed to meet the needs and characteristics of Corps aquatic plant management. After field testing of the identified or developed methods, a guidance document will be developed. The guidance document will present the valuation methods to be used in aquatic plant management and will show how valuation information can be used to improve decisions on such things as control strategies and levels of control, and to determine trade-offs of different management approaches.

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BIOLOGICAL CONTROL OF AQUATIC PLANTS

Biological Control Technology Development: An Overview

by
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DIRECT ALLOTTED RESEARCH FOR FISCAL YEAR 1988

The FY 88 direct allotted biological research was apportioned among seven work units, which are described below.

- ***Biological Control of Hydrilla Using Insects.*** *Hydrellia pakistanae*, which was first released in Florida in 1987, has been distributed to five sites, while *Bagous affinis*, also released in 1987, has been distributed to seven sites in Florida. Quarantine research has been completed on *Hydrellia balciunasi* and *Bagous* n. sp. from Australia. We are awaiting a response from the US Department of Agriculture Technical Advisory Group (USDA-TAG) on Biological Control of Weeds so that a release schedule can be finalized for FY 89. The overseas research aspect of this work unit has been examining the host specificity of three moths that feed on hydrilla in running waters. Two of these species appear specific, and work will continue on these moths in FY 89.

- ***Biological Control of Eurasian Watermilfoil Using Plant Pathogens.*** The Waterways Experiment Station (WES) and EcoScience Laboratories have produced numerous commercial formulations of the fungus *Mycoleptodiscus*. Testing was conducted at WES to determine the most effective commercial formulation. A large-scale field study will be conducted in FY 89 to test the most effective formulation under field conditions.

- ***Biological Control of Hydrilla Using Plant Pathogens.*** Laboratory and greenhouse studies have indicated that the fungus *Rhizoctonia* sp. collected in Texas is extremely effective in reducing hydrilla. A small-scale replicated field test was conducted in October 1988 to evaluate this fungus under field conditions. A significant reduction in biomass was documented 4 weeks after inoculation, when treated plots were compared with control plots.

- ***Biological Control of Waterlettuce with Insects.*** Quarantine research has been completed on specificity of the moth *Namangana pectinicornis*. A petition for the release of this biocontrol agent is being submitted to the USDA-TAG. This insect is extremely specific and should be available for field release early in the FY 89 growing season.

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- ***Management of Submersed Aquatic Plants with Genetically Engineered Microorganisms.*** The University of Wisconsin has completed research on a profusion apparatus that tests for the specificity of microorganisms on particular host plants. A report was prepared and has been submitted for review. In addition, the lectin research has identified a mechanism of specificity at the molecular level. Hydrilla plant protein extracts have demonstrated an affinity for alpha-L-fucose.

- ***Management of Waterhyacinth Using Insects and Herbicides.*** The laboratory research on the toxicology of the herbicides and adjuvants has been completed, and only minimal direct impact was documented; however, the indirect impact appears to be substantial. A field study will be conducted in FY 89 to evaluate techniques developed in the laboratory on the utilization of herbicides and biocontrol agents.

- ***Determining Feasible Integrated Control Combinations for Aquatic Plant Management Using Expert Systems.*** This was a small developmental project to demonstrate the use of expert systems. A first-generation system was developed that would examine all integrated control combinations for five nuisance aquatic plants. In FY 89, additional work will be conducted to include new criteria for eliminating, retaining, or prioritizing certain combinations of integrated control technology into the existing expert system.

SUPPORT PROJECTS FOR FISCAL YEAR 1988

Two projects were addressed in support of Corps of Engineer Districts:

- ***Biocontrol of Waterlettuce.*** *Neohydronomus pulchellus* has been established at eight locations in Florida. The insect population at the first release site, Kramer Island, has been expanding rapidly. In FY 89 the first release of *Namangana* will be conducted in Florida, as soon as approval is received from the USDA-TAG.

- ***Texas Waterhyacinth Study.*** Monthly data were collected from Wallisville, Texas, on the waterhyacinth population and the associated biocontrol insects. Predictions from the model INSECT have been made, and variations in the collected data and the predicted data have been noted. Additional studies in FY 89 will examine ways to reduce detected variations.

NEW RESEARCH UNITS FOR FISCAL YEAR 1989

Two new work units have been proposed for FY 89:

- ***Temperate Biocontrol Insects for Eurasian Watermilfoil and Hydrilla.*** This work unit's objective is to develop an operational capability for use of insects for the management of Eurasian watermilfoil and hydrilla in temperate regions.

The establishment in 1988 of a USDA Laboratory in Beijing, China, will greatly assist this project.

- ***Biological Management of Aquatic Plants with Allelopathic and Competitive Species.*** This work unit will examine the mechanisms of allelopathy in aquatic plant species and evaluate their potential for aquatic plant management.

Testing Suitability of Australian Bioagents for Control of *Hydrilla verticillata*

by
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and F. Allen Dray, Jr.*

OBJECTIVES

We established several major objectives for Fiscal Year 1988. The first was to import the *Hydrellia* fly to the United States for further host-testing by Dr. Buckingham. Next, we needed to clarify the taxonomic status of this fly. We also needed to clarify questions concerning the host range of *Bagous* n. sp. (hydrilla stem-boring weevil, HSB) which arose during quarantine testing, and to forward this information to Dr. Buckingham. Finally, we were to continue studying the field biology and testing of three stream-dwelling moth species, *Aulacodes siennata*, *Nymphula eromenalis*, and *Strepsinoma repititalis*.

RESULTS AND DISCUSSION

Field efforts at the Townsville, Australia, laboratory were severely hampered by the impacts of Cyclone Charlie, which nearly hit Townsville in February. The heavy rains accompanying this massive storm caused flooding of streams, which greatly disrupted the plant communities where we conduct research. The flooding made collecting stock to feed our laboratory colonies very difficult. Also, insects were not as abundant during this period, so examination of host ranges was slowed. Progress in Townsville was also hampered by turnover in the assistants working on the hydrilla project.

Field sites studied by our Brisbane assistant, Matthew Purcell, were pummelled by heavy rains in March. Severe flooding resulted, and virtually all of the study sites suffered a complete loss of aquatic vegetation. Matthew was unable to resume field collections until August, when macrophytes were moderately abundant at some of the study sites.

Hydrilla stem-boring weevil

This new species of *Bagous* weevil, discovered and tested in Australia during 1985-86, was shipped to quarantine facilities in Gainesville, Florida, in 1987 for further evaluation. An additional shipment was sent in January 1988 to increase

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laboratory colonies. Questions arose concerning the host range in the field of this weevil in Australia. So, we are reexamining data from our previous Australian studies, and additional field and laboratory host-specificity studies have been conducted in Australia. These data show that HSB feeds on a narrow range of related plants and clearly prefers hydrilla.

Laboratory studies determined the species of plants present in local aquatic communities that were possible field hosts of this weevil. Field surveys were then conducted to determine if the weevils actually used these species under natural conditions. In Townsville, 875 aquatic plant samples from northeast Queensland have been screened to date. Over a third, 294 samples, were hydrilla. Only 30 (10%) hydrilla samples yielded HSB (675 specimens).

Of 69 samples of *Vallisneria* from northeast Queensland, four (6%) have yielded HSB. Populations on *Vallisneria* were low, averaging less than one individual (adult or larva) per sample, as compared with over two on hydrilla. Interestingly, none of over 50 samples of *Blyxa* (another Hydrocharitaceae that resembles *Vallisneria*) has yielded HSB. Only four other plant species contained HSB larvae: three (3%) of 96 samples of *Ceratophyllum demersum*, three (5%) of 59 samples of *Najas tenuifolia*, and two (4%) of 46 samples of *Nymphoides indica*. None of the 36 other aquatic macrophytes (over 200 samples) collected in northeast Queensland have yielded HSB.

A cumulative total of 399 aquatic plant samples have been collected in southeast Queensland, as well as 75 samples from northeast New South Wales. These include 190 samples of submersed hydrilla, of which 46 yielded 1,005 adults and 3,032 larvae of HSB. An additional 12 samples consisted of hydrilla fragments stranded on shorelines. These produced 710 adults and 764 larvae.

The HSB occurred often in *Vallisneria* samples at Brisbane localities; 19 (28%) of 69 samples contained 130 adults and 1,233 larvae. This average (19 per sample) is similar to the average (21 per sample) from submersed hydrilla, but less than the 27 per sample including strandline samples. Moreover, a single collection of *Vallisneria* from Lake Borumba accounted for 750 (61%) of the larvae found. Drought had exposed *Vallisneria* beds along the shoreline of this site, and the exposure multiplied their vulnerability to the weevils. Exclusion of this sample reduced the average to 7 per sample, less than a third of that found in a typical hydrilla sample.

Egeria densa, which closely resembles hydrilla, is seldom attacked by HSB, even though in laboratory studies it was accepted as readily as hydrilla. Only two adults and one larva were found in three of 91 samples (average 0.03 HSB per sample).

Only a few other plant species collected in the southern area contained HSB. One of eight samples of *C. demersum* contained nine larvae. The 14 samples of *Potamogeton crispus*, the 17 samples of *N. indica*, and the 18 samples of *N. tenuifolia* collectively produced 21 larvae (average 0.6 larva per sample). One

of the 11 samples of *Potamogeton perfoliatus* produced a single larva. Another 44 samples of a dozen additional aquatic plant species produced no HSB.

Field data indicate an intimate relationship between this weevil and hydrilla. The weevil has never been found at a site that did not contain hydrilla. Extensive hydrilla windrows occur on shorelines, but usually only traces of other plant species are present. A dense windrow is often indicative of high weevil population levels. This circumstantial evidence suggests HSB larvae float on hydrilla fragments to pupate on shore, but other aquatic species do not provide this conveyance.

Of the species studied, the only probable alternate host for HSB is *Vallisneria*. This should be of little concern except during periods of drought. In the United States, hydrilla is displacing *Vallisneria* at many sites, and successful biological control of hydrilla would likely reverse this trend.

Recently, biological control scientists in Australia encountered a similar dilemma. The moth species proposed as a control agent for the rubber vine, *Cryptostegia grandiflora*, also attacked the closely related native *Gymnanthera nitida*. In petitioning to release this moth, these scientists emphasized that failure to control the rubber vine would lead to enormous economic and environmental losses, and that the greatest threat to the native *G. nitida* was replacement by the exotic rubber vine. The relevant Australian authorities found these arguments compelling, and the moth *Euclasta whalleyi* was released last year.

Hydrellia n. sp. flies

Permits to import *Hydrellia* n. sp. were received at the end of 1987. On 29 January 1988, over 1,000 larvae and adults were sent to Gainesville. Unfortunately, delays at Miami caused the death of these insects. A second shipment sent on 26 February 1988 arrived in good condition. A quarantine colony is now well established.

An Australian taxonomist determined that this species was not, as he previously thought, *Hydrellia ceramensis*, but possibly was conspecific with *Hydrellia pakistanae*, the Indian species recently released in the United States. Specimens of *H. pakistanae* from Florida were therefore sent to him for comparison. He has now determined that the Australian *Hydrellia* is a new species and is now describing and naming it.

Aquatic moths

The three stream-dwelling moth species, *A. siennata*, *N. eromenalis*, and *S. repititalis*, have continued to be the focus of laboratory and field research in Townsville during 1988. These three species have become uncommon as our field sites suffer the effects of the drought Australia has experienced during the past several years. In addition, they are difficult to rear in the laboratory. Our research to date has focused largely on *N. eromenalis*, the most common of the three. Live adults of all three species were collected monthly at a blacklight trap to supplement laboratory populations. Three attempts in April and May provided

moderate numbers (20 to 40 individuals) of both *N. eromenalis* and *S. repititalis*, but only 10 *A. siennata*. Colonies are still too small for feeding tests, but they are being maintained at a constant temperature (25° C) to monitor developmental rates. Work with *S. repititalis* has been halted since it is more commonly collected on *Vallisneria* and *Blyxa* than on hydrilla.

Life history studies of *N. eromenalis* indicate that developmental times within instars are variable. Eggs from the same egg mass hatch over a period of 3 to 4 days. Differential larval developmental rates produce three or four different instars after a month. At 25° C a flush of adults emerges after 6 to 7 weeks, but others emerge weeks later. Under cooler, fluctuating laboratory temperatures (20° to 27° C), complete development requires at least 9 weeks. Coupled with the short adult life span (5 to 7 days), this results in the presence of only a few adults of *N. eromenalis* at a time. Cultures subsequently die out after several generations. Field surveys show *N. eromenalis* is more common on hydrilla than on other plants, but is fairly common on *Vallisneria* as well.

Data on *A. siennata* are less extensive, but larval development is also highly asynchronous. Development takes considerably longer, with no adult emergence after 3 months, although numerous larvae remain. From our surveys to date, *A. siennata* appears to be the most specific and most highly damaging of the three moth species. While it has been difficult to collect at field sites, data from over 100 specimens of *A. siennata* in the collection of the national museum at Canberra indicate that it has been collected as far south as 600 km south of Townsville and as far north as Tully. However, it has been collected most commonly near Cooktown, about 200 km north of Cairns.

FUTURE PLANS

During these closing months of the Australian hydrilla project, considerable effort will be spent compiling and analyzing the field and laboratory data collected over the past 4 years. Initially, work will concentrate on the weevils. This will hopefully lead to the publication of several journal articles during 1989-90.

Laboratory studies will continue on the moths *N. eromenalis* and *A. siennata*, and Matthew Purcell is already developing colonies in Brisbane to assist with this work. We will collect frequently at field sites with large populations of these moths, with the intent of increasing our laboratory colonies. While laboratory colonies are currently too small to permit extensive testing, we hope to use field-collected larvae to initiate some multiple-choice feeding tests.

Quarantine Work--Insect Biocontrol for Hydrilla

by
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INTRODUCTION

The hydrilla quarantine research program is responsible for proving the safety of insects introduced for hydrilla control. Insects are imported from overseas, colonized, studied for their biologies, and tested against native aquatic plants and cultivated plants. Last year at the Portland meeting, I discussed general principles and procedures for biological control programs and for quarantine research (Buckingham 1988). Today, I would like to reiterate several of the points made last year, but principally I would like to introduce the two Australian insects we are now studying. One of these is a weevil, the hydrilla stem borer, which we have been studying for almost 2 years, and the other is a leaf-mining fly, which we have imported, colonized, and tested since the Portland meeting. Last week I submitted a request to the Federal Technical Advisory Group for permission to release the fly from quarantine.

QUESTIONS ASKED OF BIOLOGICAL CONTROL WORKERS

The first question asked biocontrol workers by aquatic plant managers is "Do any insects attack this weed?" More often than not, our answer is "We don't know." This is especially true for exotic weeds, but it is also true for many native weeds. Why do we know so little? Insects and their relatives comprise about 53 percent of the world's known organisms. This percentage is more than all other organisms combined. The plant kingdom includes about 39 percent of the organisms, and the vertebrates, the most well known and studied group, include less than 3 percent. Estimates of known insect species range from 750,000 to 1 million, with estimates of still undescribed and undiscovered species ranging from 1 to 7 million.

Compounding the problem of dealing with the overwhelming number of organisms to be studied is the technical difficulty biologists have in studying insects that attack aquatic plants. Boats or other methods are needed to collect the insects; the aquatic host plant must be collected frequently or grown, which is

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usually very difficult; and new techniques must be developed to rear and study the semiaquatic insects. Large volumes have been published on rearing techniques for both terrestrial and true aquatic insects, but not for the semiaquatic species usually associated with aquatic plants. Because of these difficulties, biologists, especially those in developing nations, have studied few insects and other organisms that attack aquatic plants. The overwhelming majority of exhaustive surveys, biological studies, and host-range tests with insects that attack aquatic plants have been conducted by biological control workers during the last three decades. Taxonomists cooperating with them have described countless new species. It is thus not surprising when biological control workers shrug their shoulders in answer to questions about potential control agents for new, as yet unstudied, weeds.

Another common question is "Will biocontrol work?" Do we have credibility? The answer to this is a resounding "Yes!" Pemberton (1981) listed 41 countries that have introduced organisms for control of weeds, both terrestrial and aquatic. Many of the target weeds have been completely controlled, while others have been partially controlled by reducing their spread into new habitats or by stressing them so that other controls are effective. The first program in the United States for biocontrol of weeds, and still our most successful, was the St. John's-wort or Klamath weed (*Hypericum perforatum* L.) program in California, Oregon, and Washington during the 1940s (Buckingham 1984). The most successful international control program was against prickly pear cacti (*Opuntia* spp.) in Australia, Hawaii, Africa, and many other countries. A variety of organisms, but primarily moths and scale insects, destroyed enormous acreages of these large spiny rangeweeds during the 1920s to 1950s (Buckingham 1984).

Success with control of an aquatic weed was demonstrated by the program against alligatorweed (*Alternanthera philoxeroides* (Mart.) Griseb.) in the US Gulf Coast States during the 1960s and 1970s (Haller 1988). The success of this program is readily apparent to anyone visiting waterways in Florida where alligatorweed flea beetles (*Agasicles hygrophila* Selman and Vogt) are present, and waterways in the Carolinas where insects are absent. Control of giant salvinia (*Salvinia molesta* Mitchell), an extremely invasive floating fern, is the most recent large-scale aquatic weed control success. Extensive waterways in Australia, Papua New Guinea, and India were cleared of this weed during the 1980s by a small weevil (*Cyrtobagous salviniae* Calder and Sands) (Joy et al. 1984). Native insects also control weeds in our waterways (natural control or natural biocontrol). Each summer, the native floating fern *Azolla caroliniana* Willd. is destroyed in our laboratory pools and in many waterways by insects. Large acreages of cattails in Florida are damaged often by caterpillars. We know that insects can and do control aquatic weeds. In fact, all four aquatic weeds against which insects have been introduced around the world have been controlled in one or more countries. This record seems to guarantee success against hydrilla if sufficient effort is made.

The most common question asked the biocontrol worker is "Is it safe?" What other plants will the insect or other organism attack? Although concern is valid,

no pest organisms have been released during classical weed biocontrol programs. "Classical" biological control is the name applied to programs involving the importation and release of control agents from the native home of the pest or from other areas where the pest occurs. Approximately 192 organisms were introduced throughout the world from 1832 to 1979 for control of 86 weed species. An additional 120 organisms were introduced from 1980 to 1985 against many of the same weeds and against new weeds (Julien 1987). None of these 312 organisms, most of which are insects, has become a pest. Our quarantine research is designed to determine which plants hydrilla insects will eat and develop upon. Insects commonly feed in the laboratory on plants ignored in the field, and it is our job to evaluate the risk that the hydrilla insects pose to these plants. The insects which we have already introduced against hydrilla and which I will discuss today are new to the United States, but they are not new types of organisms for this country. Both genera, *Bagous* weevils and *Hydrellia* flies, have many close native relatives.

THE AUSTRALIAN HYDRILLA FLY

The Australian leaf-mining fly, *Hydrellia* n. sp. A, is small--about the size of the head of a pin--and easy to overlook. Up close, it appears similar to the Indian fly released during 1987 and to native algae-eating relatives (Figure 1). Both hydrilla flies (and native *Hydrellia*) can be distinguished from most algae-eaters by their shiny metallic gold faces. Adults mate within hours of their emergence from the water, and females glue their white eggs lightly to the surface of exposed hydrilla leaves or other vegetation (Figure 2). Eggs float if dislodged. Larvae crawl or drop to hydrilla and mine into the leaves, whose entire contents they eat (Figure 3). Each larva destroys four to nine leaves. Stems are not damaged, but in our rearing jars they often broke down after the empty, transparent leaves disintegrated. Pupae, the stage during which larvae transform to adults, are formed in leaf axils so their breathing tubes can be inserted into the undamaged stem.

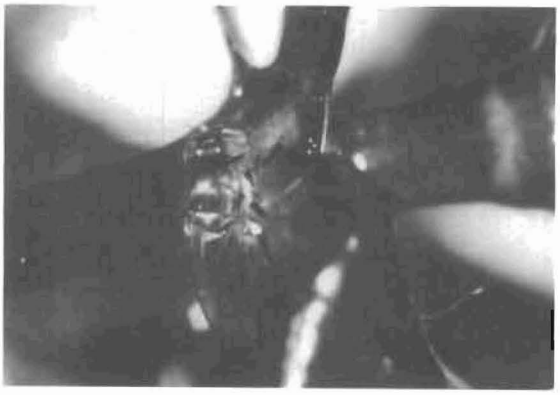

Figure 1. Australian hydrilla fly, *Hydrellia* n. sp. A, female

Life history parameters (27° C) are similar to those of the Indian fly, but there are some differences. The egg stage lasted 3 days, and complete development took 23 days in both species. Adult longevity averaged about 18 days, which was about 7 days longer than the Indian fly; however, a female laid only 36 eggs, or about 30 eggs less than an Indian fly. The Australian fly might not be as damaging as the Indian fly in a laboratory jar because it lays fewer eggs and

Figure 2. Eggs of the Australian hydrilla fly on a hydrilla leaf

Figure 3. Leaf-mining larva of the Australian hydrilla fly in hydrilla

destroys fewer leaves. In the field, however, its longer adult longevity might favor it. Together, the two species should be more effective throughout the range of habitats invaded by hydrilla than either species alone.

Rearing and host-range testing methods were the same as those for the Indian fly (Buckingham 1988). A simple technique we used to extract older larvae from leaves would be useful for those wishing to confirm the presence of either species in the field. Large mesh nylon netting was tied over the top of a plastic dishpan that contained 2 to 3 cm of water and a handful of hydrilla leaves stripped from the stem (Figure 4). A pile of hydrilla was placed on the mesh, and the pan was placed in the sun. Larvae dropped from the hydrilla as it dried and mined into the leaves in the pan. They could be easily seen when the leaves were backlighted by sun or a light. Small numbers of a native *Hydrellia* fly might be present in hydrilla collected in Florida and could be confused with the imported species. The native species, however, is principally a stem miner, and the mines can be distinguished because the stem turns black in that area.

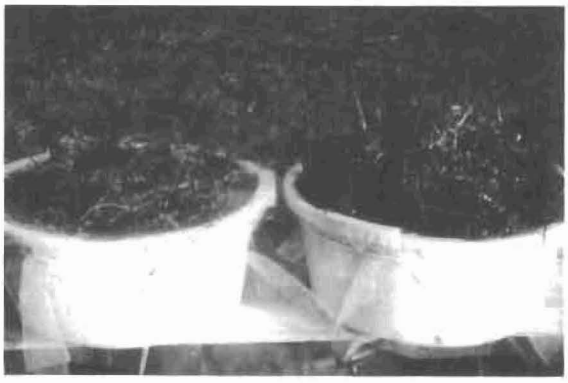

Figure 4. Technique for extracting Australian hydrilla fly larvae from hydrilla leaves

The Australian fly was even more specific in laboratory host-range tests than was the Indian fly. Only two adults developed from a total of 2,245 eggs tested on 41 plant species. Those two adults emerged from curlyleaf pondweed, *Potamogeton crispus* L., an introduced weed. A few larvae extracted from hydrilla when they were 4 days old or older were also able to complete development on curlyleaf pondweed. If this occurred in the field, it might allow small numbers to survive

complete destruction of the hydrilla so they would be available to attack the regrowth.

AUSTRALIAN HYDRILLA STEM BORER

The Australian hydrilla stem borer, *Bagous* n. sp. Z (formerly called *Bagous australasiae*), looks similar to the Indian tuber weevil, but is about half the size (Figure 5). Eggs are placed in holes eaten into the stem by the females, usually near a node. Both submersed stems and stems stranded alongshore are attacked. Larvae bore through the stems (Figure 6), which turn black in the area of the larval gallery. Adults cut through the stems when feeding (Figure 7). The fragmented stems, which contain the developing larvae, float to shore where the larvae either exit to pupate in the soil or pupate in the stranded stems. In our laboratory, eggs hatched in 2 to 3 days, and adults developed in 15 to 20 days. Biological studies and initial host-range studies were conducted in Australia by Balciunas (1987); we have conducted only host-range studies. The principal damage to hydrilla in the field would probably be caused by adult feeding, since the stranded stems with larvae would probably die anyway from exposure. Dr. Balciunas has observed a "mowing effect" in Australia due to heavy adult feeding. In our laboratory studies, this weevil has developed on several species in the hydrilla family, Hydrocharitaceae, and on southern naiad, *Najas guadalupensis* (Spreng.) Magnus. In Australia, larvae have only been reared from other plants on a few occasions and only when they were growing along with

Figure 5. Australian hydrilla stem borer, *Bagous* n. sp. Z, adult on submersed hydrilla stem

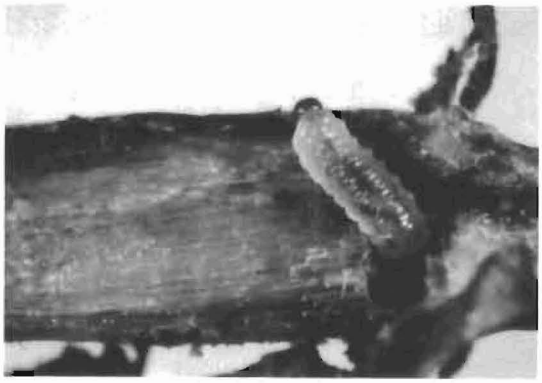

Figure 6. Larva of Australian hydrilla stem borer in hydrilla stem

Figure 7. Feeding scar of adult Australian hydrilla stem borer on submersed hydrilla stem

heavily damaged hydrilla. Because larvae develop only on stranded, not submersed, plants and because we do not believe that adult feeding threatens plant populations other than hydrilla, we consider this species to have potential as a biological control agent.

FUTURE STUDIES

We plan to continue rearing the Australian fly until we receive a decision from the Technical Advisory Group on whether it can be transferred to Fort Lauderdale or whether more research is needed. Before it can be released from quarantine, however, permission will also be needed from the Florida Arthropod Introduction Committee. We hope that this species will be available for releases in spring or summer 1989. Experiments will be completed with the hydrilla stem borer, and a request to release it will be submitted to the Technical Advisory Group. If permission is received to import two hydrilla-eating moths from Australia, they will be carried to quarantine in March or April by Dr. Balciunas. Biological and host-range studies will be initiated after the moths have successfully colonized.

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Release and Establishment of Insect Biocontrol on Hydrilla

by
Ted D. Center*

INTRODUCTION

A collaborative effort between the US Department of Agriculture and the US Army Corps of Engineers to provide biological control agents for control of aquatic weeds began 30 years ago. Six insect species were released between 1963 and 1977 for control of waterhyacinth and alligatorweed. No additional biological control agents were forthcoming until 1987. This lull was due to the lack of foreign surveys on new target weeds following termination of previous projects. The introduction approach to biological control necessitates the deliberate introduction of host-specific natural enemies for control of alien pests. This requires the conduct of foreign research that is designed to find, test, and import candidate bioagents from the native range of the target weed. Thus, the closure of the foreign studies on waterhyacinth in 1975 and the failure to initiate new foreign research created a decade-long hiatus in the biological control effort.

Fortunately, Lewis Decell recognized this serious gap in our ability to provide much needed technology for control of submersed aquatic weeds. The Corps of Engineers' Aquatic Plant Control Research Program subsequently provided support to begin faunal inventories of hydrilla on a worldwide basis. In 1981 Dr. Joe Balciunas began to explore the native range of hydrilla in the hope of finding new and unknown natural enemies. During the following 3 years he spent about 15 months abroad and searched a large portion of the tropical and subtropical range of hydrilla (Balciunas 1982, 1983, 1984, 1985). In doing so, he rediscovered a number of insect species that had been found during earlier studies (Baloch and Sana-Ullah 1974; Baloch, Sana-Ullah, and Ghani 1980). Two "rediscovered" species that he considered to be important have, in fact, now been released in the United States. He also discovered a number of other species, most of which remain to be studied. Following completion of these preliminary surveys, he established a field station in Australia where he has spent the past 4 years studying several new species of hydrilla insects. Two of these are in quarantine and could be released in the United States within the next year.

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OBJECTIVES

The objective of this project was to develop and maintain small laboratory colonies of new biological control agents of hydrilla, specifically the tuber-feeding weevil *Bagous affinis* Hustache and the hydrilla leaf-mining fly *Hydrellia pakistanae* Deonier. Although funds were not specifically provided, we also attempted to release these insects at as many sites as possible and to conduct limited follow-up visits to the sites to determine if populations had established. Monitoring of efficacy was not an objective of this project.

PROGRESS REPORT

First releases of hydrilla bioagents

During Balciunas' surveys, some of the insects originally listed as promising candidates in Pakistan were found in India. Field observations reaffirmed their candidacy, and two species were referred to US quarantine. These were a bagoine weevil, *B. affinis*, and an ephydrid fly, *H. pakistanae*. Both species were proven to be host specific by Dr. G. R. Buckingham (1988) and were approved for release.

Bagous affinis is, unfortunately, not aquatic, although adults will feed on hydrilla at the water surface. Larvae feed on the subterranean hibernacula (tubers), but only in dry soil. Females oviposit on moist, rotting wood near the edge of a receding water body. Larvae burrow through the dry soil until they encounter a hydrilla hibernaculum, then burrow into and destroy it. Because these hibernacula enable the plant to survive unfavorable periods (e.g., drought, herbicide exposure), their destruction could enhance other control measures.

These weevils will be most useful where water levels can be manipulated. We envisioned their implementation in conjunction with lake or canal drawdowns, especially in arid areas such as southern California where irrigation canals can be dewatered. Plans are presently being made for the release of *B. affinis* in that area. The first release of about 1,200 adults was made 30 April 1987 at Lake Tohopekaliga in central Florida (Table 1). This lake had been partially drawn down to expose a wide swath of shoreline. Additional releases made in Florida include wetter habitats, but no effort has been made to determine if populations have established.

Hydrellia pakistanae was first released on 29 October 1987 at Lake Patrick in Polk County, Florida. Subsequent releases were made at other south Florida sites (Table 2). Adults recovered from Lake Patrick on 29 January 1988 proved to be a species other than *H. pakistanae*. Four plant samples were collected and then held to permit emergence of any flies that were present. Two specimens emerged, and both appeared to be *H. pakistanae*. However, the containers had been opened in the laboratory in the vicinity of fly colonies and could have been

Table 1
Information on Release of *Bagous affinis* in Florida, April 1987-September 1988

Site	*Date*	*Number/Stage Released*
Lake Tohopekaliga, Osceola County	30 Apr 1987 20 May 1987	1,117 adults 370 adults
Sunshine Parkway, Exit 28, west side, Palm Beach County	22 Jun 1987	250 adults
St. Johns River at S.R. 46, Volusia County	8 Jul 1987	110 adults + eggs
Lake Okeechobee, Harney Pond Canal, Glades County	4 Sep 1987	101 adults
Conservation Area 3A, L-68A Canal, Broward County	30 Sep 1987	100 adults
Sunshine Parkway, Exit 28, east side, Palm Beach County	7 Oct 1987	203 adults
Lake Osborne, Lake Worth, Palm Beach County	18 Nov 1987	200 adults
St. Johns River near S.R. 46, Hatbill Park, Brevard/Volusia Counties	2 Jun 1988	250 adults + eggs
Lake Harney near S.R. 46, Seminole County	22 Jun 1988 24 Jun 1988 8 Jul 1988	435 adults 618 adults 990 adults
West side of US 27, 3 miles north of US 27 & I-78 intersection	14 Oct 1988 21 Oct 1988 8 Nov 1988	1,060 adults + eggs 465 adults 301 adults
Statewide total	15 releases	6,570 adults + eggs

contaminated by escapees. We were therefore unable to conclude with certainty that *H. pakistanae* had persisted at the site. Adult *H. pakistanae* were recovered from Everglades Holiday Park (Broward County) on 26 April 1988, 3 months after their initial release. Since funds have not been identified for follow-up and monitoring, efforts to verify establishment of these insects have been minimal.

Possible impacts on hydrilla populations

Coincidentally, hydrilla populations decreased dramatically at three of the first four sites within a year of the release of the fly. In fall 1987, the extent of the hydrilla infestation on Lake Patrick was estimated at 300 acres and was at problem levels (i.e., growing to the lake surface). In October 1988, Terry Sullivan, a biologist with the Florida Department of Natural Resources, visited this lake

Table 2
Information on Release of *Hydrellia pakistanae* in Florida, October 1987-September 1988

Site	*Date*	*Number/Stage Released*
Lake Patrick (Lenore) near Frostproof, Polk County	29 Oct 1987	600 adults 2,600 larvae 6,000 eggs
Rodman Reservoir, Spike Club, Marion County	9 Nov 1987	1,000 larvae
Conservation Area 3-A, Broward County	26 Feb 1988 28 Jun 1988	4,035 eggs 2,142 eggs
Lake Hicpochee, Glades County	17 Mar 1988	4,505 eggs
Lake Osborne, Lake Worth, Palm Beach County	23 May 1988	3,870 eggs
Lake Okeechobee, 26 59 14 N, 80 58 72 S, Glades County	18 Nov 1988	1,368 eggs
Statewide total	7 releases	21,920 eggs 3,600 larvae 600 adults

during the conduct of his annual plant surveys. He discovered almost a total lack of "topped-out" hydrilla. Although he estimated that about 200 acres remained, it was deemed to be at nonproblem levels, confined mostly to the bottom. He subsequently contacted Polk County aquatic weed control specialists and learned that the hydrilla mat had collapsed during July or August. After further checking, he learned that no control measures had been implemented at the lake and nothing comparable had happened at other lakes in the area. With the knowledge that topped-out mats had been present there year after year, he concluded that this sudden demise must have been due to the release of *H. pakistanae*. He immediately contacted our laboratory and, within a few days, we met with him and David Eggeman (Florida Game and Freshwater Fish Commission) at the site. The hydrilla that we collected from the bottom was completely brown and deteriorated but with green sprigs growing from a few nodes. Decomposition was too extensive to ascertain the cause of death. We collected 45 *Hydrellia* spp. flies from the surfaces of yellow waterlily pads and other floating plant material but, unfortunately, most proved to be *Hydrellia bilobifera*, a closely related native species. Two specimens appeared to be *H. pakistanae*, but upon closer examination proved to be a third species, undoubtedly another native. While there, we also collected four samples of hydrilla. These were held and checked periodically for several days. No *H. pakistanae* emerged.

We were forced to conclude that although the collapse of the hydrilla population might have been caused by an infestation of the fly, evidence was insufficient to prove that this was true. It was apparent that close monitoring would have been needed if the fly was indeed so effective in such a short period of time.

A similar pattern occurred at Lake Hicpochee. Flies were released there in March 1988, but no ensuing effort was made to determine if a population established or persisted. The mat on which the release was made all but disappeared by fall. Nearby mats persisted, however, indicating that whatever had impacted the mat at the release site was only locally active.

Flies were also released in Everglades Holiday Park, where a population had established, was persisting, and was beginning to disperse. A small mat located across a canal from the airboat concession was chosen as the first release site. Flies were released on 26 February 1988, and a single adult *H. pakistanae* (from six *Hydrellia* spp. specimens) was recovered on 26 April. A second release was made on 28 June on another mat that was very near the first. Six specimens of adult *Hydrellia* flies were collected at the original release site on 1 September, but none proved to be *H. pakistanae*. By 22 September the hydrilla mat was no longer present within the original release area, but extensive mats persisted elsewhere in the area. At that time, hydrilla samples were collected from three different areas within the vicinity of the release sites. One *H. pakistanae* was reared (emerged 4 October 1988 from a sample collected from an old mat directly across the canal from the original release site. About 100 m west of the site, two *H. pakistanae* adults (from nine specimens) were field-collected, and one adult was reared from a plant sample (emerged 4 October 1988). A total of 25 *Hydrellia* spp. adults were collected, but only two were *H. pakistanae*. Again, although the only mat to disappear was at the initial release site, we have little evidence to connect the two events.

CONCLUSIONS

During the past few years we have repeatedly emphasized the importance of foreign surveys and quarantine studies in providing biological control agents. Without these two aspects of the program, the supply of new biological control agents would dwindle and eventually cease. This would cause further delays in the development of already long-term research that is critically needed to pursue biological control objectives. However, after these agents are available, we must not neglect the need to release and establish them at field sites. This involves a great deal of effort and is often accomplished only after repeated attempts. Yet, thus far, this phase of the program against hydrilla has been underemphasized. More releases are needed at more sites with much larger numbers of insects. If populations fail to establish, it must not be due to a lack of effort. The need to move these insects out of the lab and into the field is becoming especially critical with the imminent release of new insects from quarantine. Laboratory colonization and care of additional species will stretch present resources to their limit.

Without additional resources, we will be unable to maintain colonies of some agents.

The evaluation of the impact of these agents is secondary to the primary objectives described above. However, biological control agents can have subtle but quite important impacts that are indiscernible to the casual observer. It is important to document these effects if, for no other reason, than to justify future programs and to derive cost:benefit estimates. The need for detailed studies of the plant and bioagent populations goes well beyond bureaucratic economic analyses, however. These biological systems must be thoroughly understood in order to make the most of the benefits that they offer. Only through acquisition of appropriate data bases can aquatic plant control ever consist of strategic management practices. Evaluation and monitoring of the effectiveness of biological control agents needs greater emphasis than it has had in the past.

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Biological Control of *Hydrilla verticillata* (L.f.) Royle Using an Endemic Plant Pathogen

by
Gary F. Joye*

INTRODUCTION

From a survey in the southern United States, a fungus identified as *Rhizoctonia* sp. (FHy18) was isolated from apparently healthy foliage of *Hydrilla verticillata* (L.f.) Royle collected in the fall of 1987 in Lake Houston near Houston, Texas. Based on results from these studies, this pathogen has shown great potential for use as a biocontrol agent. These studies were conducted to evaluate the virulence, host range, and toxicity of this fungus. The objective of this study was to discover and develop a plant pathogen for use as a biocontrol agent of hydrilla. To our knowledge, this is the first endemic pathogen to successfully control hydrilla in both greenhouse and field tests.

MATERIALS AND METHODS

Pathogen isolation and culture

The bacterial and fungal isolates tested in this study were obtained by random sampling of hydrilla populations in the southern United States (Table 1). Approximately 100 g of plant tissue was collected at each sampling site. The samples were placed in plastic bags and stored at 4° C until ready for use. The samples were divided into 25-g subsamples, sterilized for 20 sec in 10-percent sodium hypochlorite, washed in sterile distilled water, placed in 200 ml of sterile distilled water, and blended in a Waring blender for 30 sec. The puree was serially diluted from 10^0 to 10^{-5}. A 0.1-ml sample was taken from each dilution and spread over potato dextrose agar (PDA) + 3 μg/ml of streptomycin or nutrient agar (NA) + 3 μg/ml benomyl. Most fungi and bacteria could be separated using these growth media.

Two to five days after plating out the puree dilutions, individual isolates of bacteria and fungi were taken from the dilution plates. Fungal and bacterial isolates were placed on PDA and NA, respectively. Pure cultures of each isolate were stored in a skim milk/glycerol solution at -80° C.

Test tube assays

All microorganisms collected from hydrilla were screened for pathogenicity in

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

Table 1
Survey Sample Sites Where Hydrilla Was Collected for Isolating Endemic Microorganisms

State	*Sample Site*
Texas	Sheldon Reservoir
	Lake Somerville
	Lake Limestone
	Fairfield Lake
	Lake Athens
	Lake Palestine
	Lake Nacogdoches
	Sam Rayburn Reservoir
	Toledo Bend Reservoir
	Lake Conroe
	Lake Lewis
Louisiana	Bayou Lafourche
	Lake Theriot
Florida	Lake Seminole
	St. Johns River
	Chasakowhitzka River
	Oklawaha Lake

test tube assays. Test tubes (20 × 2.5 cm) were filled with 60 ml of a nutrient solution ($Ca(NO_3)_2$, 0.179 g/ℓ; $CaCl_2$, 0.092 g/ℓ; $MgSO_4$, 0.033 g/ℓ; $KHCO_3$, 0.015 g/ℓ; $NaHCO_3$, 0.059 g/ℓ), after which a 7-cm hydrilla sprig was placed in each tube. The test fungi were grown in potato dextrose broth on a shaker at 160 rpm. Bacteria were grown on NA. Hydrilla plants were inoculated with 1×10^6 colony forming units (cfu)/ml of each fungal or bacterial isolate and placed in a growth chamber (adjusted to 25° C with 12/12 hr light/dark periods). The plants were monitored weekly for symptoms of disease. Dry weight was taken after 6 weeks. Ten replicates were made for each isolate. The experiment was conducted twice.

Greenhouse tests

The best isolates from the test tube assay were used in the greenhouse test, with one additional isolate on loan from Dr. Charudattan (isolate 621P). Clear plastic columns (150 × 13.75 cm) were used for this test. Unsterilized lake sediment (20 cm) was placed in the bottom of each column and overlaid with 7.5 cm of fine washed silica gravel. Three 15-cm sprigs of fresh hydrilla were planted in each column, after which 16 ℓ of nutrient solution ($CaCl_2$, 0.092 g/ℓ; $MgSO_4$, 0.033 g/ℓ; $KHCO_3$, 0.015 g/ℓ; and $NaHCO_3$, 0.059 g/ℓ of deionized water) was added. The hydrilla columns were maintained at 25° C under natural greenhouse lighted conditions (Figure 1).

The inoculum for each fungal isolate was grown in V-8 broth. The fungi used in this study were *Cladosporium eladosporiodes* (224), *Fusarium moniliforme* var. *subglutinans* (236), *Fusarium roseum* var. *culmorum* (621P), *Rhizoctonia* sp. (FHy18), and *Rhizoctonia* sp. (FHy20). Isolates 224 and 236 were collected from hydrilla in previous studies (Pennington 1985). Isolate 621P is an exotic species

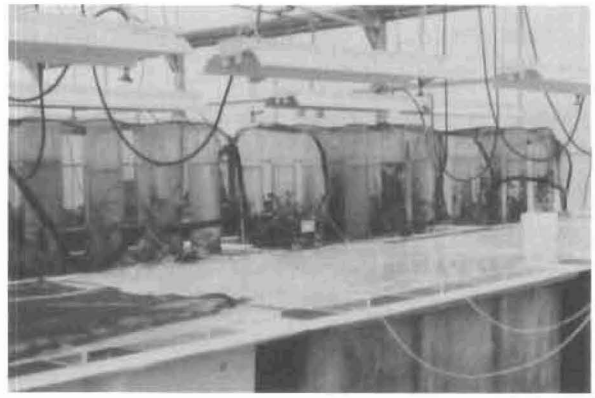

Figure 1. Greenhouse test. Clear plastic columns were used for evaluation of potential biocontrol agents. Columns were placed in 1,000-ℓ water baths to maintain a constant temperature of 25° C

that is known to impact hydrilla (Charudattan et al. 1980). FHy18 and FHy20 were collected from hydrilla in 1987.

After the hydrilla had grown to the top of the water column (100 cm), the treatment columns were inoculated with 350 ml of inoculum, which resulted in a dilution concentration of 1×10^6 cfu/ml. Control hydrilla columns were treated with 350 ml of deionized water. The plants were observed daily for any disease symptoms. Dry weight was collected 3 weeks after inoculation. All treatments were replicated five times. The experiment was conducted twice. Mean comparisons were made using Tukey's Test.

Host specificity

A host range study was conducted to determine the host specificity of FHy18. Plants used for this study included emergent and submersed aquatic plants, wetland terrestrial plants, and crop plant species. A humidity chamber (100 × 100 × 240 cm) was constructed to accommodate approximately 20 test plant species. Styrofoam cups (90 ml) were filled with potting mix (Sunshine No. 3). Either seeds or plant sprigs of each species were planted in each cup and watered as needed. Relative humidity was maintained between 90 and 99 percent using a cool water vaporizer. Temperature was maintained between 25° and 35° C. There were five to eight replicates for each treated and nontreated plant species.

Inoculum was grown in V-8 broth on a shaker (160 rpm). After the plants were established, treated terrestrial plants were dipped in the inoculum with a concentration of 1×10^8 cfu/ml. A surfactant (Tween 20 at 0.5 ml/ℓ) was added to ensure coverage of terrestrial plants.

Controls were dipped in water and surfactant only. Aquatic plants were inoculated with 150 ml of concentrated inoculum of 1×10^8 cfu/ml. The plants were maintained in the humidity chamber 14 days after inoculation and observed daily for any disease symptoms. Data were reported as plants being resistant or susceptible.

Animal toxicity

The triploid white amur, which has been used as a biological control agent of aquatic weeds (Miller and King 1984), was the test species for this study. The test procedure used is described in Standard Methods for the Examination of Water and Wastewater (American Public Health Association 1980). Ten fish were placed in each of ten 8-ℓ aquaria. After the fish were acclimated to the aquaria, liquid inoculum of isolate FHy18 was prepared as described previously and placed into each aquarium. For this initial testing, 3× the rate used in the greenhouse test was used. After inoculation, mortality was recorded every 24 hr for 96 hr. Data are reported as percent mortality.

Field test

A small-scale field test was conducted in a hydrilla mat at the Sheldon Reservoir, northeast of Houston, Texas. Plots (1 × 1 × 2 m) constructed of polyvinyl chloride tubing and polyethylene were secured in the sediment (Figure 2). Plots were established 1 month prior to inoculation to allow the plants to naturalize the plots (Figure 2). On September 29, 1988, plots were inoculated with 8 ℓ of concentrated inoculum (1×10^9 cfu/ml). Plots were observed 2 and 4 weeks after inoculation. Biomass was collected at 4 weeks. Treated and control plots were replicated five times.

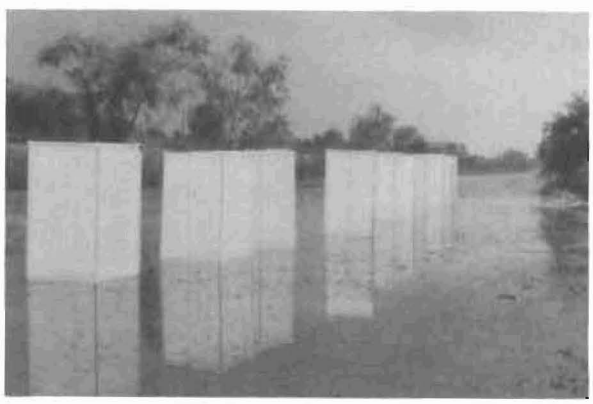

Figure 2. Field test. Established field plots at drawdown 1 month prior to inoculation with the biocontrol agent, Sheldon Reservoir, Texas

RESULTS

Pathogen isolation and culture

More than 500 isolates of bacteria and fungi were collected from hydrilla foliage in the 17 lakes in which samples were taken.

Test tube assays

A wide range of pathogenic effects among fungal isolates were exhibited in these assays with correlation coefficients (r) of 0.39 and 0.32 for each test. Isolates FHy18 and FHy20 were significantly ($P > 0.04$) more damaging than other isolates (Figure 3). No bacterium was pathogenic to hydrilla.

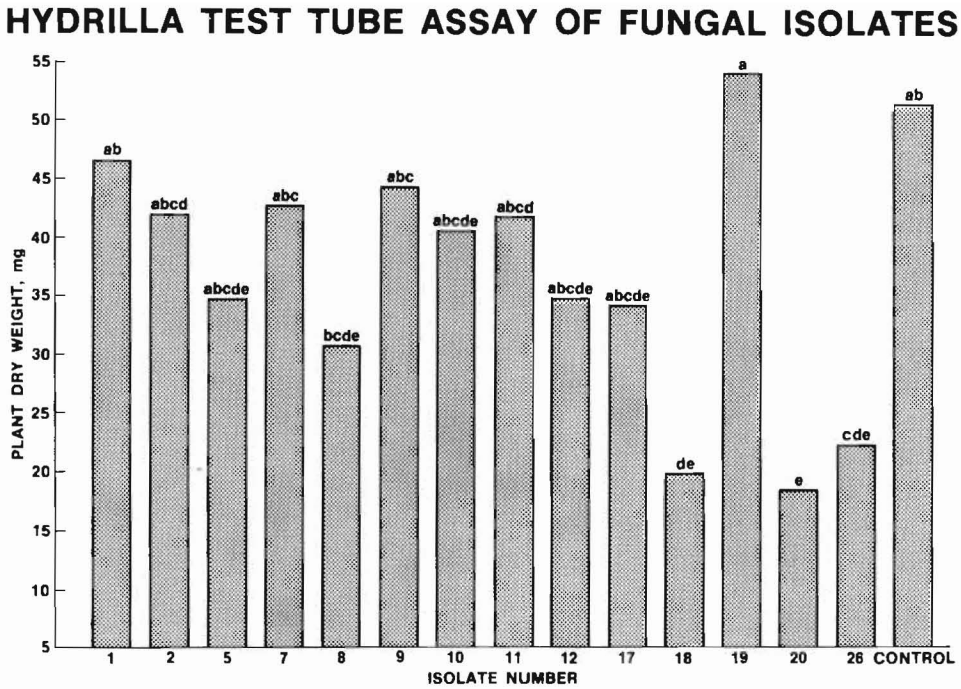

Figure 3. Test tube assays. Effects of fungal isolates collected from hydrilla. Tukey's Test ($P < 0.05$). Bars with the same letter are not significantly different

Greenhouse tests

Isolates FHy18 and FHy20 damaged hydrilla significantly ($P > 0.0001$, $r = 0.92$) more than the other isolates (Figures 4 and 5). Based on observations on the morphology of these two isolates, these fungi are now considered to be two cultures of the same fungal isolate (Joye, unpublished data); hence, only FHy18 will be used to designate this pathogen's identity. Isolate 621P, an exotic species from The Netherlands known to impact hydrilla, was significantly more damaging than isolates 224 or 236 but not FHy18 or FHy20.

Host specificity

Isolate FHy18 was nonpathogenic to 44 of 46 species and cultivars within 22 families (Table 2). Only hydrilla and *Ottelia alismoides* (duck lettuce) were susceptible. Duck lettuce is an introduced Afro-Asian species that is known only to occur in Cameron Parish, Louisiana, of the United States (Correll and Johnston 1970).

Animal toxicity

Isolate FHy18 was nontoxic to the white amur in the acute toxicity tests. There was a 20- and 22-percent mortality for the nontreated and treated grass carp, respectively. These studies are inconclusive and will be continued.

Field test

Observed symptoms between hydrilla in treated plots 2 weeks after inoculation

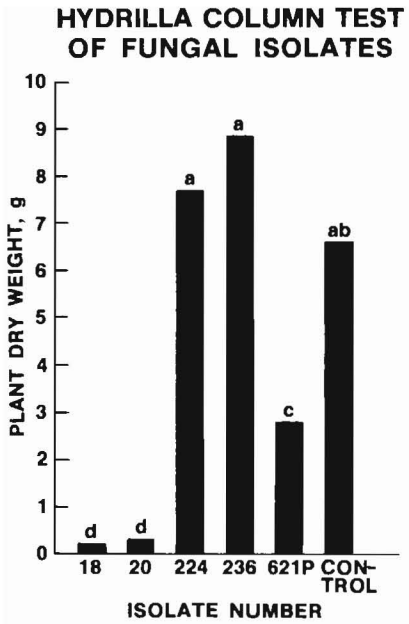

Figure 4. Greenhouse column assay. Effects of selected fungal isolates on hydrilla. Tukey's Test ($P < 0.05$). Bars with the same letter are not significantly different

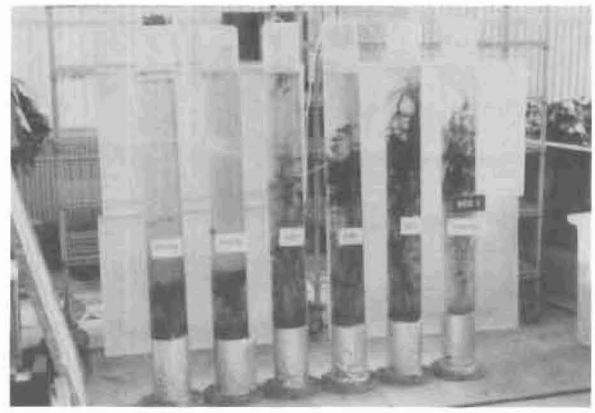

Figure 5. Representative greenhouse columns to illustrate the effects of the different fungal isolates on hydrilla

were similar to those observed in the greenhouse studies. The foliage of hydrilla became chlorotic and was disintegrating. Control plants were healthy and vigorous. There was a very highly significant difference in dry weight between controls and treatments ($r = 0.99$, $P > F = 0.0007$). Treatment dry weights were 61 percent less than controls 4 weeks after inoculation with mean dry weights of 137.18 and 354.16 g for treated and control plots, respectively (Figure 6).

DISCUSSION

A fungal plant pathogen identified as a *Rhizoctonia* sp. (isolate FHy18) was collected from apparently healthy hydrilla. In laboratory, greenhouse, and field tests, this fungus was able to evoke disease symptoms in hydrilla. In both the greenhouse and field tests, this plant pathogen showed dramatic and lethal results over a very short period when properly applied as a mycoherbicide.

In host-specificity tests, only hydrilla and duck lettuce were susceptible to *Rhizoctonia* sp. isolate FHy18 in a field of 46 species and subspecific taxa within 22 families. Although it is possible that other susceptible plant species do occur, this organism has shown a high degree of specificity. Host range studies will continue to expand the list of resistant and susceptible plant species.

Table 2
Reaction of Various Plant Species to FHy18 Isolated from Hydrilla*

Family Species	*Disease Reaction***
Aceraceae	
Drummond's red maple (*Acer rubrum* L. var. *drummondii* (Hook & Arn.) Sarg.)	R
Alismataceae	
Arrowhead (*Sagittaria latifolia* Willd.)	R
Amaranthaceae	
Alligatorweed (*Alternanthera philoxeroides* (Mart.) Griseb.)	R
Araceae	
Waterlettuce (*Pistia stratiotes* L.)	R
Ceratophyllaceae	
Coontail (*Ceratophyllum demersum* L.)	R
Commelinaceae	
Dayflower (*Commelina* sp.)	R
Cucurbitaceae	
Cantaloupe (*Cucumis melo* L.) 'Halesbest'	R
Meloncito (*Melothria pendula* L.)	R
Squash (*Cucurbita pepo* var. *melopepo* (L.) Alef.) 'Yellow summer crookneck'	R
Watermelon (*Citrullus vulgaris* Schrad.)	R
Cyperaceae	
Spikerush (*Eleocharis* sp.)	R
Fabaceae	
Alfalfa (*Medicago sativa* L.) 'Pioneer'	R
Red clover (*Trifolium pratense* L.)	R
Soybean (*Glycine max* (L.) Merr.)	
'Bedford'	R
'Braxton'	R
'Coker 368'	R
'Davis'	R
'Forrest'	R
'Hartz 8112'	R
'H6385'	R
'H7110'	R
Haloragaceae	
Parrotfeather (*Myriophyllum brasiliense* Camb.)	R
Eurasian watermilfoil (*Myriophyllum spicatum* L.)	R
Hydrocharitaceae	
Hydrilla (*Hydrilla verticillata* (L.f.) Royle)	S
Duck lettuce (*Ottelia alismoides* (L.) Pers.)	S
Eelweed (*Vallisneria americana* Michx.)	R
Limnaceae	
Duckweed (*Lemna minor* L.)	R

(Continued)

*Ten plants of each variety were dipped in inoculum containing 1×10^8 cfu/ml and surfactant. Controls were dipped with surfactant and water only. Plants were evaluated daily for 14 days.
**R = resistant; S = susceptible.

Table 2 (Concluded)

Family Species	*Disease Reaction*
Malvaceae	
Okra (*Abelemoschus esculentus* (L.) Moench.) 'Clemson spineless'	R
Najadaceae	
Southern naiad (*Najas guadalupensis* (Spreng.) Magnus)	R
Nyssaceae	
Tupelo gum (*Nyssa aquatica* L.)	R
Onagraceae	
Water primrose (*Ludwigia peplioides* H.B.K.) Raven.)	R
Water primrose (*Ludwigia* sp.)	R
Poaceae	
Wheat (*Triticum aestivum* L.)	R
'Coker 762'	R
'FL302'	R
'McNair 1003'	R
'Rosen'	R
'Terra 1812'	R
Rice (*Oryza sativa* L.)	R
'Lemont'	R
'Mars'	R
'Mercury'	R
'TeBonnet'	R
Polygonaceae	
Smartweed (*Polygonum* sp.)	R
Rubiaceae	
Buttonweed (*Diodia virginica* L.)	R
Saururaceae	
Lizardtail (*Saururus cernuus* L.)	R
Solanaceae	
Tomato (*Lycopersicon esculentum* Mill.) 'Sweet million 5352'	R
Taxodiaceae	
Bald cypress (*Taxodium distichum* (L.) Rich.)	R

Figure 6. Representative samples of remaining hydrilla tissue 4 weeks after inoculation with isolate FHy18. Sample on left was treated; sample on right was untreated

Rhizoctonia sp. isolate FHy18 was nontoxic to the white amur in replicated experiments. From these results and the general knowledge that biocontrol agents are less harmful to the environment than chemicals, we expect no major difficulty in this area of research. However, to ensure the safety of wildlife, future toxicity studies will include fish species of more economic value.

Other reports have been made of organisms with potential value as plant pathogens of hydrilla, including species of *Rhizoctonia* (Joyner and Freeman 1973; Freeman, Charudattan, and Conway 1975; Freeman et al. 1976; Charudattan and McKinney 1977; Charudattan et al. 1980). However, to the author's knowledge, this is the first report of an endemic plant pathogen that has been effective as a mycoherbicide against hydrilla.

ACKNOWLEDGMENTS

The author thanks Mr. Robert Comstock of the Texas Parks and Wildlife Department for permission to establish field tests in the Sheldon Reservoir, Texas, and Dr. James K. Mitchell and Mses. Jan Freedman, Ramona Warren, and Laura Bailey for their assistance in these studies.

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Annual Report - Microbiological Control of Eurasian Watermilfoil

by
Haim B. Gunner*

My presentation today is really the record of the transition of this project from the academic laboratory and research level to the stage of commercial development and product formulation. As many of you who have been listening to reports from me for the past half dozen years know, we have developed a strategy for the control of Eurasian watermilfoil which is based on a microbial agent derived from the environment of the target species. Our approach was to search among microorganisms associated with the plant ecosystem for those which have the capacity to produce enzymes destructive to plant tissues, in particular cellulases and pectinases. After identifying and isolating such microorganisms, we would culture them in media, which would maximize induction of these enzymes, and then apply these cultures directly to the plants. Since the plant is already home to them, it was postulated that none of the constraining mechanisms which would prevent their proliferation in a new environment would be present to inhibit their activity, and that cellulytic and pectinolytic attack on the plant would bring about the plant's demise.

Both cellulytic and pectinolytic organisms were in fact identified and shown to be effective in concert to bring about milfoil decline. Successive tests were scaled up from jar to aquarium to children's bathing pools and finally, for the past 2 years, in a natural setting at Stockbridge Bowl, Massachusetts. In the process, modification and fermentation techniques have permitted us to use only one culture, the organism *Mycoleptodiscus terrestris*, which has now been independently tested and its efficacy confirmed in the Environmental Laboratory at WES.

The past year has seen the transfer of this work which is now, as mentioned, at the stage of product development, from my laboratory at the University of Massachusetts to EcoScience Laboratories, Inc., a company which I have established with the help and encouragement of the University.

Our current strategy is to develop a formulated product that will be tested in the coming season in small-scale field trials in cooperation with WES. In advance of this, we are developing various formulations of the product which we are sending for bioassay to the WES group. Figure 1 shows one formulation that was developed as a flowable powder. Figure 2 shows an alginate preparation that has

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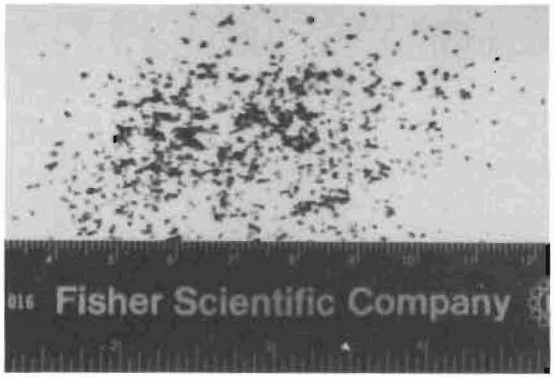


Figure 1. Flowable powder formulation

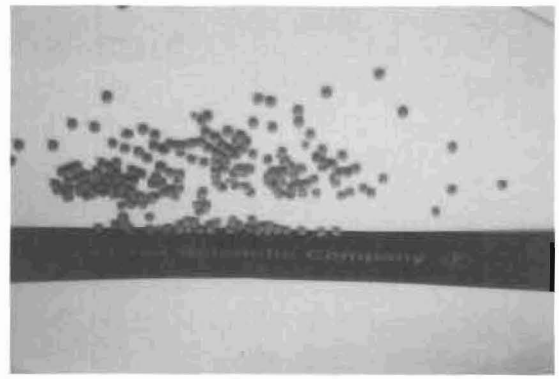


Figure 2. Alginate pellet formulation

demonstrated particular efficacy (see paper by Winfield in this proceedings). This already appears to be close to the formulation of choice for next spring's trials.

It appears to us that, as well as pioneering a novel ecosystems-compatible approach, the transition from basic research in an academic setting to commercial production in an entrepreneurial framework that we have demonstrated offers a positive pattern for the rapid translation of research results into an effective product.

Biological Control of Eurasian Watermilfoil with *Mycoleptodiscus terrestris*

by
Linda E. Winfield*

INTRODUCTION

The development of an effective biological control for Eurasian watermilfoil would aid efforts to improve navigation, flood control, and the recreational usage of water systems impacted by this obnoxious aquatic weed. We have been working toward this goal since 1986 when this work unit was initiated. Since that time several organisms have been found that showed promise as a plant pathogen of Eurasian watermilfoil. We believe that one of these, a fungus, *Mycoleptodiscus terrestris* (Gerdemann) Ostazeski, satisfies our requirements. This fungus has been found to have a high degree of specificity for and efficacy in decimating populations of Eurasian watermilfoil.

The results of several years of work with this organism were discussed at our last annual meeting (Gunner et al. 1988). Some aspects of our work this year included:

a. Verification studies.

b. Development of and experiments with alginate formulations.

c. Viability and shelf-life determinations.

d. Study of effects of aging on virulence and toxic formation.

METHODOLOGY

Several kinds of experiments have been conducted using similar protocols. The inoculum was either broth culture or alginate pellets. Liter-sized glass jars served as mini-test units. Three 15-cm-long meristematic sprigs of Eurasian watermilfoil were planted in plastic starter pots. The potting medium was a 3:1 sterile sand:soil mixture. Plants were grown in aerated aquaria which contained reverse osmosis water. The plants were allowed to grow and develop a root system for 10 days, and then were placed into the glass jars, which also contained reverse osmosis water. The jar test units were placed into an environmental chamber, and the plants were acclimated for a 24-hr period. Growth conditions were monitored and kept constant at 18° C (±0.5° C) with a photoperiod of 8:16 (light:dark). Each jar was covered with a plastic petri dish to prevent excess evaporation.

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

After the acclimation period, plants were inoculated with the fungus. Five replicates of each treatment were used, except in one study where the volume of inoculum was insufficient to permit this. Three replicates were run in that study. Biomass was determined for each replicate after a 21-day test period. An index of the percent biomass loss was calculated on each replicate and on the pooled values for each treatment. The following formula was used:

$$\text{Percent biomass loss} = \frac{\text{Weight control - Weight test}}{\text{Weight control}} \times 100$$

Controls were included with each test to check for any effects from the broth medium or the alginate formulations. Also, uninoculated controls were run with each experiment. No effects on the health or biomass of the plants were noted in the controls.

VERIFICATION STUDY

Our initial studies were done to verify results and conclusions obtained by our coresearchers at the University of Massachusetts. The inoculum consisted of shake cultures of the fungus *Mycoleptodiscus terrestris* (*M. t.*). The fungus was grown for 8 days in a potato dextrose broth to which a mineral salts solution was added. The use of mineral salts was found to enhance the pathogenicity of the fungus (Gunner et al. 1988). The shake cultures were homogenized in a commercial blender prior to inoculating the plants.

The number of colony forming units (CFU) per milliliter was determined in a cell counter and verified by growth on Martin's agar. (The number of CFUs varied from 3×10^5 to 7×10^5/ml.) To establish the optimum dose, 1-, 2-, and 5-ml aliquots of inoculum were used. Verification studies were repeated three times.

The results obtained were similar to those derived by researchers at the University of Massachusetts. Biomass reductions of 70 to 80 percent were noted depending on the type and volume of inoculum used.

ALGINATE FORMULATIONS AND EXPERIMENTS

The necessity for transporting large quantities of broth culture packed in ice complicates the logistics of conducting field studies (Gunner 1984, 1985, 1987; Gunner et al. 1988). Therefore, we decided to make changes in our formulations. The use of sodium alginate as a carrier for mycoherbicides has been reported in the literature by several researchers (Walker and Connick 1983, Lewis et al. 1985).

We began experimenting with different alginate formulations. The pellets were simple to make and proved to be much lighter and less bulky than our previous inoculums. We used varying rates and formulations, whole versus crushed, and

pellets made by our coresearchers at the University of Massachusetts. The pellets were a mixture of the following: inert clay carrier (10% w/v), sodium alginate (1.33% w/v), carbohydrate source (1% w/v), fungal culture, and distilled water.

Using the apparatus shown in Figure 1, the alginate mixture is pumped through

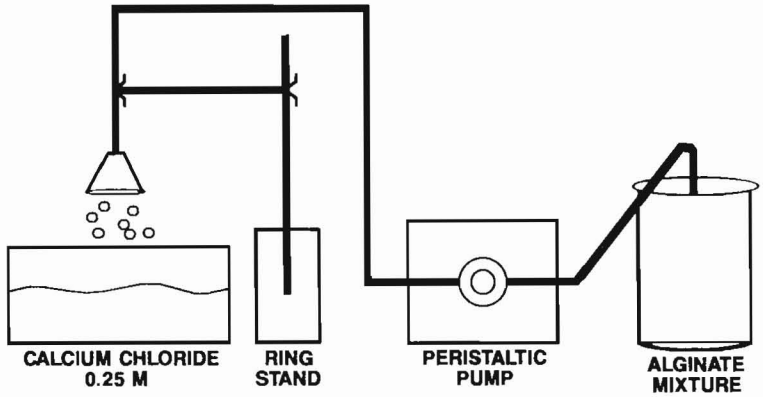


Figure 1. Apparatus for making alginate pellets

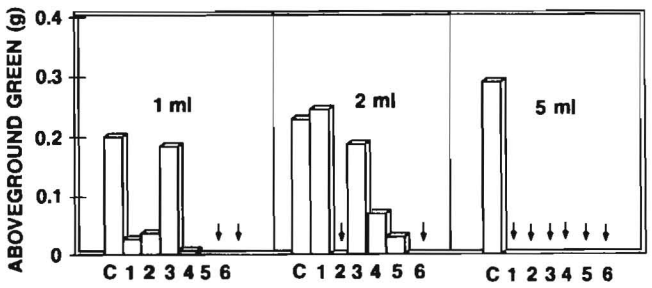
some flexible tubing with the aid of a peristaltic pump. The mixture drops slowly through a perforated plastic cap into a solution of calcium chloride (0.25 M). The drops congeal into a biodegradable gel upon contact with the salt solution. The freshly formed pellets resemble fish eggs. The wet pellets are then spread onto frames of nylon netting and air-dried with the aid of fans.

The pellets shrink to about 2 to 3 mm in size when dry and can be stored in vials, bottles, or any suitable containers. The buoyancy of the pellets can be affected by the amounts of dry or liquid ingredients used. They can be made to float on the surface or sink in the water column. We tested the pellets on Eurasian watermilfoil using the jar mini-test unit. Tests were allowed to run for 21 days using the protocol described above. We obtained between 80 and 85 percent reduction in biomass, as illustrated in Figure 2.

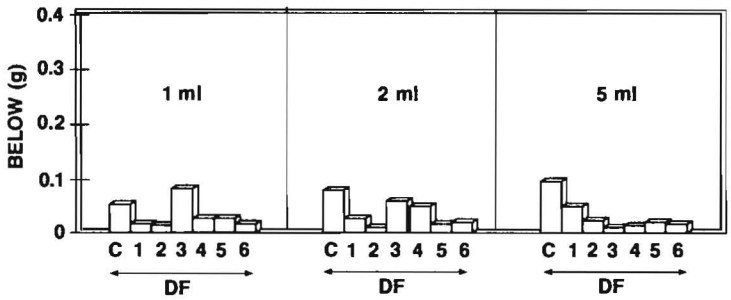
VIABILITY AND SHELF LIFE

In an effort to optimize the effects of *M. t.*, we are currently using various inert clay amendments and several carbohydrate sources (Table 1). Our objective is to determine their effects on the viability and shelf life of our formulations. This is vital for planning future field studies and for commercialization of the formulation. (To determine if the method was applicable to other fungi, two fungi were used--*M. t.* and *Alternaria* sp.)

Each batch of formulation was divided. Half was stored at 4° C and half at room temperature (25° C). Ten pellets from each portion were placed on water agar plates, incubated for 3 to 4 days at 25° C, and checked for growth. Pellets were plated on the water agar at day zero and at varying intervals thereafter.



a. Aboveground green



b. Below ground

Figure 2. Biomass of Eurasian watermilfoil after 21-day treatment with *M. t.*

Table 1
Alginate Pellet Combinations

Clay Carrier	*No Carbohydrate*	*Corn Meal*	*Rice Flour*	*Potato Flour*
		Mycoleptodiscus terrestris		
Kaolin	1	6	11	16
Montmorillonite	2	7	12	17
Bentonite	3	8	13	18
Atta gel	4	9	14	19
No clay	5	10	15	20
		Alternaria sp.		
Kaolin	21	26	31	36
Montmorillonite	22	27	32	37
Bentonite	23	28	33	38
Atta gel	24	29	34	39
No clay	25	30	35	40

Viabilities for periods of 6 to 8 months have been reported by other researchers (Walker and Connick 1983). We have been conducting our viability experiments for 12 weeks, as of this writing. As would be expected, we have noted the greatest decreases in viability in those pellets stored at room temperature and those with no carbohydrate source. Viability of those stored at room temperature (25° C) ranged from 0 to 80 percent, while those stored at 4° C had viabilities of

40 to 100 percent. Some variations have also been noted with different carbohydrate sources. This study is still ongoing.

EFFECTS OF AGING AND DETERMINATION OF TOXIN FORMATION

Concurrent with the alginate viability studies, we are examining the effects of aging on the virulence of *M. t.* cultures. We are also trying to determine if this fungus produces exotoxin, which aids in the degradation of Eurasian watermilfoil. We have inoculated pots of milfoil in the jar mini-test units with 8-, 10-, 14-, and 16-day-old shake cultures of *M. t.* The fungus was grown in potato dextrose salt and corn starch dextrose salt broths.

We also inoculated pots of milfoil with the supernatant from shake cultures (grown in potato dextrose salt broth only) and with the washed mycelium. Half of the supernatant and half of the mycelium were heat treated; the remainder was not. Both of these studies were allowed to run for 21 days, after which dry weight biomass was determined.

Our preliminary results are indicative of a decrease in virulence as the culture ages. No appreciable difference in virulence, as indicated by biomass reduction, was noted in the 8- and 10-day-old cultures. We obtained a 75- to 80-percent reduction in biomass with these cultures. However, with the 14-day-old culture, we obtained only a 50-percent reduction in biomass and a 14-percent reduction with the 16-day-old culture. This illustrates the need to inoculate (or make pellets) during the interval of peak pathogenicity of the fungus.

Our preliminary results also indicate that *M. t.* produces a substance that has toxic effects on Eurasian watermilfoil. When using the nonheat-treated mycelium and supernatant, we obtained biomass decreases of 77 and 30 percent, respectively. Biomass reductions of 40 and 14 percent were noted with the heat-treated mycelium and supernatant, respectively. These results, although still in the preliminary stages, imply that the substance aiding in the degradation of the Eurasian watermilfoil is probably a toxin as opposed to an enzyme. Although the degradative effects were less notable with the heat-treated inoculum, it was still present. If the substance was enzymatic in nature, it should have been completely denatured by the heat treatment (it was heated to 212° C for 10 min), and this was not the case.

FUTURE WORK

This year we have been studying several facets dealing with the control of Eurasian watermilfoil. We plan to initiate large-scale field studies next summer. Several areas are under consideration as possible sites.

We are currently testing several promising alginate formulations. We have obtained decreases in Eurasian watermilfoil biomass of 70 to 85 percent depending on the method being used. We intend to utilize the formulation that shows the greatest efficacy in controlling the milfoil, yet is most cost effective.

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Update on Biological Control of Waterlettuce

by
Dale H. Habeck,* Catherine R. Thompson,** F. Allen Dray,†
Ted D. Center,†† and Joe K. Balciunas†

INTRODUCTION

Waterlettuce, *Pistia stratiotes* L., continues to be a problem, particularly in south Florida waterways. The weevil *Neohydronomus affinis* Hustache (formerly called *N. pulchellus* Hustache) was released in the late spring and early summer of 1987 at five locations in south Florida. The weevil has become established at all release sites.

A colony of a second potential biological control agent, *Namangana pectinicornis* Hampson, was maintained in quarantine. Newly hatched larvae of this noctuid moth native to southeast Asia were unable to complete development to the second larval stage on any of the 60 plant species in 29 families that were tested (Habeck et al. 1988).

Studies during the past year were concentrated on completion of host-specificity tests to determine whether the moth was safe to release.

METHODS AND MATERIALS

Monitoring of the sites where *N. affinis* was released continued throughout the year. Counts of weevil adults, larvae, and waterlettuce plants were made monthly. General observations on waterlettuce condition were also made.

No-choice host-specificity tests were completed on third instar larvae. Ten larvae were placed in 1-oz (0.03-cu dm) plastic cups along with leaves and/or stems of the test plant. From two to six replicates were run on each plant. Each cup was checked daily to determine the number of live larvae and whether or not feeding had occurred. Dead larvae were removed, and fresh plant material was provided as needed. Check replicates of waterlettuce were set up each time other plants were tested. Twenty-four species of plants in 14 families were tested. A small second test involved only impatiens: three potted plants about 4 to 5 in. high were placed in a cage. Ten third instar larvae were placed on each plant.

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Plants were observed frequently for feeding damage and, after 5 weeks, were closely examined for larvae.

An oviposition choice test was also conducted. Stems and leaves of test plants were placed in 4-dram homeopathic vials that were placed at random in a vial rack. A few plants, including waterlettuce, Carolina mosquitofern (*Azolla caroliniana* Willd.), and water fern (*Salvinia minima* Baker), were placed in small petri dishes. Three replicates, each consisting of a cage with 35 test plants and 30 unsexed moths, were run. After 24 hr, any dead moths were replaced. After 48 hr the plants, as well as the vial racks, vials, and cage interiors, were carefully examined for egg masses.

RESULTS AND DISCUSSION

At the weevil release sites, the numbers of weevils and the areas infested continued to increase, but no significant impact on the plants was observed. The weevils are not having the rapid impact on waterlettuce in Florida that was reported in Australia (Harley et al. 1984) and South Africa (Cilliers 1987). Monitoring is continuing, and further releases are anticipated.

Third instar *N. pectinicornis* larvae were not as host specific as newly hatched larvae. Of the 24 plants tested, some feeding occurred on at least 13 species (Table 1). However, none of the larvae survived more than 6 days on any of the plants except waterlettuce and impatiens. Although larvae fed extensively on impatiens and a few developed into nearly mature larvae, none completed development or even pupated. The last larva died after 24 days.

In the second test involving only impatiens plants, a few larvae were observed feeding on the leaves. In a few days, some larvae bored into the stems and continued to feed. Plants were observed frequently but were not dissected until after 5 weeks. No larvae or pupae were found in the stems or soil, and there was no evidence of recent feeding, indicating that the larvae had perished much earlier. The plants had fully recovered and had grown to a height of 8 to 10 in.

The oviposition tests showed that the moths prefer to oviposit on waterlettuce. Of 91 egg masses, 64 (70.4%) were on waterlettuce. Another 19 (20.9%) were on the vial racks, cage interiors, and the cage sleeves. Single egg masses were on goldenclub (*Orontium aquaticum* L.), fragrant cudweed (*Gnaphalium obtusifolium* L.), beet (*Beta vulgaris* L.), and tomato (*Lycopersicon esculentum* L.), and four egg masses were on eggplant (*Solanum melongena* L.). Except for goldenclub and beet, all plants receiving egg masses had leaves with many hairs, like waterlettuce. The number of egg masses on the cage's interior and vial racks indicated that the moths clearly preferred to oviposit on waterlettuce but would oviposit on other plants or objects under cage conditions. Of the plants with egg masses, only goldenclub occurs in the same habitat as waterlettuce.

Table 1
Summary of Host-Specificity Studies of Third Instar
***Namangana pectinicornis* Larvae**

Family/Plant	*No. Replicates*	*Survival Period days*	*Feeding*
Amaranthaceae			
Alternanthera philoxeroides	3	2	None
Apiaceae			
Cicuta mexicana	1	2	None
Hydrocotyle umbellata	4	4	None
Araceae			
Arisaema dracontium	3	5	Some
Orontium aquaticum	3	5	Extensive - 1st 24 hr
Peltandra virginica	8	6	Some
Pistia stratiotes	10		Complete development
Asteraceae			
Gnaphalium obtusifolium	3	4	Very slight
Lactuca sativa	1	3	One larvae fed some
Balsaminaceae			
Impatiens sp.	5	25	Extensive
Brassicaceae			
Nasturtium officinale	2	4	None
Commelinaceae			
Commelina diffusa	4	4	Some
Cucurbitaceae			
Cucumis melo	1	4	Slight - 1st 24 hr
Cucumis sativus	1	2	Some
Malvaceae			
Gossypium hirsutum	1	3	None
Hibiscus esculentus	1	4	Some - 1st 24 hr
Poaceae			
Oryza sativa	3	6	A few feeding, one extensively
Saccharum officinarum	1	4	None
Pontederiaceae			
Eichhornia crassipes	1	4	None
Pontederia cordata	4	3	Slight
Rutaceae			
Citrus limon	1	3	None
Salviniaceae			
Azolla caroliniana	1	3	None
Salvinia minima	1	4	None
Solanaceae			
Lycopersicon esculentum	1	3	None
Solanum melongena	1	3	Very slight

CONCLUSIONS

Namangana pectinicornis appears to be able to complete its development only on waterlettuce. Including previous work in Indonesia (Mangoendihardjo and Narosh 1976, Mangoendihardjo et al. 1977) and Thailand (Suasa-ard and Napompeth 1978), this insect has been tested on 135 species or subspecies in 50 different plant families but has died on all plants except waterlettuce.

There are no reports of this insect causing problems or occurring on any other plant species in Indonesia, Thailand, or India (Sankaran and Ramaseshiah 1974). Even though the larvae fed rather extensively on impatiens, they were unable to complete their development. Impatiens are not likely to be grown in or near the areas where waterlettuce infestations occur. The moth appears to be a weak flier and probably would not venture far from aquatic habitats.

We are of the opinion that the moth is host specific, and are proceeding with an official request for permission to release.

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Genetic Engineering: Host Specificity

by
Edwin A. Theriot* and Stewart L. Kees*

BACKGROUND

The application of genetic engineering technology to a management scheme for biocontrol of nuisance submersed aquatic plants is emerging as a very promising biomanagement strategy. Genetic engineering is a new technology area that is innovative and has demonstrated the potential for tremendously broad application. Current management technologies can maintain lower populations of these submersed aquatics only for limited time periods. One of the most important reasons for application of this technology is its potential for the development of specific pathogens for specific problem plants at a number of different control levels.

To assist in the application of this technology to the development of biocontrol agents for submersed aquatic plants, a committee of experts was assembled to provide technical guidance and to ensure that all regulatory and environmental concerns are addressed (Theriot 1987). The first recommendation of that committee emphasized the availability of host-specific microorganisms as the most critical prerequisite for genetically engineering a microorganism for biocontrol.

The problem of host specificity has been addressed along two avenues of research. The first involves Dr. Craig Smith and other University of Wisconsin researchers who have developed a technique for evaluating interactions between microorganisms and submersed aquatic plants. Colonization experiments to date have involved the use of six fungi chosen on the basis of their likelihood to differ in both the degree and nature of their interaction with *Myriophyllum spicatum*. The flowchart presented as Figure 1 describes the assay technique and the microbial populations it measures.

Although the assay is simple, it evaluates several key interactions between a microorganism and the host aquatic plant. It evaluates (a) microbial attachment as superficial (unattached), casual (epiphytic colonizers) or intimate (endophytic colonizers), (b) growth on the plant surface that may have important implications for the outcome of the plant-microbe interaction, (c) host penetration as a function of the microbe's epiphytic or endophytic colonization, and (d) the development of symptoms. Perhaps more importantly, this technology allows us to depart from traditional microbial biological control (which concerns itself only with the production of disease symptoms) and consider nonpathogenic microbes that exhibit specificity for a particular target species.

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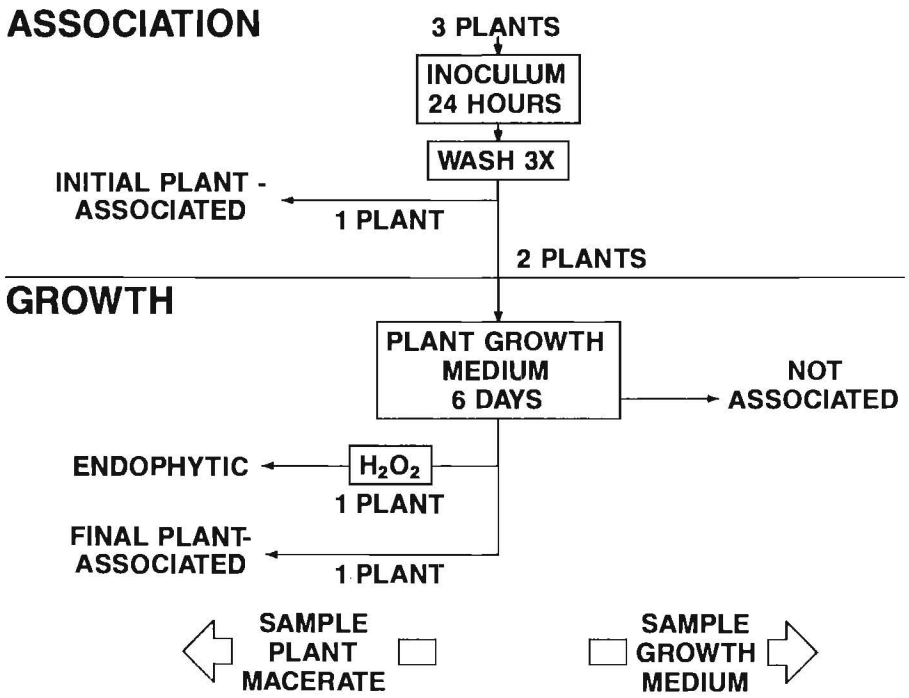


Figure 1. Flowchart of colonization assay

The second avenue of research for addressing the problem of specificity involves the lectin research being conducted at the Waterways Experiment Station in Vicksburg, Miss. The involvement of lectins in host-specific association with microbes was demonstrated as early as 1975 in the "Legume-Rhizobium" system (Dazzo and Hubbell 1975). The existence of lectins on the surface of *Hydrilla verticillata* or *M. spicatum* would mean that microorganisms that possess the complementary carbohydrate could then attach to the surface of the plant. The association is specific if the lectins are unique to each plant species. Our objectives have been to determine, through standard plant extraction techniques, whether our target species possess lectins; to identify the binding carbohydrate group; and then to use extracts of the plant(s) to demonstrate agglutination of host-specific microbes.

DESCRIPTION OF WES LECTIN RESEARCH

To identify the binding carbohydrate group, our initial affinity chromatography studies involved the use of seven sugar agaroses. These sugars are the most frequently reported carbohydrate binders in lectin literature (Goldstein, Hollerman, and Merrick 1965; Archibald and Coapes 1971; Hammarstrom and Kabat 1971; Bauer, Farr, and Horisberger 1974; Hall and Rowlands 1974; Horisberger 1976; Barkai-Golan, Mirelman, and Sharon 1978; Pistole 1981). Of the seven sugar agaroses shown in Figure 2, alpha-L-fucose is the only one for which *Hydrilla* protein extracts have demonstrated detectable affinity.

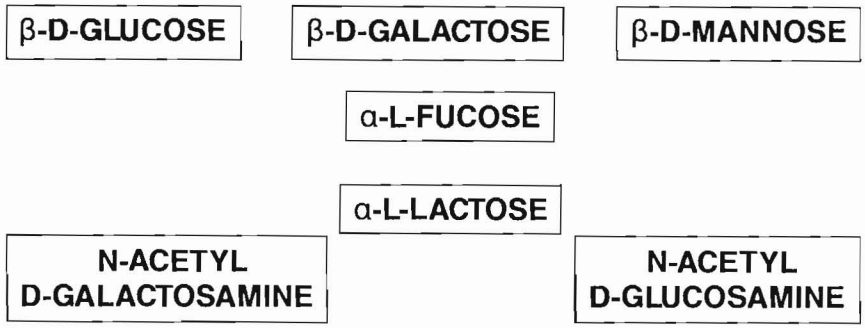


Figure 2. Sugar agaroses used in initial affinity chromatography studies

Molecular weight determinations were accomplished using standard electrophoretic techniques which consisted of both native polyacrylamide and sodium dodecyl sulfate polyacrylamide gel electrophoresis (Davis 1964, Weber and Osborn 1969, Laemmli 1970, Bryan 1977). Figure 3 is a logarithmic plot showing the four molecular weight standards used to determine the molecular weight of our fucose-binding glycoprotein, which was approximately 135,000 Daltons.

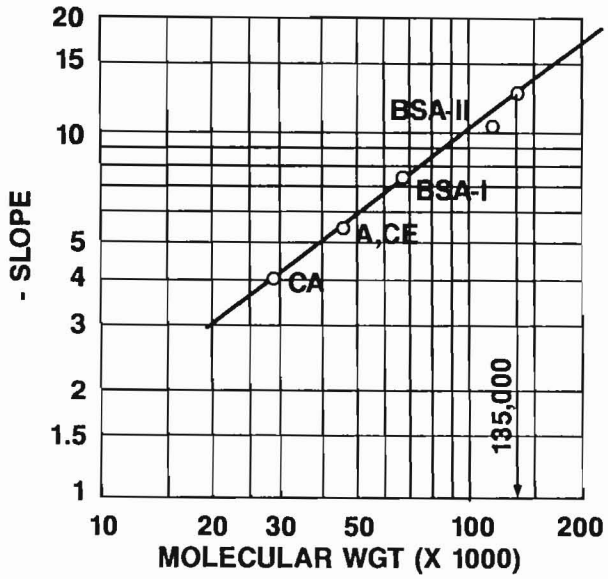
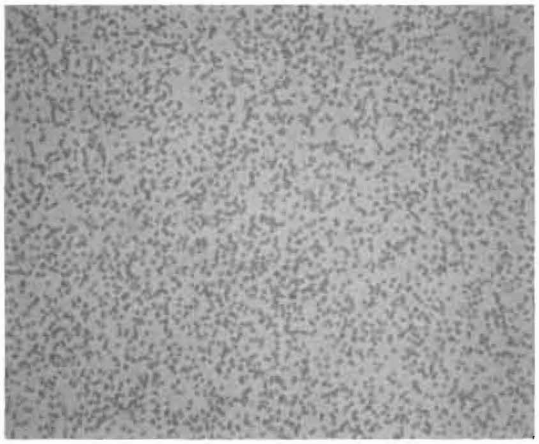
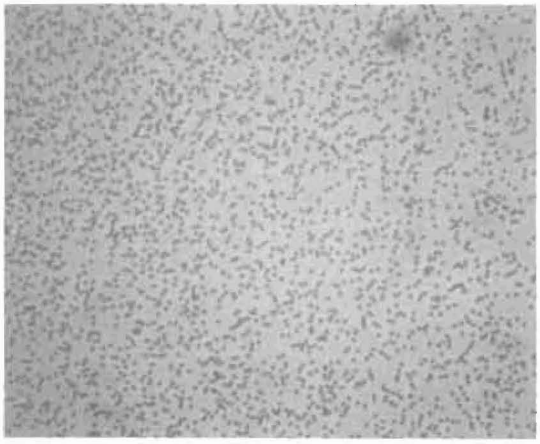


Figure 3. Native polyacrylamide gel electrophoresis data (standards: CA = carbonic anhydrase; A,CE, = albumin, chick egg; BSA-I = bovine serum albumin - monomer; BSA-II - bovine serum albumin - dimer)

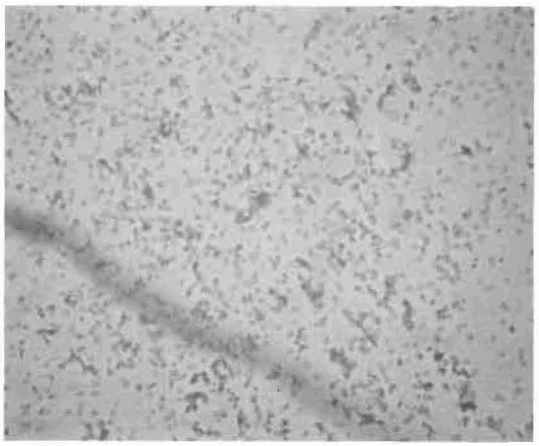
A common characteristic of all lectins is their ability to agglutinate members of the ABO blood group. All lectins reported in the literature to date which have demonstrated an affinity for alpha-L-fucose have likewise agglutinated erythrocytes of type O blood. The agglutination assay consisted of a 3-percent suspension of packed red blood cells from each of the three blood groups mixed in 50-$\mu\ell$ quantities with an equal volume of affinity chromatography-purified lectin at several twofold serial dilutions (Matsumoto and Osawa 1969; Horejsi and Kocourek 1974, 1978; Pereira and Kabat 1974; Allen and Johnson 1977; Kelly 1984). Figure 4 shows the results of the agglutination assay and confirms the presence of fucose-specific lectin.



a. Type A. Even in densely populated fields, individual cell membranes are still quite distinct after 1-hr incubation at room temperature



b. Type B. Membranes of adjacent cells are still distinct after 1-hr incubation at room temperature



c. Type O. Cell clumping or agglutination is quite evident, and individual cells are indistinguishable in agglutinated areas after 1-hr incubation at room temperature

Figure 4. Erythrocytes of human blood, group ABO

Our lectin research addresses a second recommendation of the technical advisory committee--to screen as many microorganisms as possible to enhance the possibility of identifying a suitable microbe for genetic engineering. The mechanism of specificity that has been elucidated by our research will allow us to develop a rapid screening assay (only 1 hr required to observe agglutination) and thereby screen literally thousands of microbes. Those microbes showing some agglutination will then move to the secondary screening technique developed by our colleagues at the University of Wisconsin, described earlier.

CONCLUSIONS

The WES research has led to the following results:

a. We have demonstrated the presence of lectins in the shoots, roots, and tubers of *Hydrilla verticillata*.

b. We have identified the binding carbohydrate group to be alpha-L-fucose.

c. We have characterized the molecular weight of this fucose-binding lectin and demonstrated specific agglutination with a finite number of fungal isolates.

d. Preliminary affinity chromatography data suggest that a fucose-binding glycoprotein also exists in the shoots of *Myriophyllum spicatum*; the root data are inconclusive.

These data attest to a mechanism of host specificity at the molecular level for *H. verticillata* which is present in every part of the plant; comparable data are accumulating for *M. spicatum*. This mechanism of specificity has enhanced our knowledge of plant-microbe interactions and will enable us to develop more feasible strategies for the biomanagement of submersed aquatic plants.

FUTURE RESEARCH

The two screening techniques described above will generate a list of specific microbes which can be applied in several biomanagement strategies. The diagram in Figure 5 describes three options for effective biomanagement of submersed aquatic plants with host-specific microbes. Inherent in the development of this genetic engineering biomanagement scheme is the potential to identify a sufficiently pathogenic microbe on the target species at the level of the secondary screen. If a pathogen is not found at the level of the secondary screen, the options still remain to use our specific microbe(s) in combination with lethal products such as enzymes, primary and secondary metabolites, etc., and thereby achieve plant management; specific microbe(s) may be used in an integrated approach in combination with pesticides or other allelochemicals to achieve plant management, or we may genetically engineer known lethal products into host-specific microbes and achieve plant management.

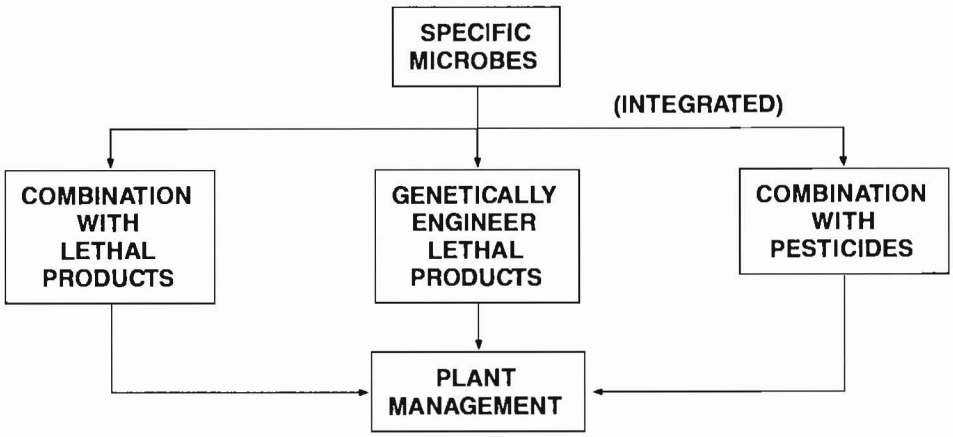


Figure 5. Future research

ACKNOWLEDGMENTS

We wish to thank Ms. Frankie Wilkerson of the Occupational Health Office, Waterways Experiment Station, for the acquisition and preparation of suitable human blood specimens.

We also wish to thank Dr. Fred Ryan of the University of California at Davis for his expert counsel in overcoming problems in our plant protein extraction techniques.

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Effects of Chemical Applications on the Biological Control Agents of Waterhyacinth

by
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BACKGROUND

Waterhyacinth, *Eichhornia crassipes* (Martius) Solms-Laubach, is a noxious aquatic plant that infests nearly all of the tropical and subtropical regions of the world (Holm et al. 1977, Penfound and Earle 1948). Because of its prolific nature and growth characteristics, it causes manifold problems, including the hindering of navigation and recreation, obstruction of drainage, and destruction of wildlife resources (Penfound and Earle 1948).

A common and currently acceptable solution for waterhyacinth management in the United States is the use of herbicide applications (Haag 1986a). For example, in 1987, more than 70,000 acres of waterhyacinth were treated in seven Corps Districts (unpublished data, 1987, Aquatic Plant Control Survey, Aquatic Plant Control Operations Support Center (APCOSC), Jacksonville, Florida). While other control practices have been used (Wunderlich 1962), chemical applications are still the most prevalent form of practiced management. Chemical control techniques have been shown to be effective; however, they are also relatively expensive. In 1987, chemical control costs ranged from $20 to $164/acre annually depending on herbicide used and mode of application (unpublished data, 1987, Aquatic Plant Control Survey, APCOSC).

Beginning in 1972, several different biological control agents of waterhyacinth were released in the United States (Perkins 1973a, Center and Durden 1981, Cofrancesco 1985). These organisms included two weevil species, *Neochetina eichhorniae* and *Neochetina bruchi*, and a moth species, *Sameodes albiguttalis* (Bennett and Zwolfer 1968; Perkins 1973b, 1974; DeLoach and Cordo 1976; Perkins and Maddox 1976; Center and Durden 1981; Center 1982; Center, Durden, and Corman 1984; Sanders, Theriot, and Perfetti 1985). Releases continued in different regions of the United States through 1984 (Table 1).

Presently, the distribution of waterhyacinth in the United States is confined to the Southeastern States, southern Texas, and portions of California (Figure 1). Coinciding with the US waterhyacinth distribution is the occurrence of all three species of biocontrol agents. However, the distribution of *S. albiguttalis* is patchy (Cofrancesco 1985), and populations of the waterhyacinth weevils become reduced within the northern range (i.e., Tennessee and North Carolina). The most

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Table 1
Initial Releases of Insect Biological Control Agents in Different Regions of the United States

State	*Release Date*
Neochetina eichhorniae	
Florida	1972
Louisiana	1976
Texas	1981
California	1983
Neochetina bruchi	
Florida	1974
Louisiana	1974
Texas	1980
California	1982
Sameodes albiguttalis	
Florida	1977
Louisiana	1979
Texas	1981
California	1984

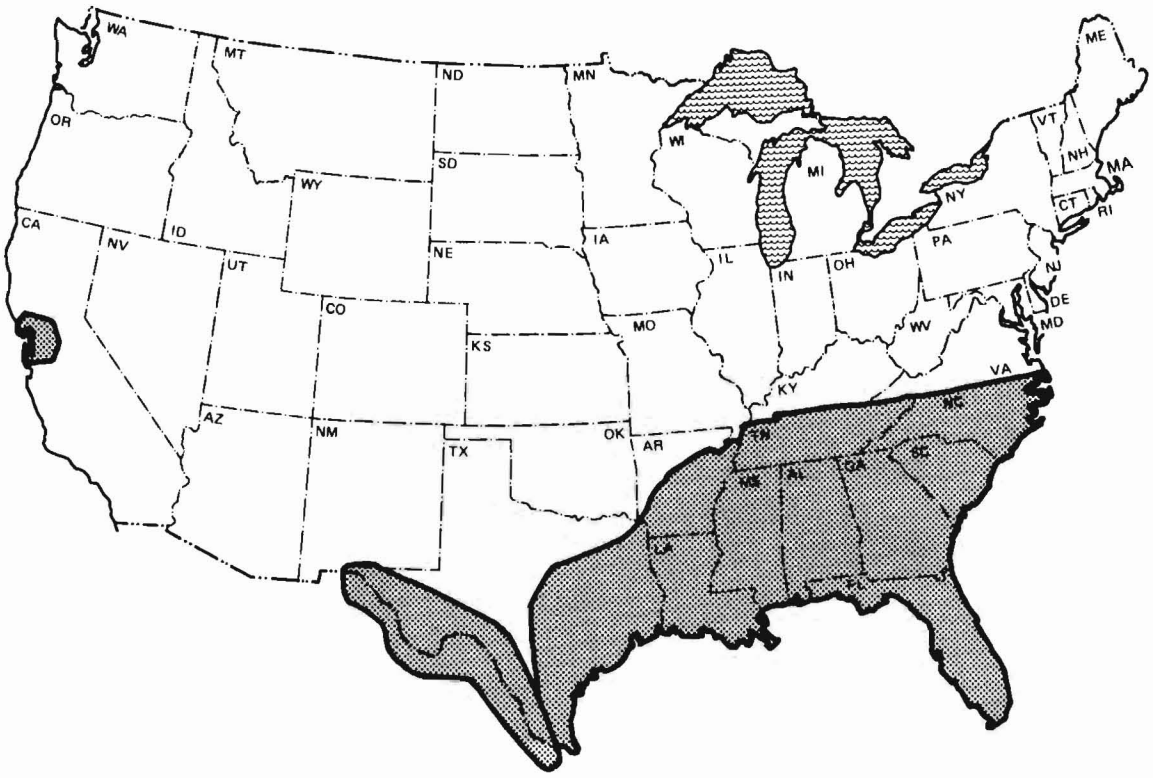

Figure 1. Map of the continental United States with regions of waterhyacinth infestation indicated by stippling

common biocontrol agents are the two weevil species. *Neochetina eichhorniae* is the most abundant weevil in areas where waterhyacinth is under the greatest stress by insect herbivory (personal communication, T. Center, USDA Aquatic Plant Management Laboratory, Fort Lauderdale, Florida). Most of our research has centered around the two weevil species (especially *N. eichhorniae*) because of their greater abundance.

While satisfactory biocontrol has been achieved at many sites using these agents (Goyer and Stark 1984; Cofrancesco, Stewart, and Sanders 1985; Center and Durden 1986), other areas have received only minimal impact. Many factors can affect the distribution, abundance, and efficacy of the weevil species. These factors include predation, disease and parasitism, environmental barriers (e.g., isolated patches of waterhyacinth), age-related mortality, and chemical application. Of all the factors that could possibly influence the biocontrol agents' efficacy, chemical application is the only factor over which operational personnel have active control.

Since chemicals are used over large geographical areas where the biocontrol agents exist, impact to the biocontrol agents may be tremendous. It is, therefore, imperative that any negative chemical effects on the biocontrol agents be quantified and understood.

To this end, we are conducting studies to characterize the direct and indirect effects of chemical usage on the biocontrol agents of waterhyacinth, specifically *N. eichhorniae*. Direct effects are those that are manifested because of the chemicals themselves and can include both mortality and behavioral changes. Indirect effects are those that are manifested because of herbicide-induced changes in the surrounding habitat. Again, increased mortality or changes in behavior can result.

EFFECTS OF HERBICIDE APPLICATIONS

Direct effects

Several commonly used herbicides, adjuvants, and herbicide/adjuvant combinations were tested for toxic effects on *N. eichhorniae* (Table 2). Commonality of these chemicals in waterhyacinth control practices was determined by questioning District and state personnel.

At 3× and 6× the recommended field rates, little mortality is observed when the chemicals are applied topically to the weevils. Significant mortality occurs only when X-77 and diquat are applied to weevils that are subsequently held under dry environmental conditions (i.e., low humidity and no access to free water; Table 3). Such dry conditions are probably never realized in the field, but additional research is needed. Haag (1986b) also found limited mortality for weevils treated with various herbicide formulations. The only significant mortality observed in her studies was with the adjuvant I'VOD, an inverting oil. However, this compound is

Table 2
Herbicides and Associated Adjuvants Commonly Used in Waterhyacinth Control. The Listed Herbicides, Adjuvants, and Associated Combinations of the Two Were Tested to Determine Their Toxic Effects on *N. eichhorniae*

Herbicides
2,4-D (2,4-dichlorophenoxyacetic acid)
Diquat dibromide (6,7-dihydrodipyrido(1,2-α:2′,1′-c) pyrazinediium dibromide)
Glyphosate (the isopropylamine salt of N-[phosphonomethyl]glycine)
Adjuvants
X-77
Nalco-Trol II
Spreader/Sticker
Sta Put
Cide-Kick
Nalco-Trol II + X-77

Note: List was derived by questioning District and state personnel.

Table 3
Percent Mortality for Male and Female *N. eichhorniae* Weevils Topically Treated with Water (Control), Diquat, X-77, and Diquat + X-77

	Male		*Female*	
Treatment	*Wet*	*Dry*	*Wet*	*Dry*
Control	10.0	0.0	0.0 (5.0)*	0.0 (5.0)
Diquat	0.0	15.0** (25.0)	5.0 (10.0)	25.0†
X-77	10.0	0.0	0.0	10.0
Diquat + X-77	5.0 (20.0)	30.0† (70.0)	0.0	70.0†

Note: Weevils were held at both wet and dry environmental conditions (n = 20 weevils). Concentration of each treatment was at 6× field rates.
*Numbers in parentheses indicate percentage of weevils showing signs of physical impairment at the termination of the experiment (96 hr).
**P = 0.07 using a chi-square row by column contingency analysis.
†P < 0.05 using a chi-square row by column contingency analysis.

rarely used in formulation with herbicides targeted for waterhyacinth control.

Although limited mortality results from chemical exposure, few if any changes in behavior are detected after direct chemical contact. Feeding apparently remains at levels comparable to those observed when no chemicals are applied. Hence, direct effects appear to have little influence on the weevils.

Indirect effects

To understand the effects of chemical treatment on plant quality as they pertain to weevil biology, a series of studies were conducted. Plants were treated with 2,4-D, diquat, or water (control), or they were allowed to desiccate (a negative control).

Changes in the biochemical/nutrient status of the plants occurred that were dependent on treatment. The desiccating and diquat treatments showed major reductions in plant quality within 2 to 4 days. Loss of turgor and browning occurred for the diquat-treated plants. These morphological changes were accompanied by significant losses of ether-extractable compounds (i.e., mainly lipids). Desiccating plants also exhibited losses in ether-extractable compounds, as well as significant reductions in moisture content. The 2,4-D treated plants degraded slowly and, correspondingly, there was little detectable change in their biochemical/nutrient status.

Weevil responses to degrading chemical-treated plant material, and to the desiccating and water-treated control plants, were quantified. Changes in weevil behavior were associated with the observed reductions in biochemical/nutrient status. Weevil feeding on desiccating and diquat-treated plant material decreased significantly in the first 2 days (Figure 2). Little if any feeding was observed 8 days after application of these treatments. Weevil feeding also decreased on 2,4-D-treated plant material, but took longer to become evident.

As feeding decreased, insect movement toward untreated plants increased (Figure 3). This movement was noted early and occurred at a high rate in relatively quick-acting treatments (i.e., desiccation and diquat). Few weevils moved off of the slowly degrading 2,4-D-treated plants within the first 5 to 6 days. However, movement subsequently proceeded at higher rates. Percent cumulative movement for both sexes off of all treatments was significantly different from movement off controls ($P \leq 0.05$). Movement off control plants proceeded at a low but relatively constant rate for the duration of the experiments. Results from these studies indicate that the indirect effects of chemical applications significantly influence *N. eichhorniae* behavior.

Essentially, these experiments indicate that changes in weevil behavior are directed by the degradation of the plant material. As feeding decreases in response to changes in plant quality, weevils move off of treated plants, presumably in search of more suitable food sources (i.e., untreated plant material).

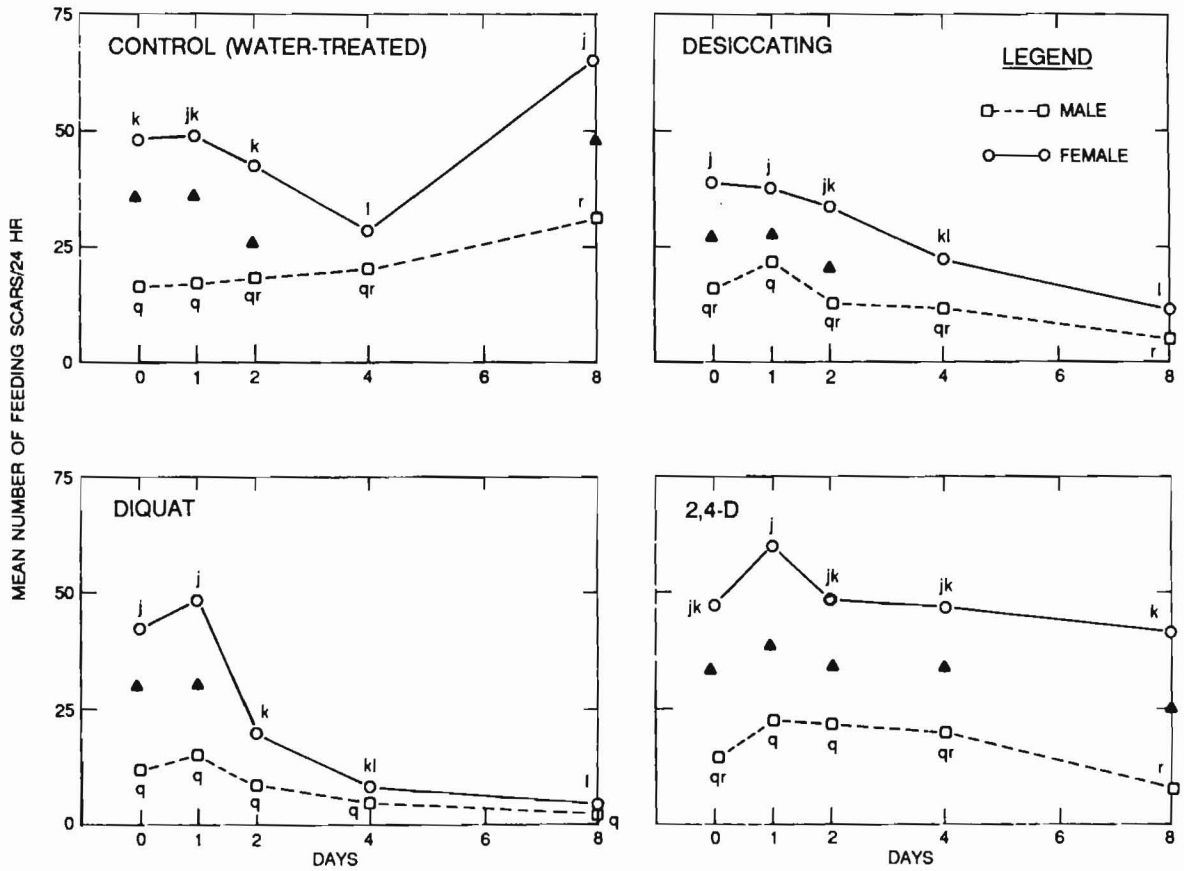

Figure 2. Mean number of feeding scars/24 hr for *N. eichhorniae* given waterhyacinth treated with water (i.e., control), drying conditions (desiccation), diquat, or 2,4-D. (Means followed by the same letter are not significantly different through time at P > 0.05 for a given sex and treatment. Means separated by a triangle indicate significant differences between sexes at P ≤ 0.05 for a given treatment and day)

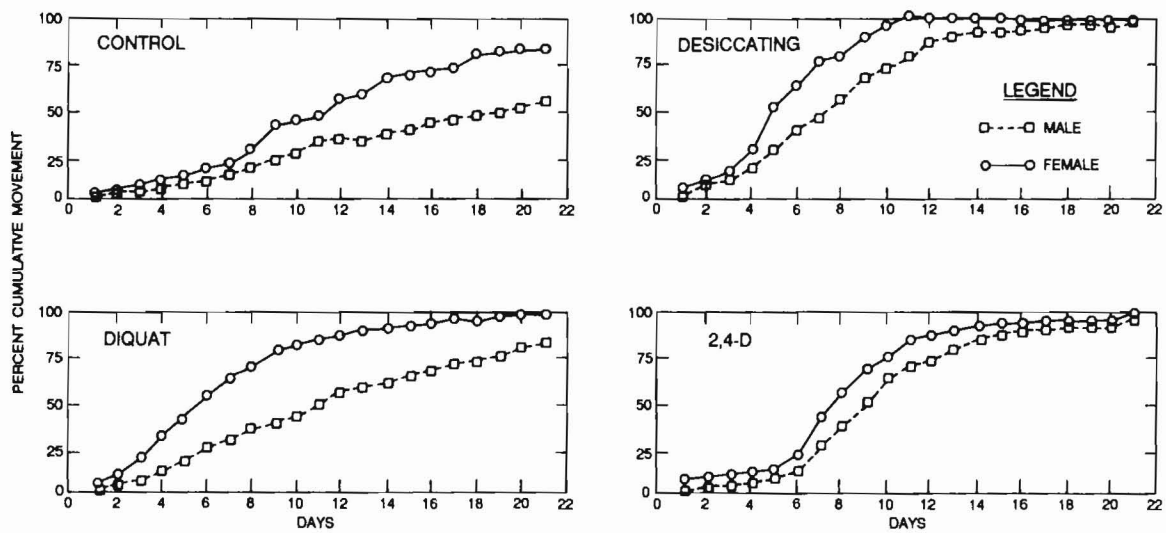

Figure 3. Mean percent cumulative movement of *N. eichhorniae* off of waterhyacinth treated with water (i.e., control), drying conditions (desiccation), diquat, or 2,4-D

Movement from treated to untreated plant material has been noted previously for *N. eichhorniae* (Haag 1986a, 1986b).

The indirect effects with the greatest impact are related to the destruction of the plant material after chemical application. These occur because the relatively nonmobile immature stages (i.e., eggs, larvae, and pupae) of *N. eichhorniae* are confined within the plants. With plant degradation and subsequential destruction of the waterhyacinth mat, high mortality of immatures occurs.

The loss of large numbers of the immatures is only one way that chemical applications indirectly affect biocontrol agent efficacy. Another, more subtle effect is the long-term change in the population structure following chemical application. Establishment of a population structure consisting of all life stages is a slow process. This is especially true for this weevil species, whose development from egg to adult takes over 90 days (Center 1982). The larval stages, especially third instar larvae, have been shown to be the most damaging (Center 1982). Considerable time is needed for the recovery of enough immature individuals to significantly impact the plants.

Residual plant populations occur at sites following chemical treatment. In the absence of stressful populations of biocontrol agents, these highly prolific plants can quickly reinfest a site (Westlake 1963, Bock 1969). Such rebound effects occur because waterhyacinth productivity exceeds that of the weevil (Wright and Center 1984). Therefore, waterhyacinth can reach economically important levels prior to the redevelopment of stressful biocontrol agent populations, which act to reduce waterhyacinth levels below their carrying capacity.

No information is available quantifying the mortality of weevil populations following plant degradation. However, a simple hypothetical example should suffice in describing this mortality. Consider that a 5-acre pond with an insect density of 75 immatures and 25 adults/m^2 would contain ca. 1.5 million immatures and ca. 500,000 adults. After chemical application and complete degradation of the plant material, 1.5 million immatures would be lost (Figure 4). A threefold reduction in hypothetical mortality to only 500,000 immatures/5 acres could be realized if the population structure consisted of 75 percent adults instead of the 75 percent nonmobile immatures cited in the example.

Shifts in population structures have also been shown to occur naturally in the field (Figure 5). In Lake Alice, Florida, changes in the proportion of adults ranged from 50 to >80 percent relative to the total number of third instar larvae and adults (Akbay, Wooten, and Howell 1988). While this proportional shift may be minimized if all life stages were considered, changes in the proportion of adults do occur seasonally (Grodowitz and Stewart 1989). In addition, population structure changes probably vary from site to site.

Population structure is only one consideration when estimating the impact that chemical applications have on weevil survival. Other factors may also be important. The potential reestablishment of weevil populations on nearby

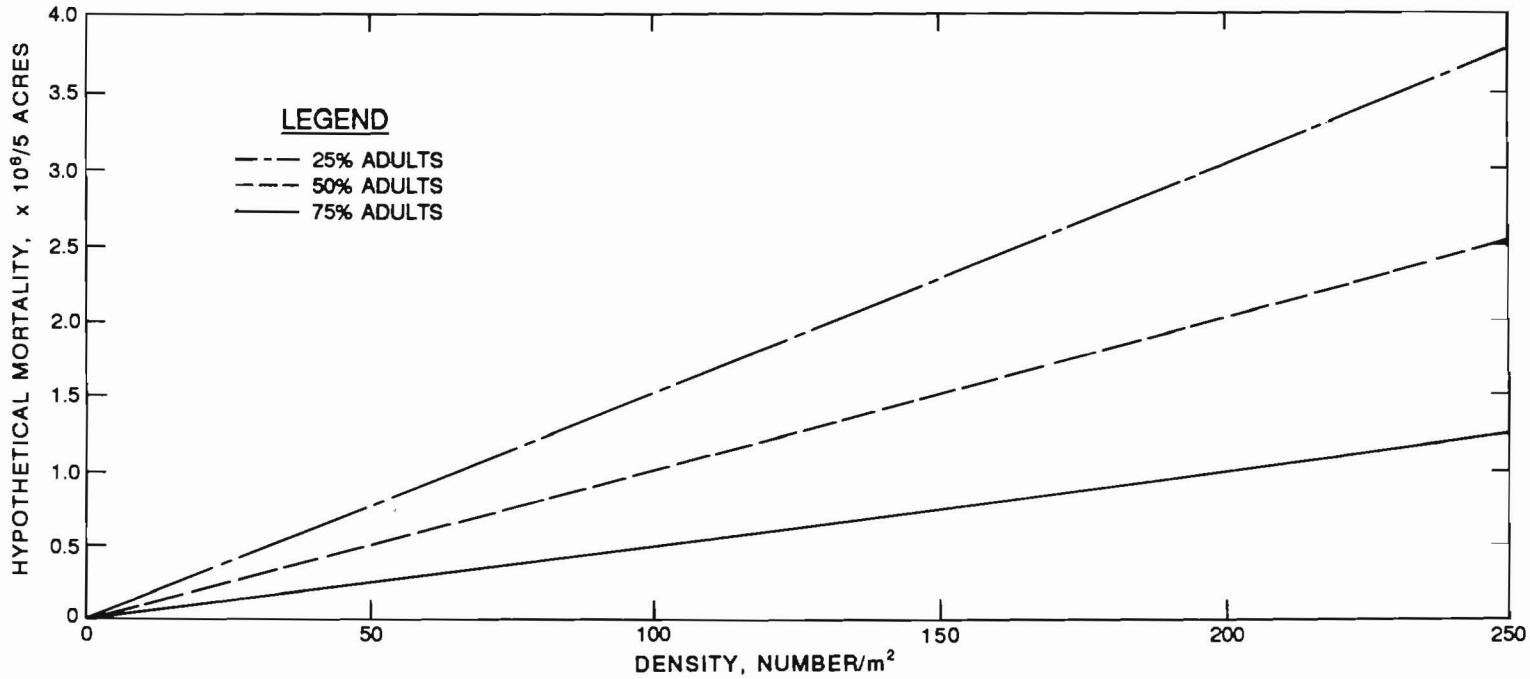

Figure 4. Hypothetical mortality of *N. eichhorniae* after herbicide application. Such mortality is shown for populations with different structures, i.e., varying proportions of adults in the population. Density per square metre represents the number of individuals of all life stages found in a square metre sample. Each line represents the number of individuals lost on a 5-acre pond after chemical application for population structures containing 25, 50, and 75 percent adults. The following assumptions apply: no mortality for the adults, 100-percent mortality for the immatures, and the insects in the square metre sample are distributed evenly over the entire 5-acre pond

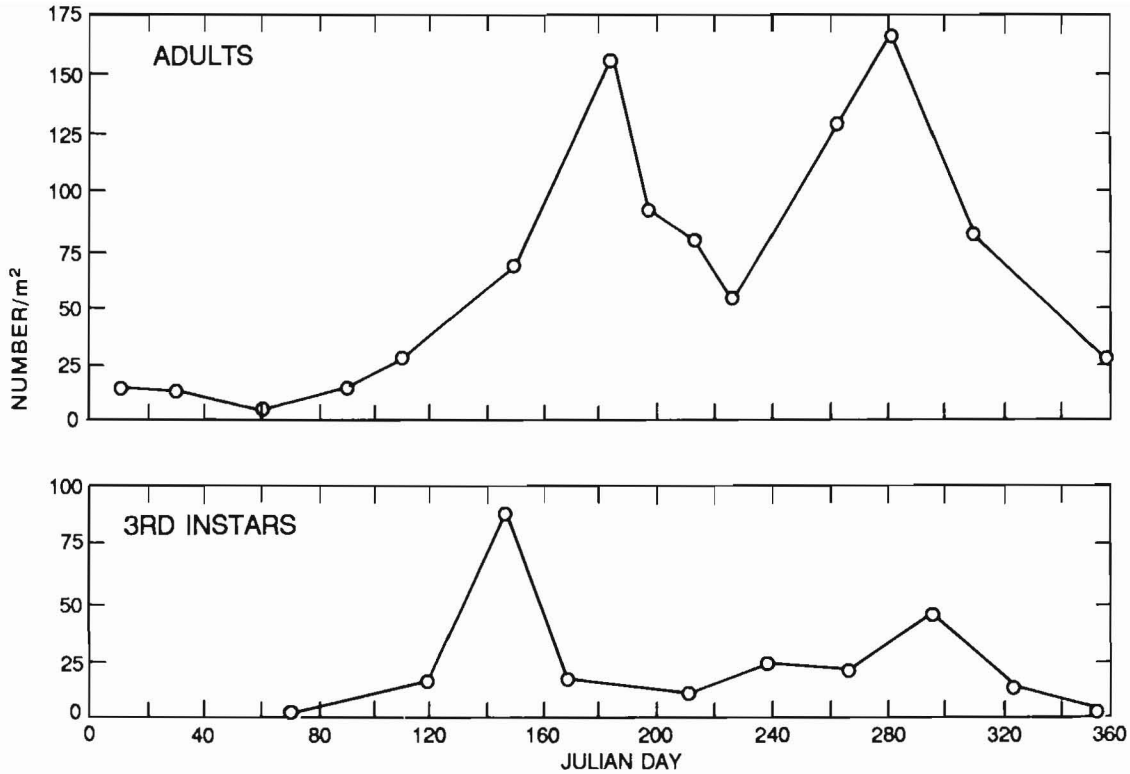

Figure 5. Numbers of adults and third instar larvae/m^2 through time at a site on Lake Alice, Florida, in 1976

untreated sites may be affected by the reproductive status of surviving weevils. The following example illustrates this concept. At a density of 50 females/m^2, a 5-acre pond would contain ca. 1 million females (Figure 6). If 25 percent of these females were fecund (i.e., actively reproducing), then only 250,000 individuals could oviposit and thus contribute to the next generation at nearby sites. A threefold increase in viable colonizers would result if the female population were 75-percent fecund.

Limited information describing the reproductive status of *N. eichhorniae* populations is available. However, preliminary information from sites in Wallisville, Texas (unpublished data) (Figure 7), and West Palm Beach, Florida (personal communication, T. Center), indicates that shifts do occur. Changes in reproductive status have been found to be related to season. Lower numbers of individuals with fully functioning reproductive organs are found during the winter months at both sites. At the start of the waterhyacinth growing season, the percentage of individuals with nonfunctioning ovaries declines, and most female weevils are again reproductively active. Hence, shifts in reproductive status do occur and could potentially influence the weevils' ability to reestablish on nearby untreated sites.

Are present chemical control technologies compatible with existing biocontrol agents of waterhyacinth? The answer to this question must be no! While only limited direct effects were documented, chemical applications do have a decided

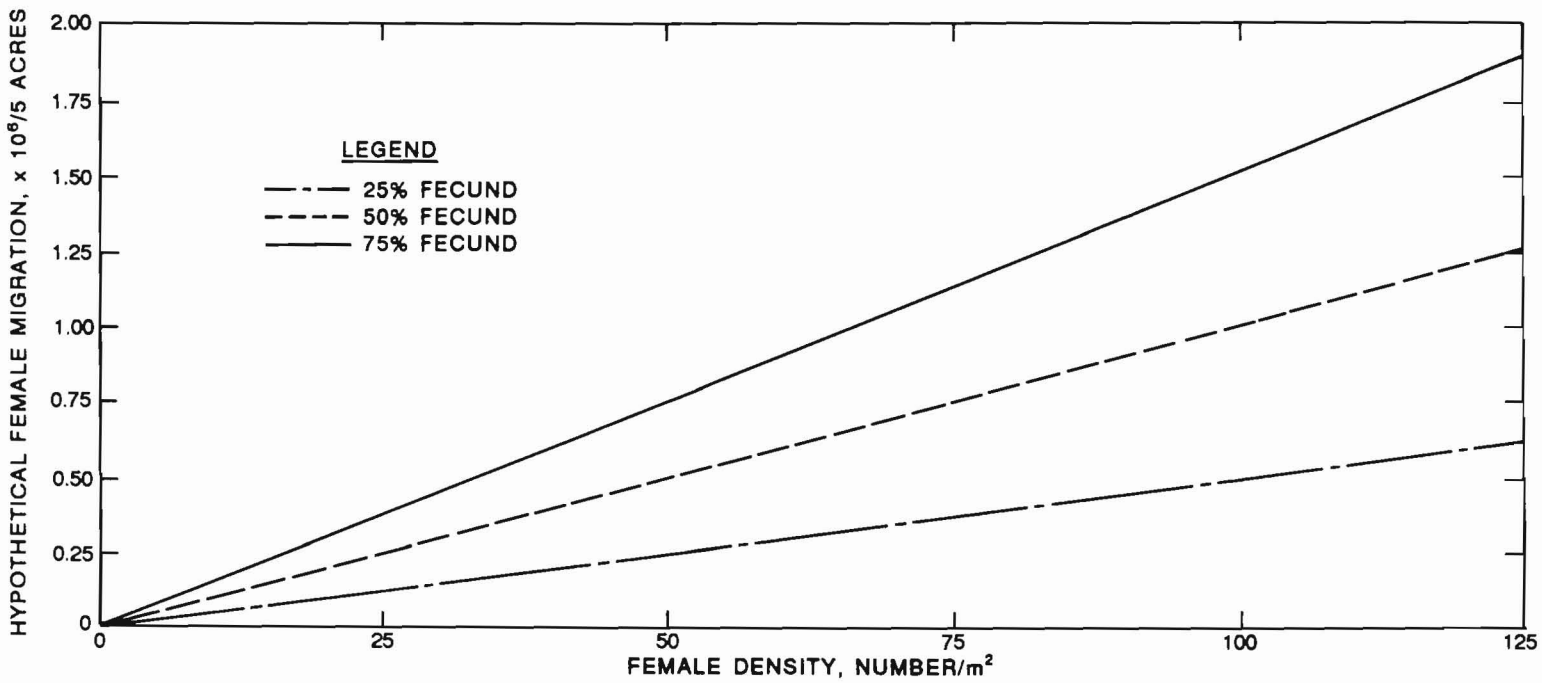

Figure 6. Effects of different proportions of fecundity (i.e., ability to produce and lay eggs) on the hypothetical number of fecund females left in a 5-acre pond after herbicide application. No mortality is assumed for the females, and all females can successfully migrate to the untreated waterhyacinth sites

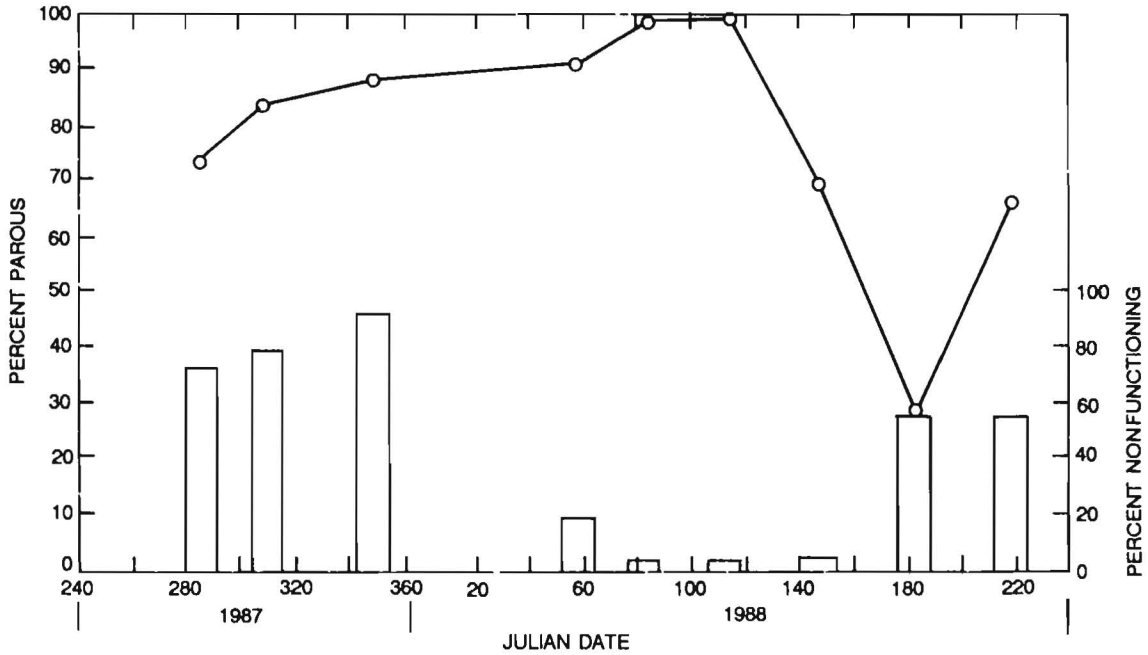

Figure 7. Percentage of females in the parous state (i.e., reproductive) collected at Wallisville, Texas, in 1987 and 1988. Histograms indicate the proportion of the parous individuals with nonfunctioning ovaries

impact. This impact is manifested in terms of the indirect effects. These include changes in weevil behavior and reductions in weevil populations due to plant degradation and mat loss. If we consider the 70,000 acres onto which herbicides were applied in 1987 and speculate that an average of only 50 immatures occurred per square metre, then ca. 14 billion individuals would have been lost to the overall effort of waterhyacinth management. This present noncompatibility must be hindering the effectiveness of the existing biocontrol agents. Present chemical control methodologies are detrimental to biological control. Also, it would be difficult to integrate the two technologies (i.e., chemical with biological control) as proposed by District personnel.

FUTURE WORK

More information is needed before solutions to this noncompatibility can be realized. One viable solution is to time herbicide applications based on the existing weevil population structure as well as the population's reproductive status. Such efforts toward timing herbicide applications should reduce the total number of weevils lost with plant degradation. Simultaneously, unsprayed waterhyacinth could be left as harborage for the migrating weevils and their potential offspring. The use of harborages (or reservoir areas) for weevil conservation has been proposed previously (Center 1982; Haag 1986a, 1986b).

Unfortunately, the suggested solution has not been adequately tested. This solution may be ineffective in reducing the total number of weevils lost after

herbicide application. If effective, it may still prove to be a very impractical solution, especially considering the logistics involved in timing herbicide applications.

Future studies are needed to determine the effectiveness of using such a system. This can be accomplished by quantifying the actual effects of chemical applications on field populations of weevils and by characterizing population structure changes, both seasonally and at various sites. The system should be implemented on a small scale to further determine its effectiveness. In addition, the practicality of using this system on a large scale in existing operational programs should be determined. Timing of herbicide applications could be facilitated by the use of simulation models that predict population dynamics (such as the model INSECT) (Howell and Stewart 1989). Until this noncompatibility is reduced, there can be no effective integration of the two technologies.

The described studies have concentrated on noncompatibility between the waterhyacinth weevils and chemical control methodologies. Similar effects could occur in other aquatic plant/biocontrol agent systems including hydrilla, water-lettuce, etc. Whenever chemical control is used, changes will occur that may reduce the biocontrol agent effectiveness. Hence, concepts developed as a result of the present research on waterhyacinth will be valuable in understanding and reducing noncompatibility in other aquatic plant/biocontrol agent systems.

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Expert Systems in Aquatic Plant Management Programs

by
Hal E. Lemmon* and Michael Jay Grodowitz**

EXPERT SYSTEMS

One method of transferring knowledge held by a specialist, or several specialists, is by means of an expert system. Expert systems are special computer software applications that are capable of carrying out reasoning and analysis functions in narrowly defined areas at proficiency levels approaching those of a human expert.

The study of expert systems is a subfield of the computer science field known as artificial intelligence. Currently there are approximately 3,000 expert systems operating in the United States.

Many expert systems are of the diagnostic type. For example, there are expert systems for diagnosing problems with an automobile electrical system, a high-performance disk drive, a diesel locomotive, and a stuck drill pipe on a drilling rig.

An expert system typically performs as follows:

- Asks questions about the problem.
- May instruct the user to perform tests and report the results.
- Diagnoses the problem.
- Recommends an action to solve the problem.

Expert systems are designed by a team consisting of:

- Experts - the person or persons who are experts in the field.
- The knowledge engineer - the person who knows how to take the knowledge from an expert and put it into a computer in such a manner as to allow reasoning and analysis.
- Users - those for whom the expert system is designed. Users use the expert system, and also play an important role in debugging the system. They often provide additional knowledge, which is then added to the expert system. For example, the principal users of the XCON Expert System (to configure VAX computers) are also the experts on how to configure computers.

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RULE-BASED EXPERT SYSTEMS

There is no limit to the variety of ways that expert systems can be developed. However, in the past few years there has been an acceleration in the popularity of rule-based expert systems.

Rule-based expert systems have many advantages:

- It is easy to think in terms of rules and facts.
- It is easy to enter rules and facts into the computer, thus eliminating time-consuming programming.
- It is easy and fast to build a prototype to test the feasibility of using an expert system to solve a problem.
- After gaining experience building a prototype, it is easy and inexpensive to start over again using another approach.
- After a satisfactory prototype has been built, it is easy to modify and extend it to a comprehensive final system.

There are three parts to a rule-based expert system (Figure 1).

- Rules (knowledge base).
- Facts.
- Inference engine.

A set of rules and facts is prepared which contains the knowledge and reasoning required for the expert system to perform. The expert system requests additional facts from the user. The inference engine applies the rules and the facts and *infers* (hence, the name inference engine) from these a conclusion and recommendations.

HYBRID EXPERT SYSTEMS

A principal disadvantage of a rule-based system is that sometimes the problem or some parts of a problem cannot be expressed in rules. For example, the rate at which aquatic plants grow is better expressed as a mathematical formula or several mathematical formulas depending on temperatures, day length, nutrients, and so forth. It would be impossible to express this as a set of rules.

In these cases, we use hybrid systems. We use rules where they are appropriate, and call in and execute mathematical subroutines when needed. Comax/Gossym, an expert system for the management of cotton, is a hybrid. Comax is the rule-based expert system, and Gossym is a model of the cotton plant. HOPPER, an expert system for control of grasshoppers on rangeland, is essentially a rule-based

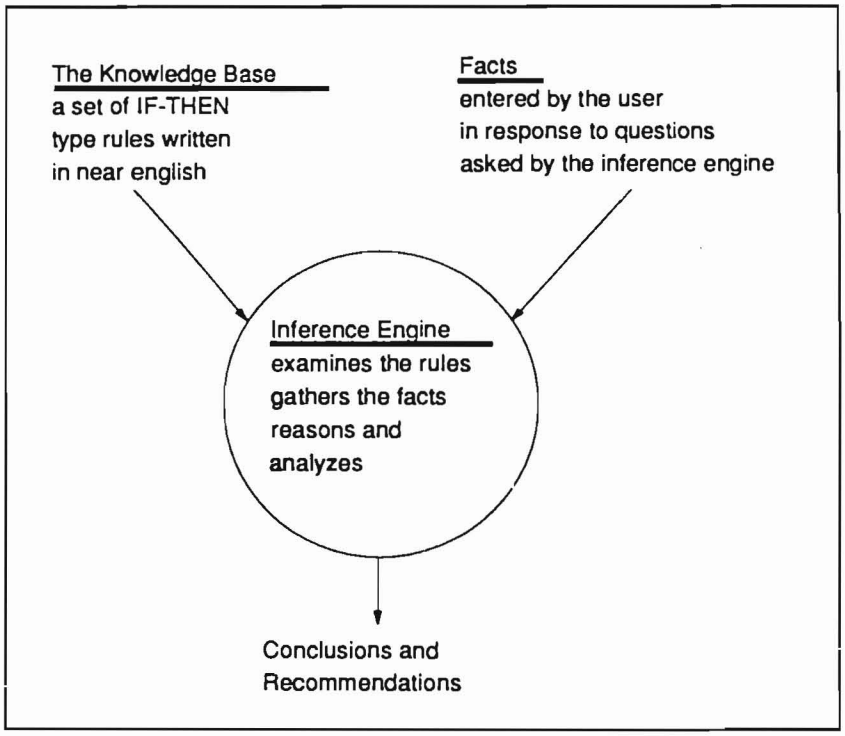

Figure 1. Graphic representation of an expert system

system but calls upon mathematical programs to compute the rates at which grasshoppers grow and the amount of forage they consume.

Rule-based expert systems also have an educational advantage. It is possible to design the system in such a way that it can explain its recommendations. For example, if the expert system recommended c as a control for alligatorweed, then you could ask it why it made this recommendation and the system would explain its reasoning. Such as, alligatorweed is an above-water plant; the controls which are applicable to above-water plants and are effective in a short period of time are a , b , and c ; and c is the least expensive.

The system can also explain why a different recommendation was not made. For example, the user might ask, why not use white amur? The expert system would reply, white amur will not control plants whose growth is above water.

Other computer systems can be programmed to explain their results, but it is easier with rule-based systems.

EXPERT SYSTEM SHELLS

A wide variety of expert system shells are available. An expert system shell is a system of programs that provide a means for entering rules and facts into the

computer plus an inference engine that executes those rules and facts interactively with the user.

Commercial shells range in price from $100 to $60,000. In 1985, the US Department of Agriculture (USDA) purchased two expert system shells (named ART) at $40,000 each. They also purchased a Symbolics LISP computer at $120,000 to run these expert system shells. These were used to develop Comax, the cotton crop management expert system.

In 1986, USDA purchased the VP-Expert package for $100, which can operate on the PC. It was used to develop HOPPER, the grasshopper management program.

There is also an excellent shell named CLIPS, developed by the National Aeronautics and Space Administration, which runs on the PC, is patterned after ART, and is free to Government agencies.

AN EXPERT SYSTEM FOR AQUATIC PLANT CONTROL

A simple expert system has been developed to assess the feasibility for providing field managers with recommendations for controlling aquatic plants. The expert system works as follows:

a. The user is asked at what level he would like to think about his problem--at the habitat level, the plant type level, or the species level.

b. If the habitat level is selected, the user is asked to select above water or below water.

c. If the plant type is selected, the user is asked if the plant type is emergent, floating, or submerged.

d. If the species is selected, the user is asked if the plant species is alligatorweed, hydrilla, waterhyacinth, waterlettuce, or watermilfoil.

e. Finally, the user is asked if he wishes to consider that the possible treatments be applied concurrently or sequentially.

There are aquatic means of controls which can be applied to the stated problem. Controls are divided into four categories, as detailed below.

a. Biological: *Agasicles*, *Neochetina bruchi*, *Neochetina eichhornia*, *Sameodes*, thrips, and white amur.

b. Chemical: 2,4-D, complexed copper, dichlobenil, diquat, endothall, fluridone, glyphosate, and triclopyr.

c. Mechanical: Dredge and harvester.

d. Physical: Barrier and drawdown.

The expert system eliminates all controls which are not effective for the problem stated. It does this by applying a set of rules which are formulated by the human experts in the field. The system then lists all possible combinations of controls which can be expected to be effectively applied.

CHEMICAL CONTROL TECHNOLOGY DEVELOPMENT

Chemical Control Technology Development: Overview

by
Howard E. Westerdahl*

The following discussion describes the interdependency of research work units within the Chemical Control Technology (CCT) area of the Aquatic Plant Control Research Program (APCRP). The overall goal of this research area is to improve management of nuisance aquatic plants in an environmentally compatible manner. The current focus of research within each work unit is on aquatic plant control in high water-exchange environments. Accomplishment of this goal requires close coordination with the chemical industry and the US Environmental Protection Agency's Registration Division.

The CCT research area (Figure 1) identifies and tests registered chemicals for aquatic use (considered on-the-shelf products). Also, we consider testing new chemical formulations for which industry has made a firm registration commitment. We select these formulations based on published reports and unpublished data provided to us by the chemical company.

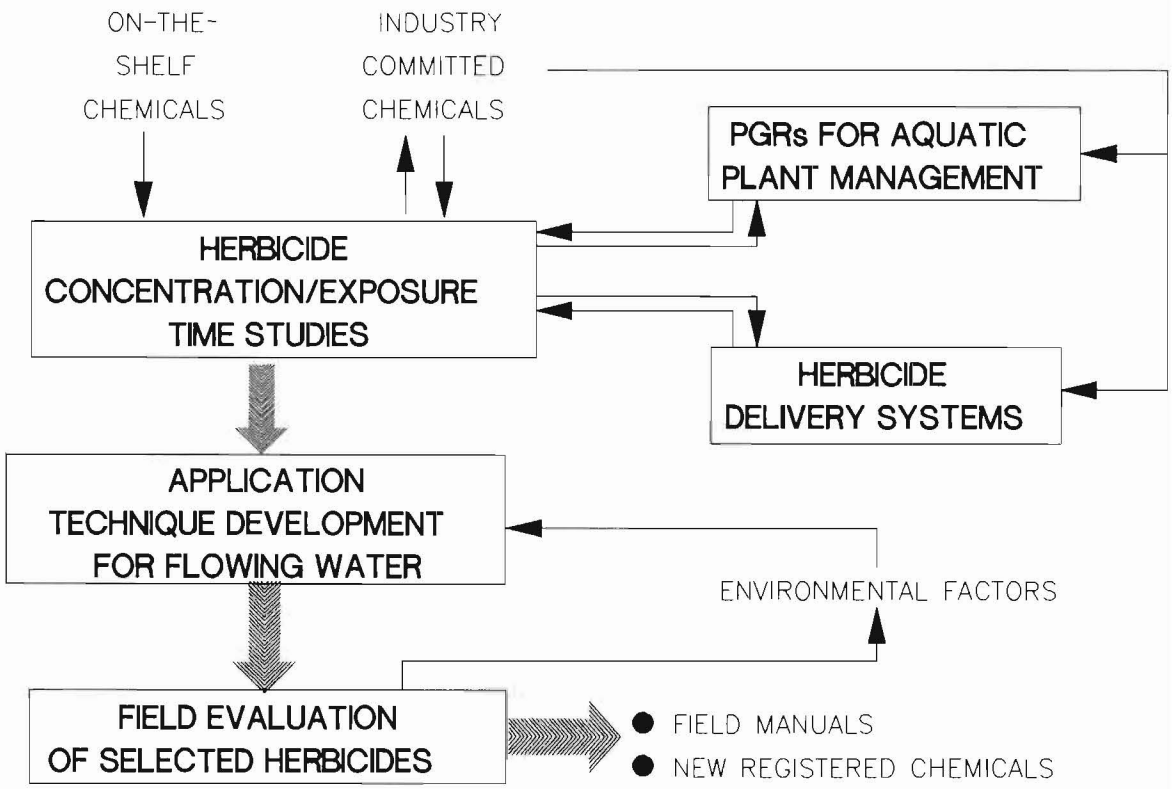

Figure 1. Interdependence of Chemical Control Technology work units

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Two new work units characterize our continued interest in improving chemical use in an environmentally compatible manner. The first, Plant Growth Regulators for Aquatic Plant Management (PGRAPM), represents our research efforts to find new approaches to managing aquatic plant growth. Current efforts to control aquatic plants reduce the standing crop. This often results in plant decomposition and disruption of the overall plant community structure. Moreover, fluctuations in nutrient levels, turbidity, dissolved oxygen, and habitat loss may impact food web relationships. Plant growth regulators (PGRs) offer the potential for slowing the vertical growth rate of submersed aquatic plants. Hence, these plants may not interfere with the intended uses of the lake or disrupt the overall plant community. Bioassay testing of PGR formulations includes their activity on vegetative growth suppression and reproduction of nuisance aquatic plants. Dr. Carole Lembi, Purdue University, Indiana, will discuss bioassay procedures and results.

The second work unit, Herbicide Delivery Systems (HDS), explores ways to improve herbicide delivery to target plants in high water-exchange environments. The HDS research focuses on development of controlled-release carrier systems, e.g., polymers, elastomers, emulsions, and fibers. These controlled-release carriers release herbicides at a slow, predictable rate to the vicinity of plants. However, the herbicide release rate and concentration-exposure times required to achieve plant control are not known. This information is not important to industrial formulation chemists during conventional herbicide formulation development. The focus in industry is on determining the cost-effectiveness of using the effective chemical concentration required to control target plants. Consequently, the rate of herbicide release from conventional formulations and the effective concentration/exposure times are most important to controlled-release formulation development. The HDS research was temporarily halted until this information was obtained.

The work unit Herbicide Concentration/Exposure Time Study (HCETS) is beginning to provide information required by the HDS and PGRAPM work units. In the HCETS, various herbicide/PGR concentrations and exposure times are tested against target aquatic plants in large-scale aquaria systems. Simply stated, the relationship between herbicide concentration and exposure time required to control specific target plants does not exist for most registered herbicides. Mr. W. Reed Green of the University of Arkansas, employed by the Waterways Experiment Station (WES) under the Intergovernmental Personnel Act (IPA), will describe research in this work unit. The results from this research may lead directly to small- or large-scale field testing or may require changes in application techniques and formulations. This information improves bioassay testing procedures and formulations. We test new herbicide formulations in this work unit to determine if repackaging of a herbicide is desirable. Repackaging of the active ingredient is expensive and may require additional testing before registration.

However, this may result in an improved product that is more cost-effective over several years of use.

The most promising chemicals proceed to the next level of testing in the work unit Herbicide Application Technique Development. The application techniques under consideration deliver chemical formulations to the target plants in high water-exchange environments. Current efforts focus on understanding the hydrodynamics in and around submersed aquatic plant stands. Application techniques receiving considerable interest are controlled-release delivery systems, conventional liquid and granular herbicides, herbicide/adjuvant combinations, and continuous injection. Drs. Kurt Getsinger, WES, and Alison Fox, University of Florida, will discuss research in this work unit.

Following evaluation in laboratory flumes and small-scale field tests, we test the most effective application techniques and chemical formulations under operational field conditions in the work unit Field Evaluation of Selected Herbicides for New Aquatic Uses. Tests of this size (>0.5 ha) usually require an Experimental Use Permit (EUP), because they involve changes in registration status, site use, or an amendment of residue tolerances. The field studies are cooperative efforts among chemical companies, other Federal/state agencies, and WES with the aim of obtaining environmental fate and dissipation data. These data are used to prepare field manuals and reports with recommendations to field offices on the chemical's activity, uses, and application techniques. The chemical companies use the information to fulfill requirements for Federal registration of the specific formulations. During Fiscal Years 1989 and 1990, WES, with DuPont, Inc., and other Federal/state agencies, will conduct a cooperative field study under an EUP, in Lake Seminole, Georgia, and Banks Lake, Washington.

The work unit Coordination of Control Tactics with Phenological Events of Aquatic Plants interfaces with all technology areas of the APCRP (Figure 2). Current management techniques do not consider the importance of physiological weak points in the growth cycle of aquatic plants. An understanding of a plant's phenology and survival strategies can be used to identify and mark weak points in the growth cycle. When used in this manner, weak points become "control points" in the plant's growth cycle. Small-scale outdoor and environmental chamber studies will be conducted to identify weak points, or control points, in the growth cycle of waterhyacinth, hydrilla, Eurasian watermilfoil, and alligatorweed. The results of these studies will be used by the various technology areas, i.e., biological, chemical, mechanical, and ecological, to improve control effectiveness. Dr. Kien Luu of the University of Tennessee (also employed by WES under the IPA) will summarize ongoing research with waterhyacinth.

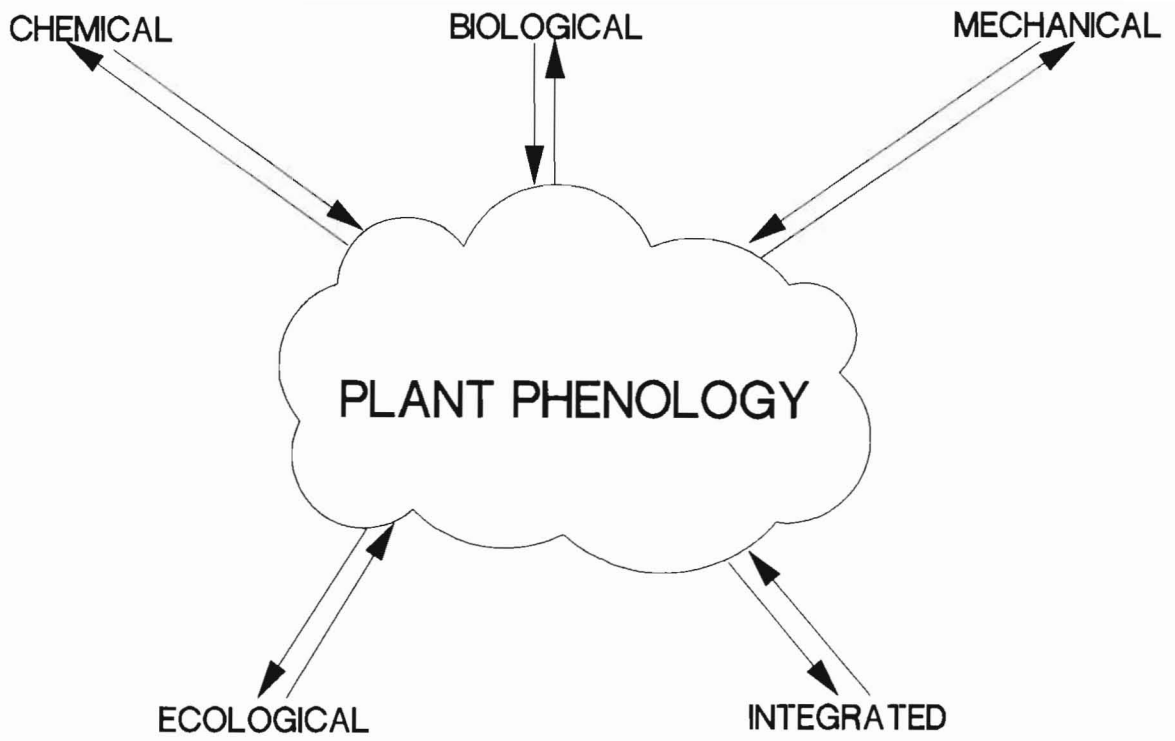


Figure 2. Work unit *Coordination of Control Tactics with Phenological Events* provides support data to all technology areas within the APCRP

Herbicide Application Techniques for Flowing Water

by
Kurt D. Getsinger*

INTRODUCTION

Although chemical control of submersed plants in static water is very effective, control of these plants in flowing-water systems (e.g., rivers, streams, canals, and tidal areas) is inconsistent. This erratic control is presumably due to hydrodynamic processes, such as water flow, wind mixing, thermal stratification, etc., which can influence herbicide concentration, contact time, and containment. Since chemical control of submersed plants is directly related to herbicide concentration/exposure time relationships, an understanding of water exchange patterns within plant stands is necessary to improve control in flowing water. This information is needed to select the season, formulation, and application technique to maximize herbicide exposure around the target plant.

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 that maximize herbicide contact time in flowing-water environments.

This article presents partial results of a dye study designed to characterize water exchange patterns in submersed plant stands of the Pend Oreille River, Washington.

STUDY SITES

A series of dye studies were conducted in submersed plant stands in the Pend Oreille River in August 1988. Study sites were located near the river towns of Usk, Cusick, and Ione (Figure 1). Three sites were selected for comparative purposes: (a) River Mile 46 (RM-46), where dense plants formed an "island" stand (~3 ha) in the center of the river (mean depth 1.2 m); (b) Calispell River (CR), where plants formed a dense, shoreline stand (~20 ha) north of the mouth of the Calispell River (mean depth 2 m); and (c) Lost Creek Bay (LCB), where plants infested a 4-ha cove at the mouth of Lost Creek (mean depth 2 m) (Figure 2). These sites represented the types of submersed plant stands that would be targeted for chemical control. Flow rates in the Pend Oreille River ranged from 113 to 170 m^3/sec (4,000 to 6,000 cfs) during the studies, or some 50 to 60 percent below normal for August.

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

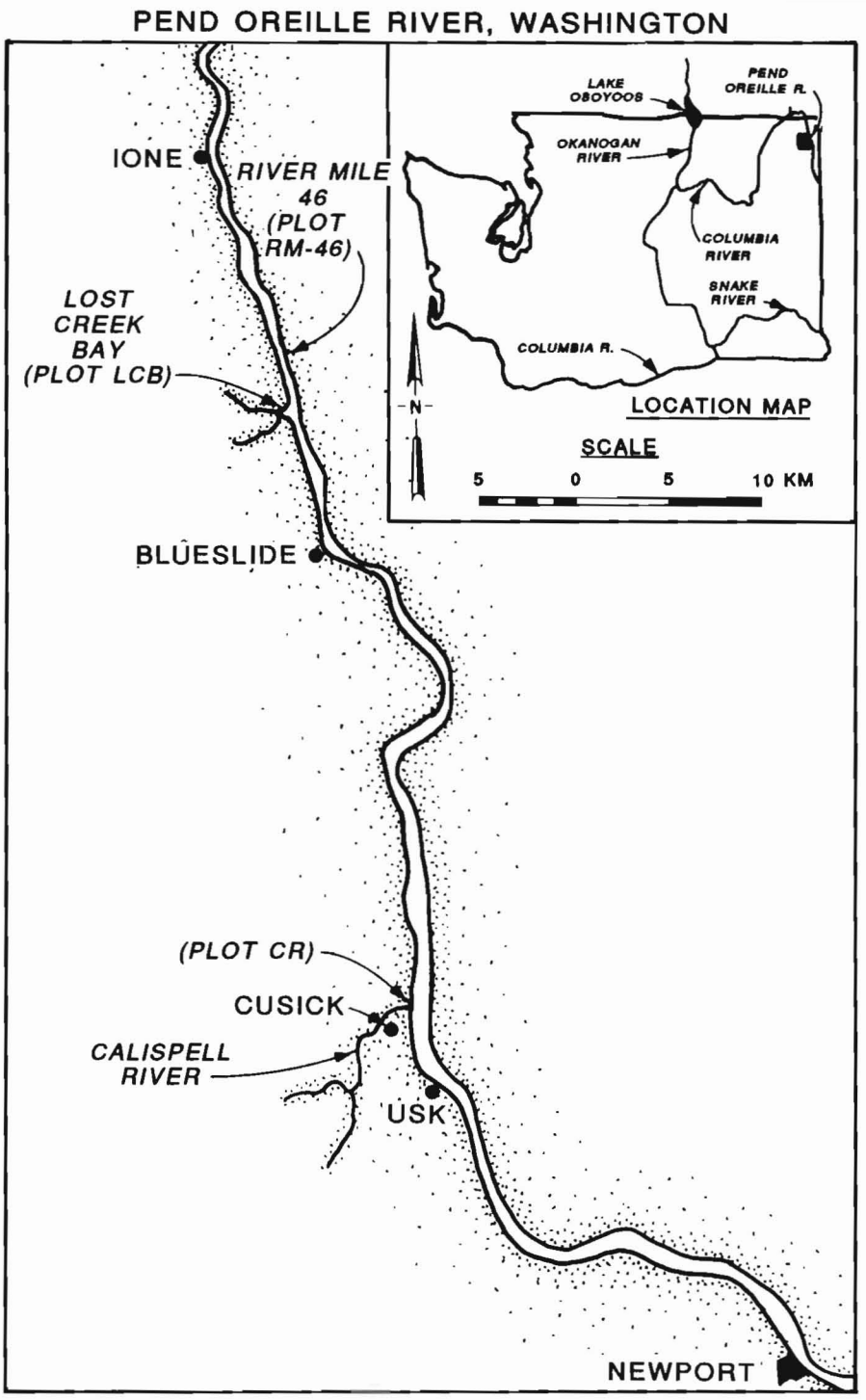
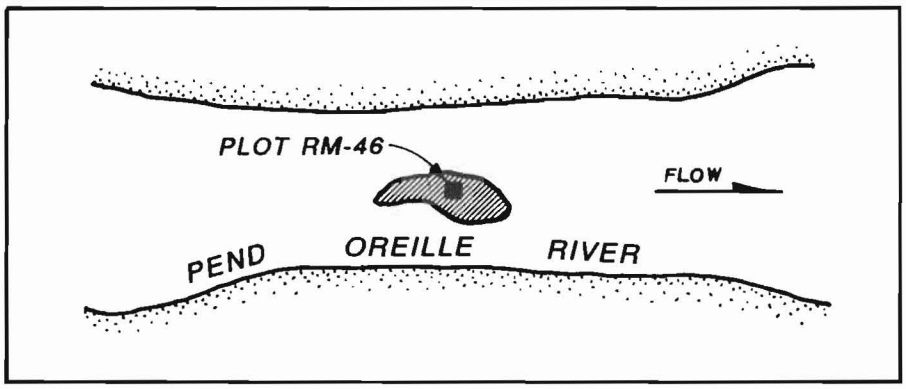
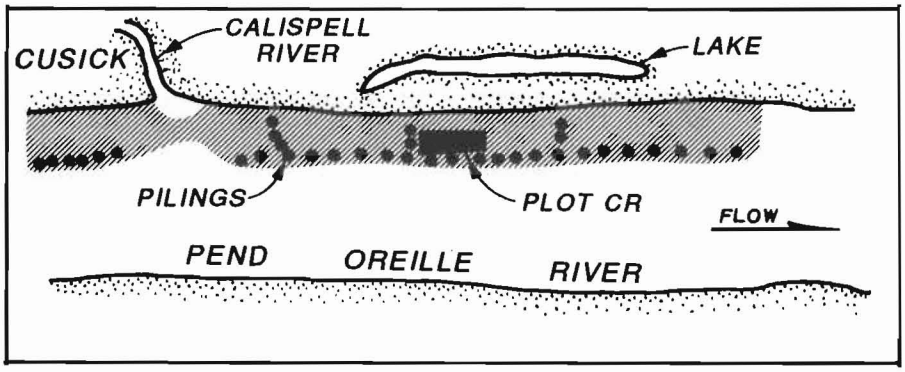


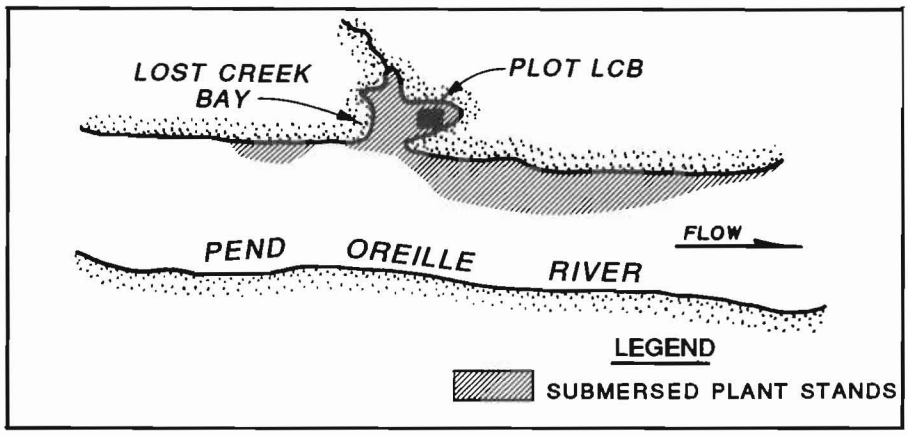
Figure 1. Pend Oreille River study sites



a. Plot RM-46



b. Plot CR



c. Plot LCB

Figure 2. Dye plots in Pend Oreille River

All sites contained mature plants, with apical tips at or just below the surface. The plant stands were dominated by Eurasian watermilfoil (*Myriophyllum spicatum*), interspersed with native watermilfoil (*Myriophyllum excalbescens*) and various species of pondweeds (*Potamogeton* spp.).

MATERIALS AND METHODS

A test plot, approximately 0.5 ha in size, was established in each of the three study sites. The fluorescent dye Rhodamine WT was applied to each plot at a rate calculated to achieve 10 $\mu g/\ell$ (ppb) in the entire water volume. Dye was injected throughout each plot using a 4.3-m boat powered by an outboard engine. The boat was equipped with a Spotlyte Sprayer (60 psi, 12-V electric diaphragm pump, 75-ℓ capacity) and a bow-mounted boom system. Attached to the boom were five weighted drop hoses (spaced at 0.6 m to provide a 2.5-m treatment swath), each fitted with a No. 6 straight-stream nozzle tip. The drop hoses reached a depth of 0.45 to 0.75 m when dragged through the plant stands. When applied in this manner, the dye treatment simulated an operational, liquid herbicide application.

Dye was monitored using a Turner Design Model 10-005 field fluorometer, fitted with a high-volume continuous flow cuvette system. Water was pushed through the fluorometer by an electric bilge pump, attached to the end of the hose which was lowered over the side of the sampling boat. An in-line thermistor was used to measure water temperature, which was recorded and used as the correction factor for the calibration temperature of the fluorometer.*

Dye was measured at fixed points along transects that were established perpendicular to the direction of flow in each plot (one transect and two sampling points in Plot RM-46, three transects and five sampling points in Plot LCB, and three transects and nine sampling points in Plot CR). Readings were taken at 0.5-m intervals at each point, from 2 cm below the surface to 0.5 m above the bottom. Initial readings were taken 0.5 hr after treatment in Plots LCB and CR, and at 2-hr posttreatment intervals until detection limits were reached (<0.01 $\mu g/\ell$). Since dye dispersion was rapid in Plot RM-46, readings were taken 0.5 hr after treatment and at 0.5-hr posttreatment intervals until detection limits were reached.

Half-lives of the dye and linear regressions of concentration against time were calculated for each treatment. Only data from sampling points located in the center of each plot are presented in this paper. Additional sampling locations are being analyzed and will be presented in a final report.

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*A. M. Fox, W. T. Haller, and K. D. Getsinger. 1988. "Preliminary Study of Dilution of Dyes in Tidal Canals of the Crystal River, Florida," *Proceedings, 22nd Annual Meeting, Aquatic Plant Control Research Program*, Miscellaneous Paper A-88-5, US Army Engineer Waterways Experiment Station, Vicksburg, Miss., pp 195-201.

RESULTS AND DISCUSSION

A comparison of mean dye concentrations and dye half-lives from the center station of each plot is presented in Table 1. Mean dye concentration in Plot RM-46 was below detection by 3.0 hr posttreatment, and the dye half-life was 0.33 hr. This short half-life was expected, since Plot RM-46 was located in a small stand of plants (~3 ha) isolated in the center of the river.

Table 1
Dye Concentrations and Half-Lives from the Center Stations of Plots RM-46, CR, and LCB in the Pend Oreille River

Plots	*Hours Posttreatment*	*Mean Concentration, µg/ℓ*	*Half-Life, hr*
RM-46	0.5	14.45	
	1.5	4.92	
	2.5	0.24	
	3.0	<0.1	0.33
CR	0.5	4.47	
	2	8.34	
	6	2.73	
	10	0.02	
	12	<0.01	1.10
LCB	0.5	16.37	
	6	10.03	
	12	8.36	
	24	4.99	
	48	0.08	
	60	<0.01	7.20

In contrast to the midriver application, mean dye concentration in Plot CR (the shoreline plot) did not fall below detection until 12 hr posttreatment. However, dye half-life in this plot was only 1.1 hr. This relatively rapid dispersion of dye was somewhat surprising, since the plot was bordered on its downstream edge by a dense plant stand spanning several hundred metres in length. In addition, virtually no dye was detected "leaking" from the edge of the plot, which was parallel to the open river channel.

Treatment of the cove plot (Plot LCB) resulted in the longest time span (60 hr posttreatment) before dye detection limits were reached, as well as the longest dye half-life (7.2 hr).

Based on dye/herbicide field studies in Florida, a 7-hr half-life provided control of hydrilla (*Hydrilla verticillata*) when treated with endothall at 3 mg/ℓ (personal communication, W. T. Haller, University of Florida, Gainesville). This suggests that the control of Eurasian watermilfoil (generally considered more susceptible to endothall than hydrilla) is possible in situations allowing an endothall half-life of approximately 7 hr. The Lost Creek Bay milfoil infestation, with a dye half-life of 7.2 hr, falls into this category. Studies are being conducted at the Waterways

Experiment Station (WES) to determine the concentration and exposure time of endothall required to control Eurasian watermilfoil and hydrilla.

Preliminary results of the Pend Oreille River dye studies indicate a need for further work in this system. A second series of dye studies should be conducted to determine the effect of plot size and riverflow on dye half-lives. Plots at least 4 ha in size should be treated with dye, during normal river discharge, to ascertain if larger plots will significantly increase dye half-life (and potentially herbicide half-lives).

ACKNOWLEDGMENTS

The Pend Oreille dye study was conducted in cooperation with the US Army Engineer District, Seattle; the Washington State Department of Ecology; the US Bureau of Reclamation; and Aquatics Unlimited, Inc. Mr. W. Reed Green of the WES assisted in data analysis.

Factors Affecting the Dispersion of Dyes in Tidal Canals in the Crystal River, Florida

by
Alison M. Fox,* William T. Haller,*
and Kurt D. Getsinger**

INTRODUCTION

A dye study, initiated in 1987 to investigate the rates of water exchange in a series of dead-end, tidal canals (Three Sisters Canals) in Crystal River, Florida (Fox et al. 1988†), was continued in 1988. The purpose of the study was to provide information that would improve the control of hydrilla (*Hydrilla verticillata*) using herbicides.

This study was designed to (a) characterize water movement using Rhodamine WT in four canals under similar tidal conditions, (b) compare the influence of spring and neap tidal cycles on the rate of dye dilution, (c) compare the rate of dye dilution from total canal treatments in unvegetated and vegetated conditions, and (d) based on the most favorable conditions predicted by the dye dispersion rates, evaluate the efficacy of herbicides applied to the canals.

This paper provides an update of the Crystal River dye study. A final report summarizing this work is in preparation.

RESULTS

Dye treatments

Prior to November 1988, 18 dye treatments were made to the four dead-end canals. Statistical comparisons were made of the rates of dye dissipation from applications conducted under conditions of spring versus neap tides, densely versus sparsely vegetated canals, and seasonal water temperature regimes. Results of these studies showed that the maximum retention time of Rhodamine WT, applied throughout each of the canals, can be estimated from the exponential dispersion curve as a half-life, measured in hours. These data would be used to predict conditions (i.e., the slowest rates of dye dilution) representing the optimum time

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†A. M. Fox, W. T. Haller, and K. D. Getsinger. 1988. "Preliminary Study of Dilution of Dyes in Tidal Canals of the Crystal River, Florida," *Proceedings, 22nd Annual Meeting, Aquatic Plant Control Research Program*, Miscellaneous Paper A-88-5, US Army Engineer Waterways Experiment Station, Vicksburg, Miss., pp 195-201.

for herbicide application. This would ensure the maximum possible herbicide contact time with hydrilla.

Spring versus neap tides. A faster rate of water exchange was expected during spring tides (when the range between high and low tides was greatest) compared with neap tides (when this range was smallest). Four comparisons between these types of tide were made when all other factors (canal, vegetation density, temperature regime) were constant.

There were no significant differences in the rate of dye dispersion from the canals when treated during spring and neap tides. Actual changes in the volume of water in the canals during these tidal cycles have been calculated from the water level data that were collected throughout the studies. These data confirmed that dispersion rates were affected by factors independent of the total volume of water exchanged on each tide.

Densely versus sparsely vegetated canals. Since it is known that dense vegetation can affect the velocity patterns of water in a channel,* it was anticipated that the density of hydrilla might affect the pattern and rates of water exchange in the Three Sisters Canals. These comparisons of the effects of vegetation were made by making simultaneous dye applications to pairs of canals, one of which was choked with plants, while the other had recently been cleared of most vegetation.

The two comparisons, made in summer, showed no significant differences in the rates of dye dispersion between the densely and sparsely vegetated canals. However, there was a significant difference in the rates of dye dispersion in a pair of canals treated during winter.

Seasonality - water temperature regimes. The greatest difference in the rates of dye dispersion was found between applications made during the three seasons of summer, fall, and winter. With the exception of the effects of vegetation in winter as noted above (and one spurious winter result), there were no significant differences in the rates of dye dispersion between treatments made within the same season, or between fall and spring treatments.

Half-lives for the dye were compared by season, as shown in Table 1. This seasonality appeared to be related to the water temperature regimes in the canals. During summer, the temperature of the surface water in the canals was 3° to 4° C greater than at the bottom of the water column, and 5° to 6° C greater than the temperature of water flowing from the nearby Three Sisters springs. In winter, similar temperature differences occurred between the top and bottom of the water column, but the surface water was 1° to 2° C colder than the spring water. During a certain period in the fall, and to lesser extent in the spring, the whole canal system, including the springs, was a uniform temperature (isothermal).

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*K. D. Getsinger. 1988. "Development of Herbicide Application Techniques for Flowing Water," *Proceedings, 22nd Annual Meeting, Aquatic Plant Control Research Program*, Miscellaneous Paper A-88-5, US Army Engineer Waterways Experiment Station, Vicksburg, Miss., pp 189-194.

Table 1
Seasonal Half-Lives of Dye in Three Sisters Canals, Crystal River, Florida

Season	*Mean Half-Life, hr (Range)*	*Applications*
Summer	18.0 (11-25)	9
Winter	44.4 (30-60)	4
Spring	63.5 (62-65)	2
Fall	104.5 (77-120)	3

Herbicide treatments

Two canals were treated with endothall in fall 1987, fall 1988, and spring 1988, when dye half-lives were over 60 hr. These treatments were effective in removing hydrilla for several months. Two unsuccessful endothall applications made in the winter (and past records of ineffective summer treatments) indicated that, for contact herbicides, the rate of water movement in the canals should be at least as slow as that found in the fall or springtime.

Two canals were treated with fluridone during fall 1988. Despite clear symptoms of herbicide uptake, no significant reduction in plant biomass was observed within 14 weeks posttreatment. This suggests that, even under optimum conditions, conventional fluridone formulations and application methods may not provide control of hydrilla in the canals.

CONCLUSIONS AND FUTURE STUDIES

A mechanism has been devised to explain the relationship between the temperature regimes in the canals and the rate of dye dispersion during the tidal cycles following application. This hypothesis has been supported by data already collected and will be further tested during the winter and summer of 1989. Dye will be applied, on an incoming tide, to the water flowing from the Three Sisters springs. Movement and circulation of the dye, over the subsequent tidal cycle, will be monitored. Fluridone formulations and application techniques that extend the period of herbicide release should be evaluated in this system.

ACKNOWLEDGMENTS

This study was conducted by the University of Florida, Institute of Food and Agricultural Sciences, Center for Aquatic Plants, and the US Army Engineer Waterways Experiment Station (WES) in cooperation with the US Army Engineer District, Jacksonville. Herbicide applications were made by the Citrus County Aquatic Plant Control Program. Much appreciated technical assistance was provided by Ms. Margaret Glenn of the University of Florida and Mr. W. Reed Green of the WES.

Herbicide Concentration/Exposure Time Relationships: Endothall, 2,4-D, and Eurasian Watermilfoil

by
W. Reed Green*

INTRODUCTION

The effectiveness of a herbicide treatment in the aquatic environment is determined by the period of target plant exposure to dissipating concentrations of the herbicide. Functional relationships exist between the degree of plant injury, the concentration at which plants are exposed, and the exposure period. Determining these functional relationships for the registered herbicides and the targeted macrophytes will provide required information for the development of new formulations and operational strategies.

OBJECTIVES

The objective of this study was to determine the concentration/exposure time relationships for controlling Eurasian watermilfoil (*Myriophyllum spicatum* L.) when exposed independently to 2,4-D and endothall.

MATERIALS AND METHODS

The plant exposure experiments were conducted in the laboratory using the setup and procedures described by Green (1988) and Green and Westerdahl (1988). The design consisted of twenty-four 55-ℓ aquaria (0.75 m $\times$ 0.3 m^2) located in a controlled-environment greenhouse. Supplemental lighting was provided at a light:dark cycle of 13:11 hr. The mean photosynthetically active radiation measured at the water surface was 1,600 $\mu E\ m^{-2}$ (Hall et al. 1982). Each aquarium was independently supplied with a continuous supply of reconstituted natural hard water. The water volume of each aquarium was displaced every 24 hr. Air was bubbled through each aquarium to provide a source of carbon dioxide and to circulate the water. Water temperature was maintained at 21° ± 2° C. The sediment used to grow the plants was collected from Brown's Lake, Waterways Experiment Station, and supplemented with macro- and micro-nutrients to negate the effects of nutrient limitation. Four Eurasian watermilfoil apical shoots were planted 5 cm deep in 300-ml beakers containing sediment. Eleven beakers of plant propagules were placed in each aquarium.

Herbicide treatment tests (Table 1) were applied when the plant foliage grew to

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Table 1
Results of 2,4-D and Endothall Concentration and Exposure Time Tests

2,4-D Concentration mg/ℓ	*Exposure Time, hr*					
	12	*24*	*36*	*48*	*60*	*72*
0.5	X	X	X	X	X	X
1.0	X	X	X	X		
2.0	X	X	X	X		

Endothall Concentration mg/ℓ	*Exposure Time, hr*						
	2	*12*	*18*	*24*	*36*	*48*	*72*
0.5					X	X	X
1.0	X	X	X	X	X	X	
3.0	X	X	X	X			
5.0	X	X	X				

approximately 0.5 m in height. One beaker of plant material was randomly removed from each aquarium prior to herbicide application to provide an estimate of treated biomass. The remaining 10 beakers remained in the aquaria for herbicide treatment. Calculated concentrations of the test herbicide were then poured into the designated aquarium and allowed to remain until the appropriate exposure time was reached. At this point, the water in the aquarium was drained and filled three times with fresh water to remove the remaining herbicide residues. Aqueous residue samples were collected for analysis immediately after treatment to verify treatment concentration, just prior to rinsing to determine residue dissipation, and after rinsing to verify residue removal.

The plants were allowed to grow for 4 weeks after treatment. Herbicide injury was evaluated based on four criteria: (a) visual injury (0 to 100 percent), (b) harvested biomass, (c) viable root frequency, and (d) viable stem frequency.

RESULTS AND DISCUSSION

Increasing concentrations and/or increasing exposure times increased plant injury to a point at which total control is achieved. Concentrations at exposure times greater than the control threshold provided total control of the exposed plant material. Eurasian watermilfoil control occurred, using 2,4-D, in those tests exposed to concentrations and exposure times within the shaded area of Figure 1. Plant injury increased as the coordinates of the tests approached the threshold.

These laboratory results coincide well with results of the field exposures reported by Hoeppel and Westerdahl (1983). Based on the dissipation estimates developed

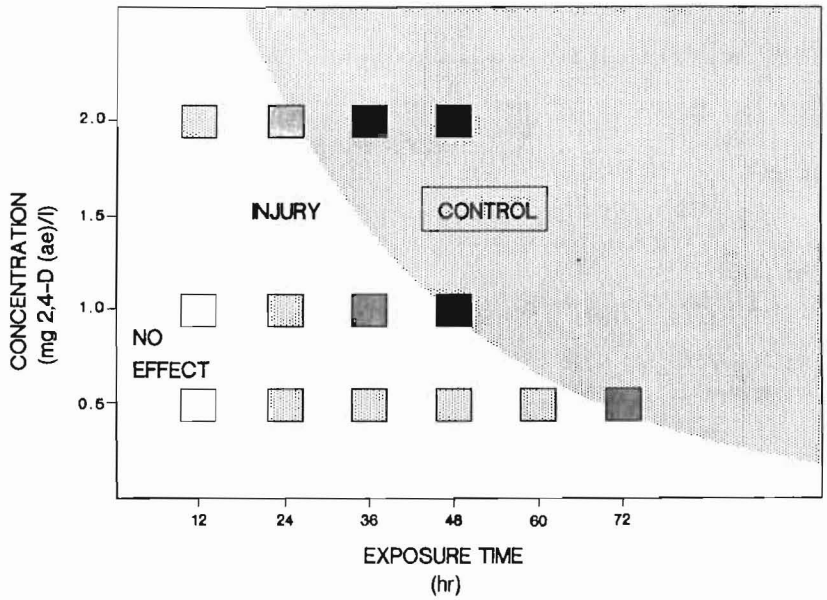

Figure 1. Eurasian watermilfoil control using 2,4-D. (The rectangles located at the various concentration and exposure time coordinates represent actual laboratory application tests. The density of shading within the rectangles represents the degree of injury incurred from the tests: no shading = no effect, light shading = marginal injury, dense shading = severe injury, and solid shading = complete control. The area shaded within the graph represents the concentration and exposure time coordinates that would be expected to provide plant control)

from their residue data (Figure 2), only one treatment would be expected to provide Eurasian watermilfoil control. The other three treatments would only be expected to provide various degrees of plant injury. Three of the four applications (represented by the lines short of the threshold, Figure 2) were reported as causing injury to the Eurasian watermilfoil standing crop (Hoeppel and Westerdahl 1983). The standing crop in these three plots was reestablished by 70 days after treatment. One treatment (represented by the line past the threshold, Figure 2) provided total plant control, which lasted the entire growing season.

Similar concentration and exposure time relationships occurred with Eurasian watermilfoil when exposed to endothall (Figure 3). If the endothall- and 2,4-D-Eurasian watermilfoil control relationships are superimposed (Figure 4), one can see that endothall would be more effective in providing control at lower concentrations and shorter periods of exposure.

The laboratory concentration and exposure time studies for nuisance aquatic plants for all registered herbicides will provide baseline information for successful management of aquatic plants in high water-exchange environments. For example, if the hydrodynamic events in the field would allow the necessary exposures of both endothall and 2,4-D (Figure 4), then 2,4-D might be selected due to its mode of action or for economic reasons. If the expected exposure fell short of that necessary for control with 2,4-D, but included that necessary for control with

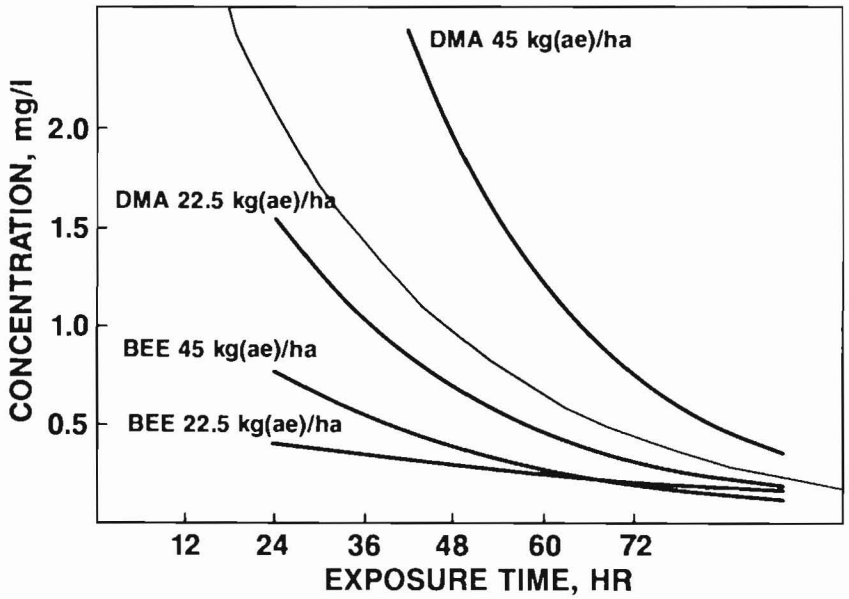

Figure 2. Estimates of 2,4-D dissipation from the field study of Hoeppel and Westerdahl (1983) superimposed on the laboratory results of Figure 1

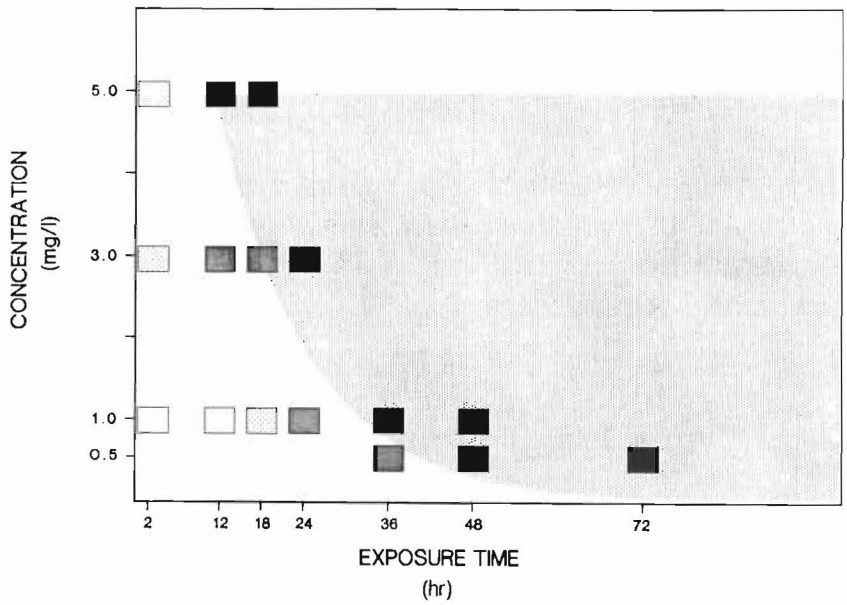

Figure 3. Eurasian watermilfoil control using endothall. (The rectangles located at the various concentration and exposure time coordinates represent actual laboratory application tests. The density of shading within the rectangles represents the degree of injury incurred from the tests: no shading = no effect, light shading = marginal injury, dense shading = severe injury, and solid shading = complete control. The area shaded within the graph represents the concentration and exposure time coordinates that would be expected to provide plant control)

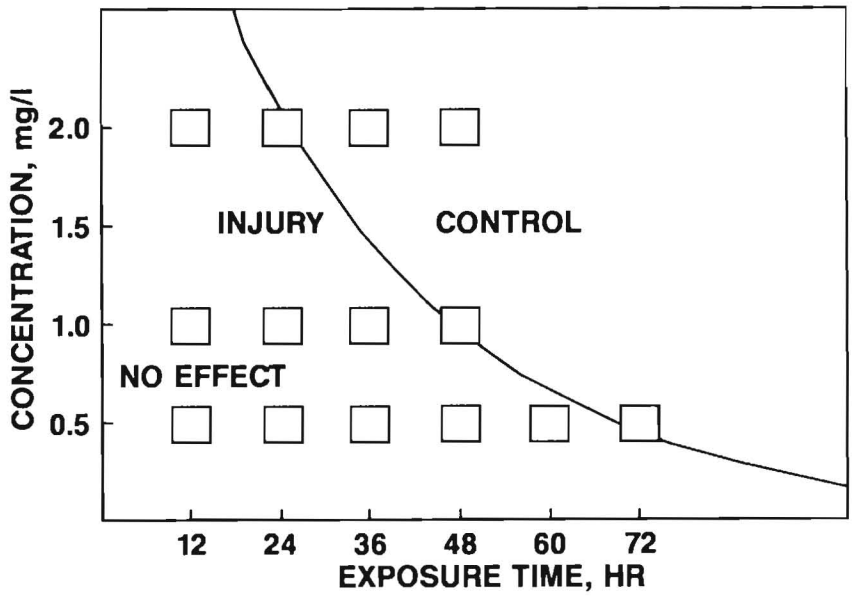

Figure 4. Eurasian watermilfoil control of both 2,4-D and endothall

endothall, then endothall could be selected for use. Knowledge of the chemical behavior of the herbicide and its potential dissipation in the field provides technology focal points from which to develop new formulations, application techniques, and delivery systems.

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Growth Characteristics and Carbohydrate Allocation of Waterhyacinth

by
Kien T. Luu*

INTRODUCTION

Improvement in the effectiveness of present plant control techniques will result from a better understanding of aquatic macrophyte growth cycles and, specifically, identification of physiological weak points in those cycles. Many studies involving terrestrial plant species have used carbohydrate allocation to identify physiological weak points in the growth cycle of plants. Carbohydrate allocation studies describe the distribution pattern of photosynthetically produced sugars in various plant tissues. A physiological weak point is a period during the growth cycle when a plant is least likely to recover following the use of a control method. Application of a control tactic during this period should increase its overall effectiveness.

The specific goals of this study are to (a) verify the important morphological and growth characteristics of waterhyacinth, (b) determine seasonal carbohydrate distribution within various plant structures, and (c) identify potential weak points in the waterhyacinth growth cycle.

This article is a synopsis of the results from the waterhyacinth studies of 1987-1988. A final report on the growth characteristics and carbohydrate allocation of waterhyacinth is in preparation.

EXPERIMENTAL APPROACH

Waterhyacinth ramets of similar size and age were cultured outdoors in 1,300-ℓ tanks. The aqueous media was supplemented with 10-percent Hoagland solution as a nutrient source.** Plant samples were taken monthly and separated into different plant structures (e.g., stem bases, roots, stolons, leaves, petioles) for the determination of carbohydrate and dry weight distributions. Plants were tagged, and leaf growth was measured. Growth rates of similar size plants were compared at different seasons. Growth characteristics included fresh weight, plant density, relative growth rate, leaf length, leaf development length, leaf longevity, and seasonal dry weight distribution of different plant structures. Carbohydrate parameters included free sugars, starch, and total nonstructural carbohydrate.

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

**D. R. Hoagland and D. I. Arnon. 1950. "The Water Culture Method for Growing Plants Without Soils," Circular 347, California Agricultural Experiment Station.

RESULTS

Spring versus summer growth rate, 1988

Plant biomass (fresh weight) increased faster in the summer than in the spring during the first 10 weeks of the growth season (Figure 1). However, in the last

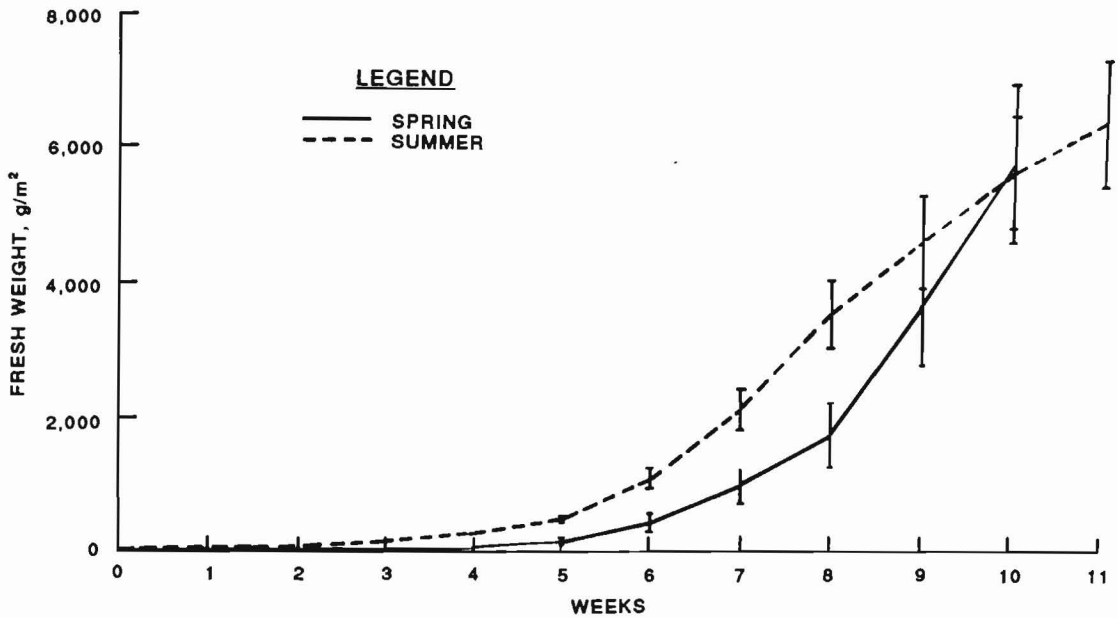

Figure 1. Plant biomass growth, spring and summer 1988 (3-g size plant)

2 weeks of the season, spring growth was faster than summer growth. Spring plants covered the experimental area (2,520 cm^2) 1 week earlier than summer plants, although all plants started with the same initial weight (3 g fresh weight). After 10 weeks growth, spring and summer biomass were similar (550 g/m^2 for spring and 540 g/m^2 for summer).

The relative growth rate of spring plants peaked (0.17 g/g/day) following 6 weeks of growth (Figure 2). However, the relative growth rate of summer plants peaked (0.13 g/g/day) much earlier, i.e., during the third week of summer. After the peak, both spring and summer growth rates declined toward the end of each season as crowded conditions occurred. The decreasing summer growth rate was faster than the spring growth rate near the end of the season. This difference resulted from the overcrowded condition and the higher proportion of old tissue at the end of summer.

Plant density of summer growth peaked at 170 plants/m^2 around midsummer (Figure 3). However, spring growth increased throughout week 10, where plant density was 450 plants/m^2. At week 10, the density of summer-growth plants (150 plants/m^2) was one third the density of spring-growth plants. It seems that spring growth tends to create a larger number of plants, while summer growth results in a larger plant size.

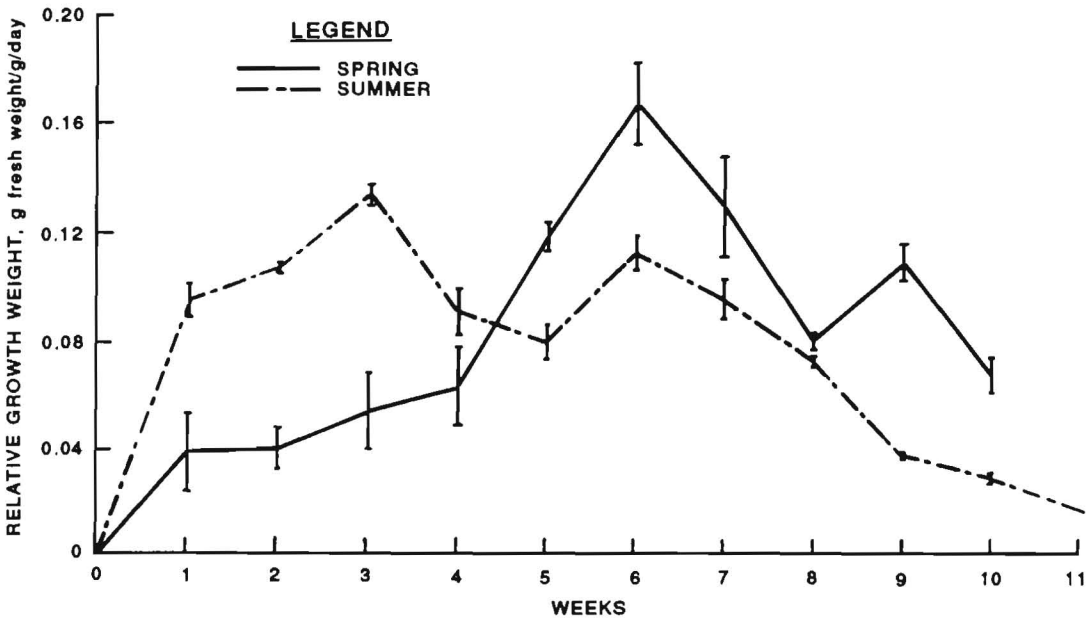

Figure 2. Relative growth rate, spring and summer 1988 (3-g size plant)

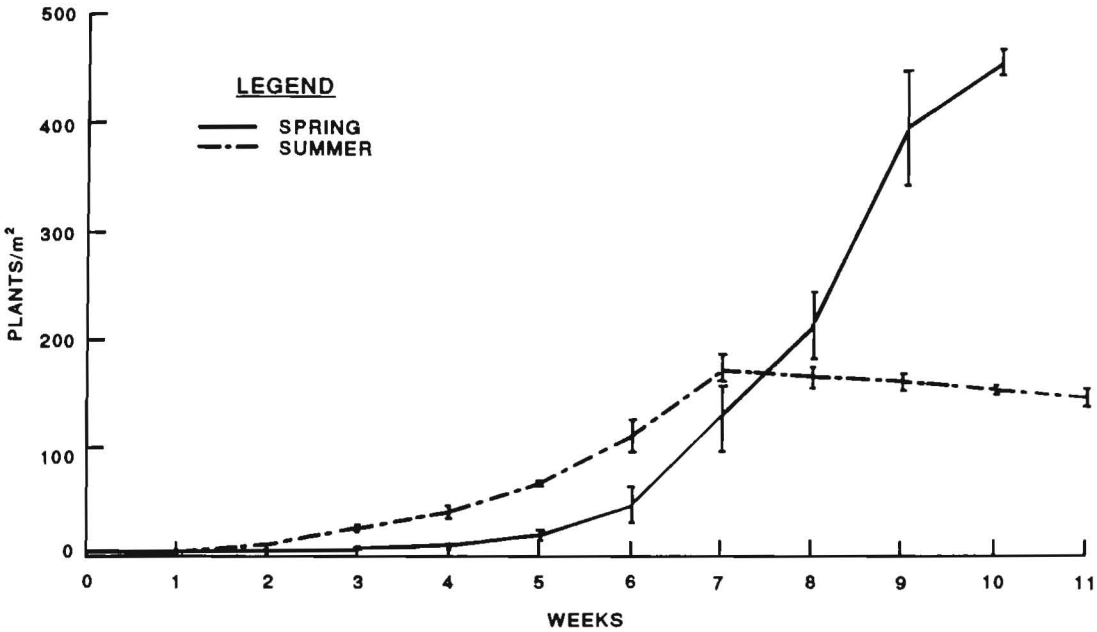

Figure 3. Plant density, spring and summer 1988 (3-g size plant)

Leaf growth

Leaf length is a combination of petiole length and blade length. Although the first leaf had an average length of 10 mm, the first 10 leaves had almost equal lengths of 125 mm each. The length of later leaves (leaves 11 to 22) gradually increased as crowded conditions increased. The longest leaf (380 mm) was leaf 22 in early August (Figure 4). Leaves 17 to 30 matured in a gradually crowded

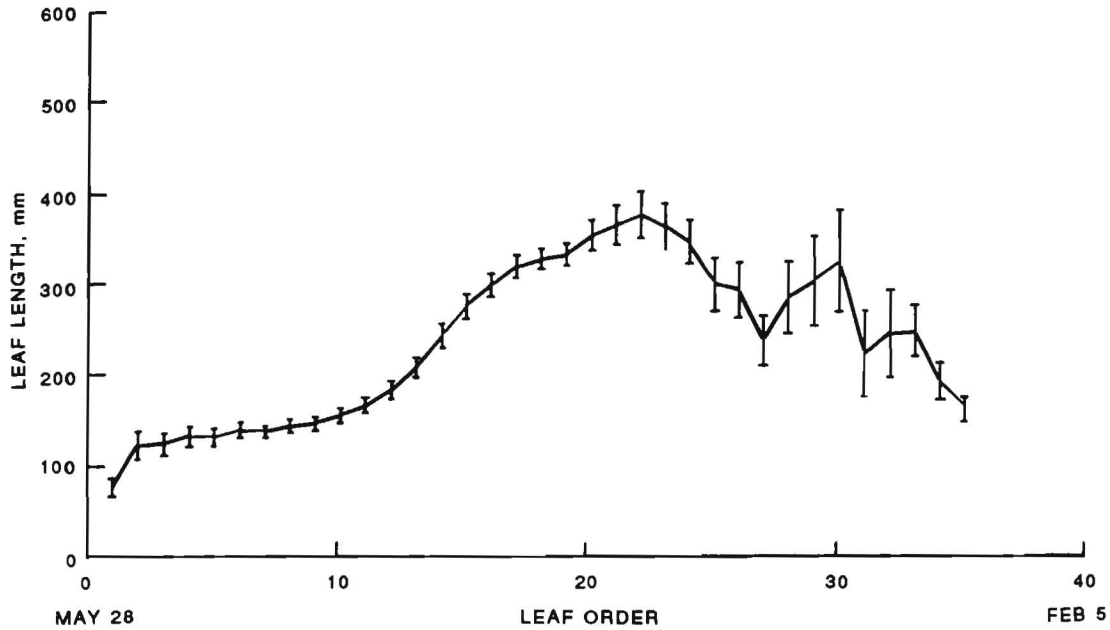

Figure 4. Leaf length, 1986-87

condition and developed partially bulbous or nonbulbous petioles. The leaves of late fall were normally shorter and developed bulbous petioles. These short bulbous leaves form in the open space occurring after the senescence of tall, nonbulbous leaves in late fall. Seasonal leaf length pattern appeared similar to seasonal biomass distribution shown by other researchers in the literature. A single plant formed in late May can have up to 43 leaves by mid-February. Leaf size may depend on available nutrients and temperature. However, the bulbous or nonbulbous character of petioles is dependent on available growing space (or distribution of light).

Leaf development span is the period from formation to maturity (maximum length). An average of 6 days was necessary for the first leaf to fully mature. Each of the next four subsequent leaves needed 2 days longer than the previous leaf to reach maturity (Figure 5). Successive leaf development required a little longer time in comparison with the previous leaf. In general, leaves 6 to 28 required an average of 16 to 18 days to reach maturity. Development of leaves 29 and above was longer and fluctuated, resulting from daily air temperature variations in late fall.

Leaf longevity is the time from formation to the day that half of the leaf turns

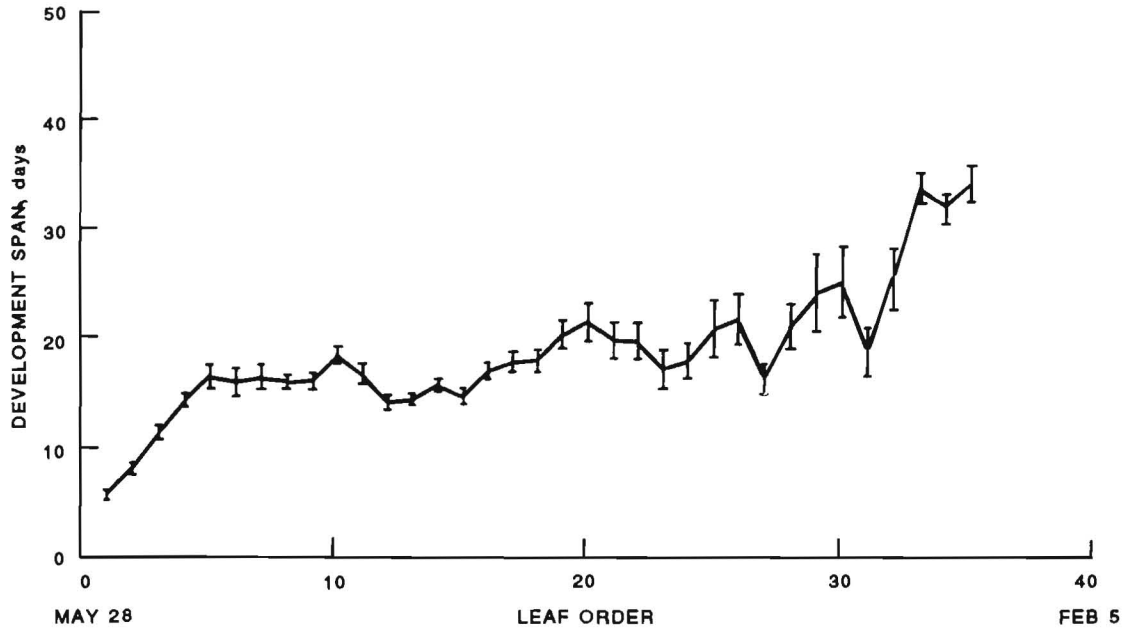

Figure 5. Leaf development span, 1986-87

yellow. Average longevity of the first leaf was 18 days. Leaves 2 to 12 had an average longevity of 35 days. Leaf 13 had a longevity of 38 days. Each subsequent leaf lived about 2 days longer than the previous one. Leaf 23 had the greatest longevity (58 days). The longevity of leaves formed after leaf 23 was less than 58 days, with a high degree of variability (Figure 6). Most waterhyacinth leaves can live longer than 1 month, but not more than 2 months under our experimental conditions.

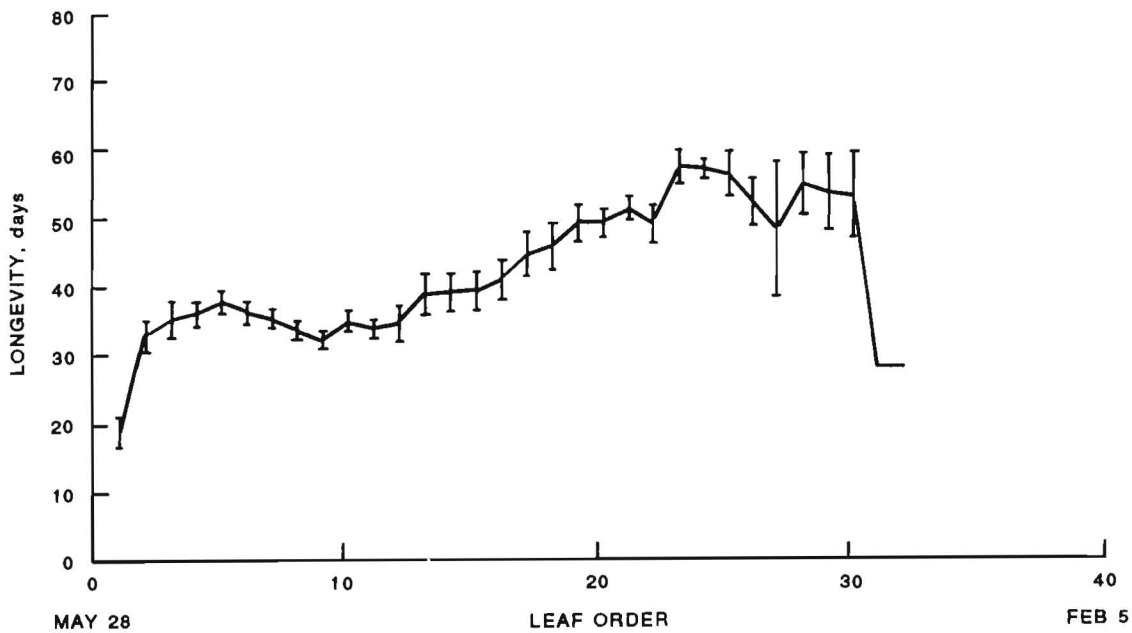

Figure 6. Leaf longevity, 1986-87

Seasonal dry weight and carbohydrate distribution

During February and March, the highest dry weight proportion (15.8 to 17.6 percent) of total plant dry weight was stem bases. Dry weight proportion of stem bases was small during other months, i.e., from 2.2 to 5.4 percent of total plant weight. Stem bases are vital structures for winter survival, because they support young buds and carry energy reserves for new growth in the spring.

Carbohydrate concentrations in stem bases were significantly greatest during September and October for both years (1987 and 1988). Carbohydrate accumulation in the stem bases initiates in July or August of each year (Figure 7). September-October is the time of the year when waterhyacinth stores maximum carbohydrate reserve in the stem bases.

Winter survival

Approximately 12 percent of plants in a population of short plants with a density of 198 plants/m^2 survived winter conditions. About 24 percent of plants survived in a short plant population with a density of 238 plants/m^2. No plants survived in a population of tall plants (year-round intact) after the 1987 winter. Winter survival of the high-density, short plant population was superior to that of the high-density, tall plant population. Secondary saprophytes and sunlight limitation probably caused the necrosis of stem base's meristematic tissues in the tall plant population.

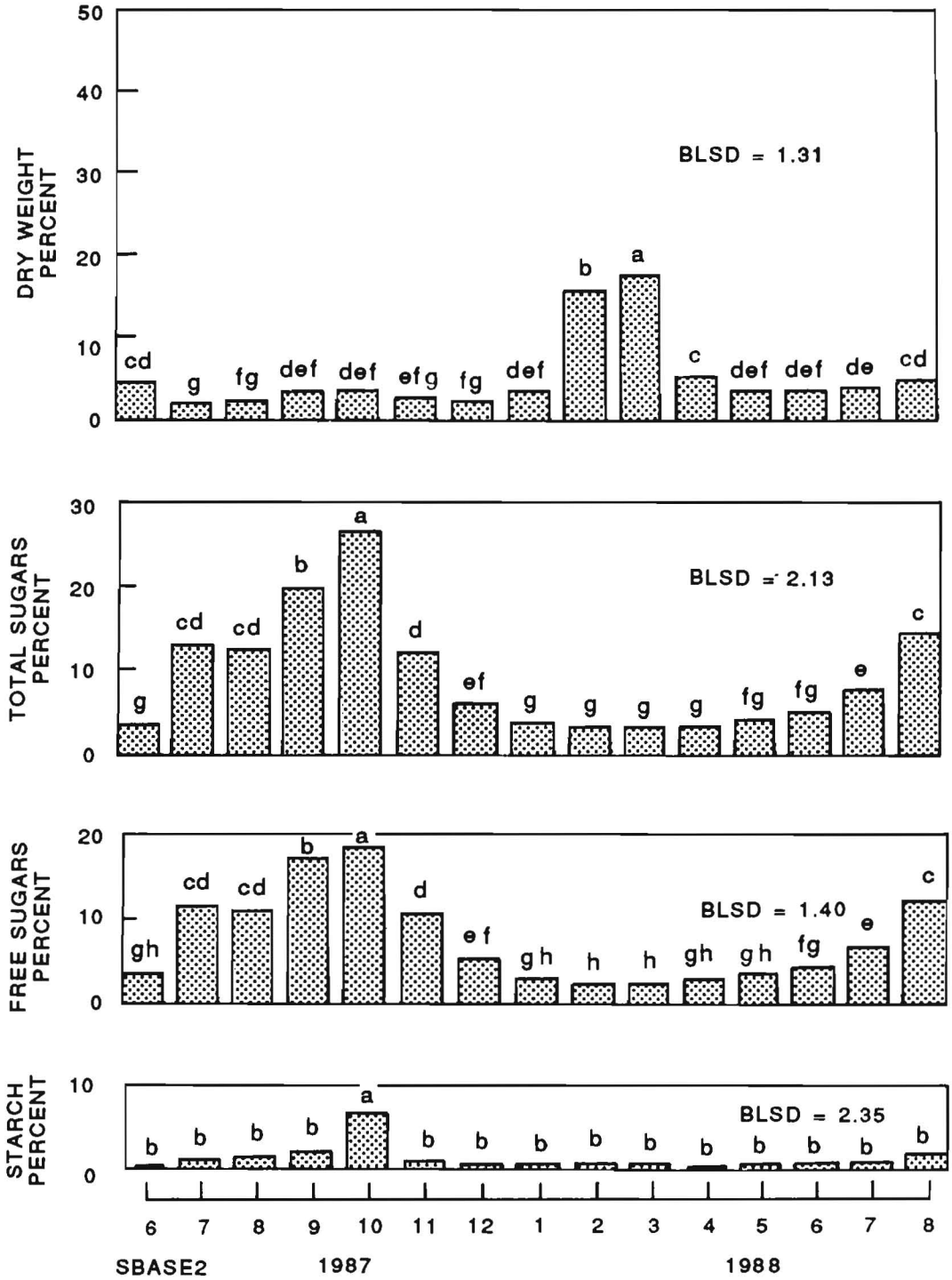

Figure 7. Seasonal dry weight and carbohydrate concentrations in stem bases

Bioassay of Plant Growth Regulator Activity on Aquatic Plants

by
Carole A. Lembi* and Michael D. Netherland*

INTRODUCTION

Submersed aquatic plants play many important roles in aquatic systems. They provide oxygen through photosynthesis, shelter and shade for fish fry, spawning beds for fish, and stabilization for sediments. These beneficial roles can be negated when the growth of aquatic vegetation becomes too dense. Such growth can impede or prevent essential uses of water such as navigation, irrigation, and recreation. Current methods for treating aquatic weed infestations rely heavily on aquatic herbicides, many of which are nonselective and result in killing most plants in the treatment area.

One potential alternative to current aquatic weed control strategies is to alter natural plant hormonal processes and thereby change the morphology of the plant. Many of the more competitive submersed aquatic macrophytes rapidly elongate from the sediments and grow to the water surface where they form a canopy, effectively shading out other, more low-growing species. It is this elongated type of growth form that is generally perceived to be problem-causing or weedy. In terrestrial plants, stem elongation is known to be regulated by the plant hormone gibberellin, and some evidence suggests that stem elongation in aquatic plants also is caused by gibberellins (Muskgrave, Jackson, and Ling 1972; Raskin and Kende 1984). Certain substituted pyrimidine (e.g., flurprimidol) and triazole (e.g., paclobutrazol and uniconazol) derivatives have been found to inhibit the synthesis of gibberellin in terrestrial plants and plant homogenates (Lever, Shearing, and Batch 1982; Rademacher et al. 1984; Hedden and Graebe 1985; Graebe 1987). These compounds reduce stem length in species ranging from grasses to trees without altering viability or morphological differentiation, such as seedhead development.

The goal of our study was to develop a simple bioassay system to determine whether gibberellin synthesis inhibitors could be used to reduce the rate of stem elongation in submersed aquatic plants without killing the plants. This presumably would lead to a lawn or "turf" at the bottom of the body of water that would not be "weedy." This turf, however, would be composed of physiologically competent plants able to provide oxygen, habitat, and bottom stabilization.

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EXPERIMENTAL APPROACH

Algal-free cultures of Eurasian watermilfoil (*Myriophyllum spicatum* L.) and the dioecious strain of hydrilla (*Hydrilla verticillata* (L.f.) Royle) were graciously provided by Dr. John Andrews, University of Wisconsin, and Dr. Steve Klaine, Memphis State University, respectively. The plants were grown in a growth chamber under constant environmental conditions of temperature (25° ± 1° C), light (400 $\mu E\ m^{-2}\ sec^{-1}$ ± 10%) and photoperiod (16:8 hr light:dark). Watermilfoil was grown in a modified Gerloff's solution (Andrews 1980), whereas hydrilla was grown in 10-percent Hoagland's solution (Hoagland and Arnon 1950). The two media were buffered after autoclaving with 10 ml/ℓ of a 2 g/100 ml stock solution of $NaHCO_3$. The sodium bicarbonate solution was passed through a 0.45-μm membrane filter (Gelman metricel membrane filter) to prevent contamination of the media. Stock cultures of milfoil were bubbled continuously with pure air enriched with 0.5 to 1 percent CO_2. Stock cultures were transferred to freshly prepared medium every 20 to 25 days.

For the bioassay, 4-cm apical shoots were removed from stock culture plants and transferred to 250-ml Erlenmeyer flasks (one apical shoot per flask) containing 150 ml of the appropriate culture medium and gibberellin synthesis inhibitor. Inhibitors tested were 50 percent wettable powders of flurprimidol (Eli Lilly Company), uniconazol (Valent), and paclobutrazol (Monsanto). The experimental flasks were then placed in the growth chambers under the same conditions described above except that CO_2 bubbling was not provided. Dose response experiments were conducted for a 4-week period with measurements taken at 0, 1, 2, and 4 weeks. Each treatment was replicated three times, and flasks within a measurement date were arranged in a randomized block design by replicate.

Growth measurements included main stem length, lateral stem length and number, root length and number, and fresh and dry weights. Physiological parameters measured were net photosynthesis, respiration, and chlorophyll content. Photosynthesis and respiration rates were monitored using oxygen evolution; total chlorophyll was measured using a DMSO extraction method described by Hiscox and Israelstam (1979). Data were subjected to analysis of variance. Means of the dosage responses of each parameter measured at each date and among dates were separated using the Student-Newman-Keul's multiple range test at a 95-percent confidence interval.

RESULTS AND DISCUSSION

The use of algal-free cultures of hydrilla and Eurasian watermilfoil resulted in good growth of untreated plants over the 4-week test period. Main stem lengths increased at mean rates of 0.53 cm/day in hydrilla and 0.41 cm/day in milfoil (doubling times of 7.4 and 10 days, respectively). Untreated plants produced lateral shoots and roots from nodal tissues but did not flower or produce tubers.

Milfoil was considerably more sensitive to the gibberellin synthesis inhibitors than hydrilla (Figure 1). After 4 weeks exposure, main stem elongation of milfoil

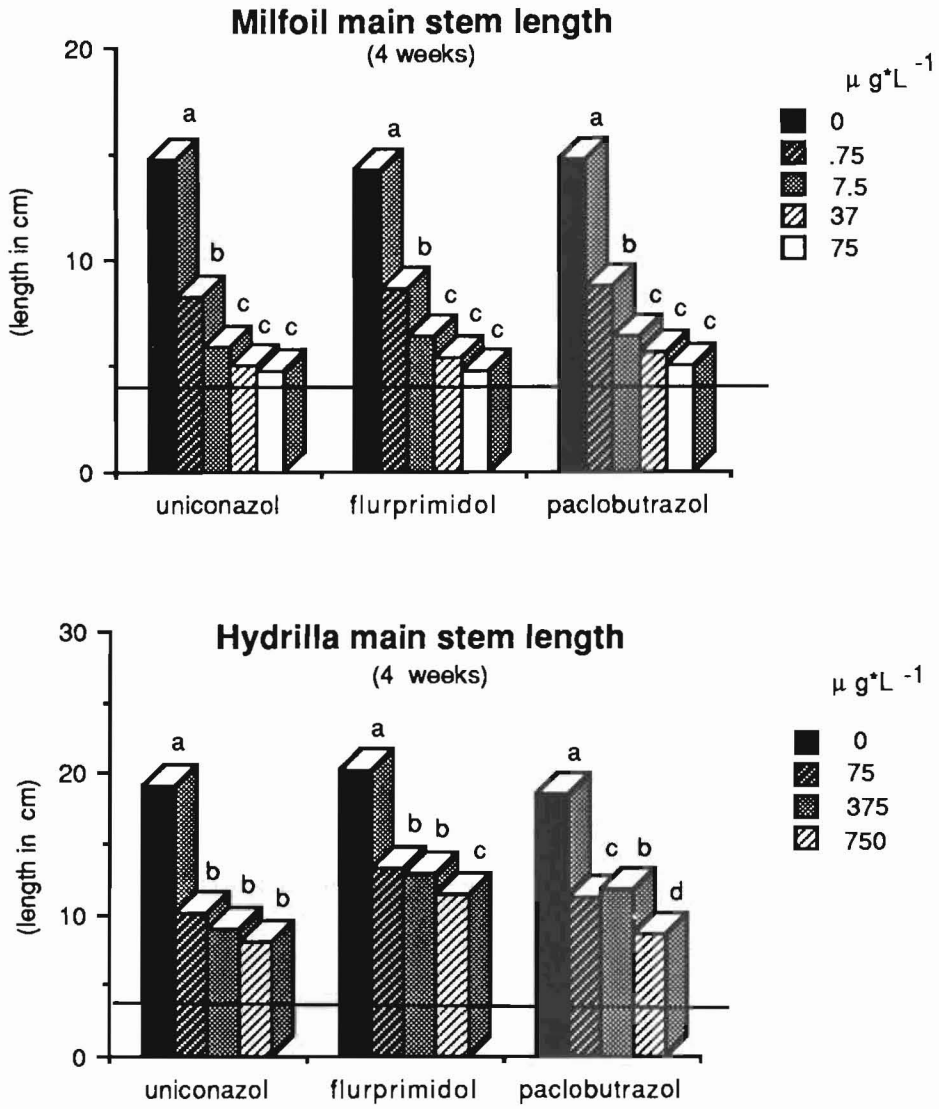

Figure 1. Effect of gibberellin synthesis inhibitors on main stem length in milfoil and hydrilla at 4 weeks. Horizontal line = initial 4-cm length. Note differences in dosage ranges and in y-axis scales between the two plants

was significantly inhibited (approximately 60% decrease in main stem length compared to untreated main stem length) at uniconazol, flurprimidol, and paclobutrazol concentrations as low as 7.5 $\mu g/\ell$. Hydrilla, although sensitive to all inhibitors and most of the concentrations tested (Figure 1), showed only a 40-percent (flurprimidol) to 58-percent (uniconazol) reduction in main stem length over untreated controls at concentrations as high as 750 $\mu g/\ell$. No effect on main stem length was detected at 7.5 $\mu g/\ell$ (data not shown). Presumed toxic effects such as total cessation of growth and photosynthesis, brittleness, and increased red pigmentation were noted in hydrilla at inhibitor levels of 1,500 $\mu g/\ell$ and above.

Milfoil exposed to dosages of 750 $\mu g/\ell$ were knotted and abnormal-looking, but still photosynthesized at a low rate.

Gibberellin synthesis inhibitor effects were visible almost immediately. As soon as untreated plants began elongating, usually within a week, elongation of treated plants was already significantly reduced.

The number and morphology of lateral stems formed during exposure to the inhibitors were quite different between the two plants. At dosages of 75 $\mu g/\ell$ or less after 4 weeks, hydrilla lateral stem production was stimulated, resulting in a mean of four lateral stems per main stem, in contrast to two lateral stems produced per untreated main stem (Figure 2). However, the length per lateral

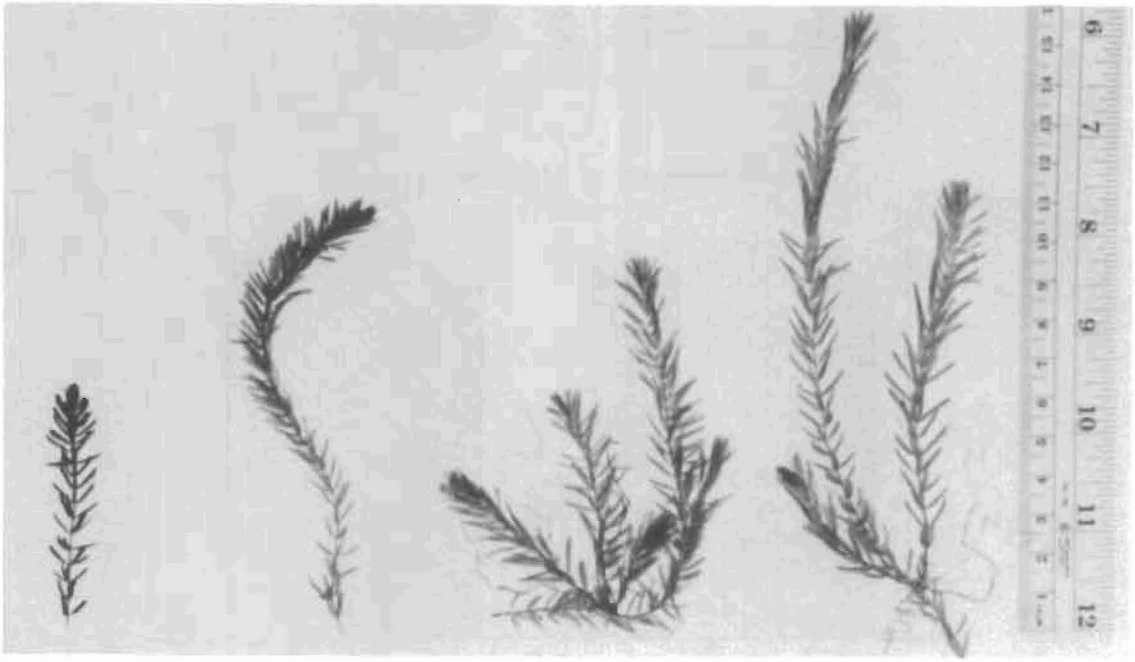

Figure 2. Effect of paclobutrazol on hydrilla at 4 weeks. Dosages (from left to right) are 1,500, 375, 75, and 0 $\mu g/\ell$

(3 cm) was lower than that in untreated controls (7 cm). If all stem lengths are added (main plus laterals), the overall length of the paclobutrazol- and flurprimidol-treated shoots was approximately the same as in the untreated shoots (Figure 3). Uniconazol treatment at 75 $\mu g/\ell$, however, produced total stem lengths significantly less than those in untreated shoots. Roots also were both shorter and higher in number per plant at these dosages. Inhibitor concentrations greater than 75 $\mu g/\ell$ of all compounds tested resulted in hydrilla lateral stem numbers, lateral stem lengths, total stem lengths, and fresh and dry weights that were significantly lower than those of the untreated controls.

In contrast to hydrilla, the number of lateral stems produced in milfoil increased with increasing concentrations (0.75 to 75 $\mu g/\ell$). As many as 13 lateral stems per

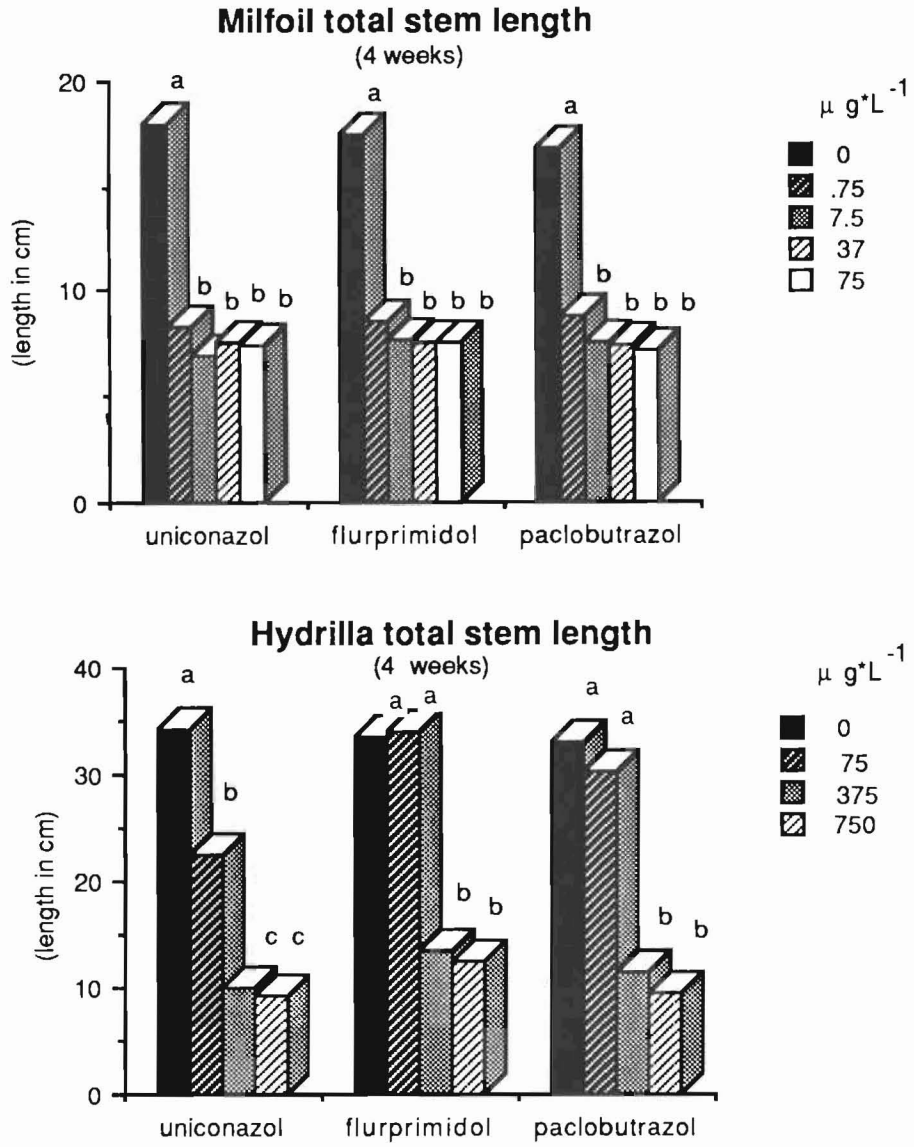

Figure 3. Effect of gibberellin synthesis inhibitors on total stem length (main + laterals) in milfoil and hydrilla at 4 weeks. Note differences in dosage ranges and in y-axis scales between the two plants

main stem were produced on uniconazol-treated milfoil plants (75 $\mu g/\ell$) in contrast to one per main stem on untreated plants and four per main stem on treated hydrilla plants. Unlike hydrilla, however, the lateral stems produced on treated milfoil remained dense and compact in the leaf axils and usually measured no more than 0.5 cm in length (Figure 4). The end result of many extremely short laterals was that total stem lengths and fresh and dry weights of treated milfoil plants were always significantly lower than those of untreated plants (Figure 3).

These response differences between hydrilla and milfoil resulted in different morphologies in the bioassay. Hydrilla plants at "low" concentrations tended to be shortened but bushy, with many lateral stems and roots and with fresh weights

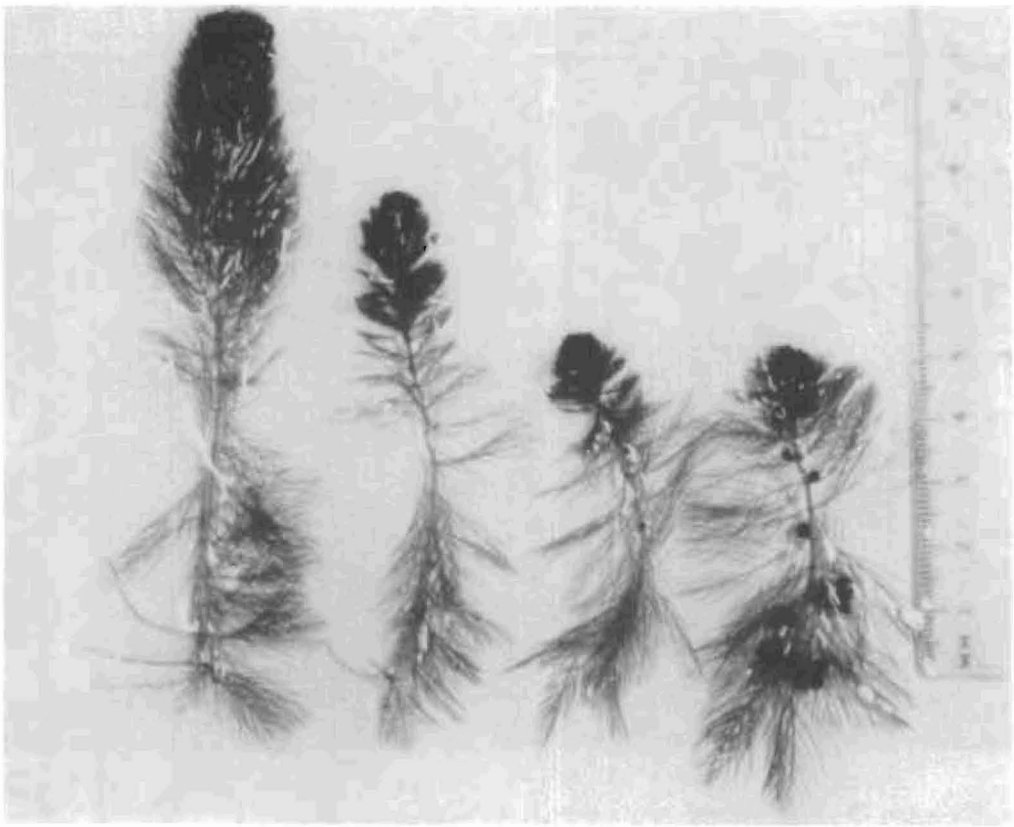

Figure 4. Effect of paclobutrazol on milfoil at 4 weeks. Dosages (from left to right) are 0, 0.75, 7.5, and 75 μg/ℓ. Note lateral buds at the 75-μg/ℓ dose

similar to or somewhat less than untreated plants. The overall appearance was one of the stoloniferous habit. As inhibitor concentrations increased, the number of lateral stems and roots decreased, leaving only single, shortened main stems and considerably less fresh weight biomass. This general tendency was also seen in small-scale field tests in which two 6-cm hydrilla segments were planted into sediment in 67-ℓ barrels and treated with uniconazol. Five weeks after treatment, the fresh weight of plants treated with 75 μg/ℓ was 72 percent that of untreated plants and averaged 469 lateral stems (compared to 181 for untreated plants). The fresh weight of plants treated with 750 μg/ℓ was 48 percent that of untreated plants and averaged 235 lateral stems, similar to the numbers for untreated plants. However, no matter how many lateral stems were produced on the treated plants, all were considerably shorter than untreated controls, resulting in a low-growing carpet-like effect on the bottom of the barrels (Figure 5).

In the milfoil bioassay, increasing dosages resulted in greater numbers of compacted buds. No stoloniferous-type growth was noted. We did not treat milfoil in our small-scale field tests; however, we were concerned that the large number of buds might increase the potential for reinfestation. We removed treated plants from the inhibitors, rinsed them, and placed them in fresh medium without inhibitor. The buds did not elongate or grow even after 8 weeks exposure

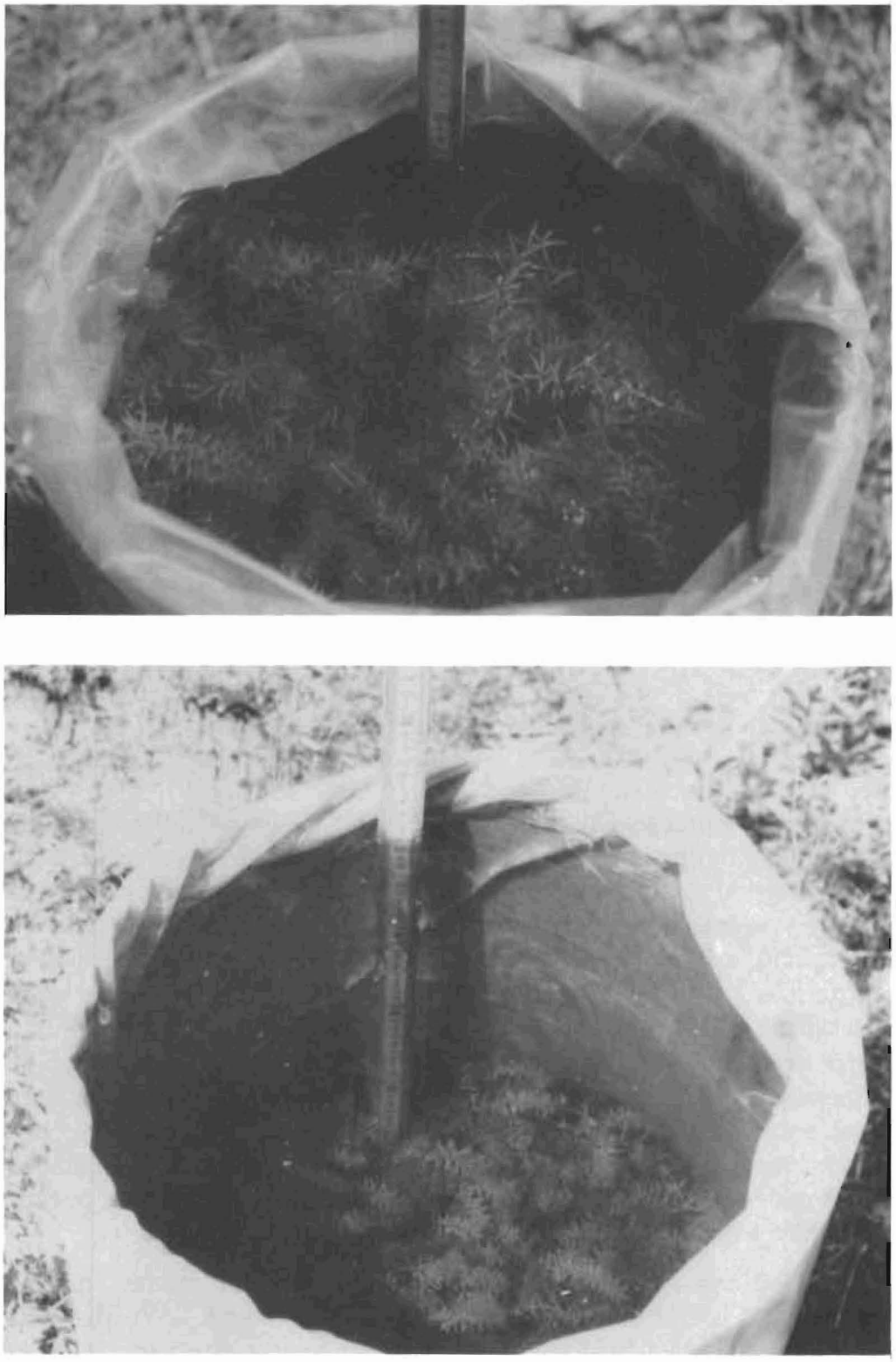

Figure 5. Hydrilla treated with 75 $\mu g/\ell$ uniconazol (bottom) and untreated (top) in small-scale field tests

to untreated medium. This was also the case when buds were excised from the plants and placed in untreated medium. The only way in which these compacted buds could be induced to elongate was by the addition of gibberellic acid (10^{-5} M), either during inhibitor exposure or after the inhibitor had been removed. This suggests not only that the gibberellin synthesis inhibitors are indeed affecting gibberellin synthesis, but also that excised buds from milfoil plants in the field are unlikely to sprout. It is interesting to note that buds are not formed at the lower end of the concentration range that is effective on milfoil.

Physiological parameters were not affected at inhibitor concentrations that produced shortened, healthy-looking plants. Net photosynthesis was not significantly affected until concentrations of 1,500 $\mu g/\ell$ for hydrilla and 375 $\mu g/\ell$ for milfoil were used. Chlorophyll content per gram fresh weight also was not significantly affected at any concentration or by any compound at the range in which plants appeared to be healthy.

We also tested hydrilla and milfoil for recovery potential by rinsing and transferring the plants to untreated medium after exposures of 1, 3, 7, and 14 days to the inhibitor (Figure 6). Hydrilla recovered to untreated control total stem lengths after a 6-week recovery period, no matter how long the plants had been exposed to the inhibitor. Milfoil, on the other hand, remained suppressed, even after only a 1-day exposure to the inhibitor.

CONCLUSIONS

The bioassay used in this study is appropriate for rapidly screening gibberellin synthesis inhibitors at a variety of concentrations and exposure times. Uniconazol, paclobutrazol, and flurprimidol were all effective at reducing stem length and other growth parameters in hydrilla and Eurasian watermilfoil. Milfoil appeared to be more sensitive to these compounds and exhibited different morphological effects than hydrilla. In fact, it appears that concentrations at the low end of the dosage range for milfoil and at the high end of the dosage range for hydrilla may be required to reduce the number of lateral buds produced and the risk of increased infestation. Bioassay results also suggest that hydrilla must be kept in contact with the inhibitor for growth to be suppressed, whereas milfoil requires only a short exposure to the compound for continued suppression of growth. This requires verification in the field; inhibitor residues in water or sediment may be sufficiently high enough to prevent hydrilla regrowth in a growing season.

Our preliminary small-scale field results to date support what we have observed in the bioassay; i.e., uniconazol at bioassay dosages altered the regular growth form of hydrilla by keeping the plant in a low, rug- or carpet-like form. We believe our bioassay has been useful in predicting the dose range, morphological responses, and potential for regrowth from treated plants or buds and that the results provide a firm basis on which to plan an extensive field-testing program.

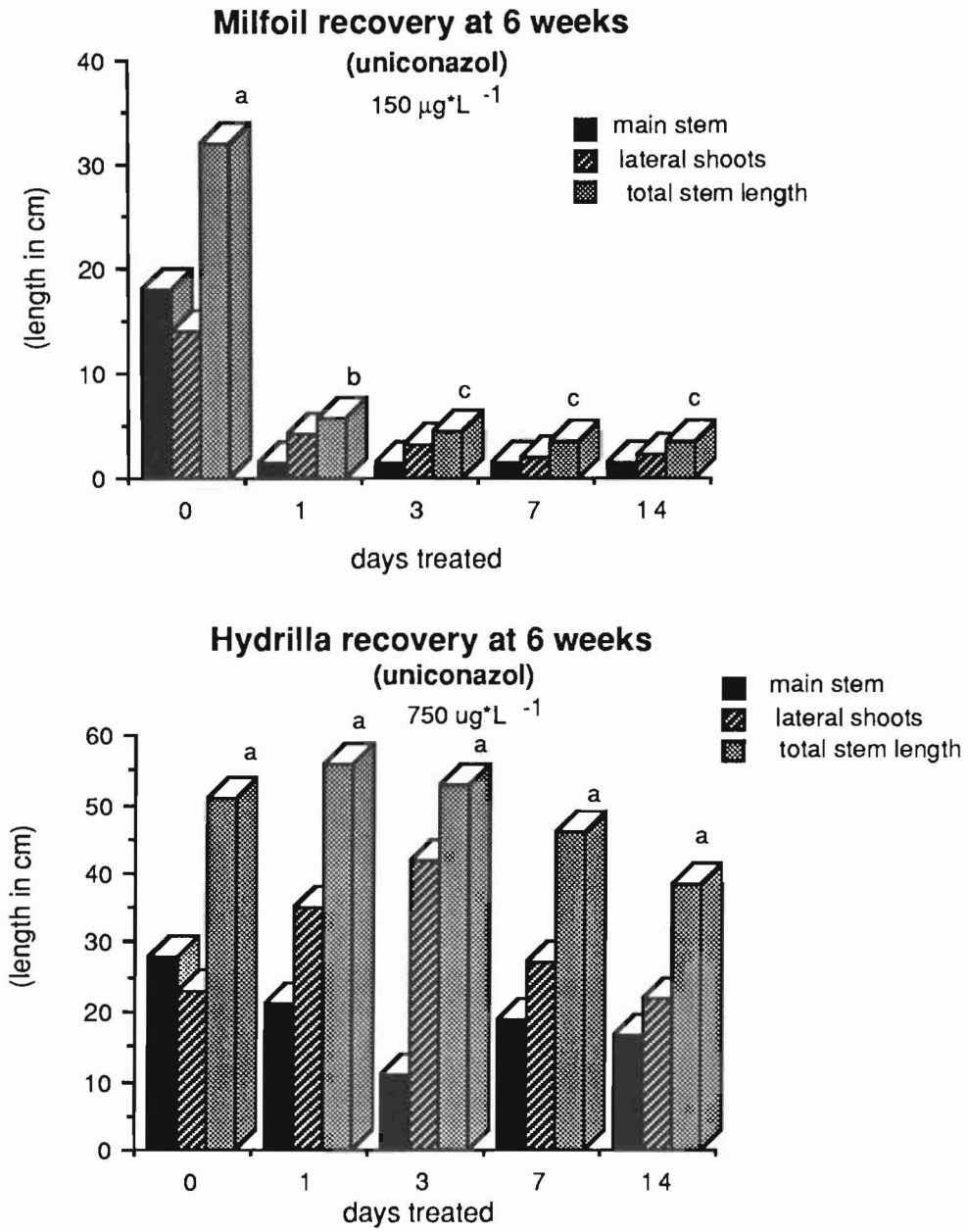

Figure 6. Six-week recovery of milfoil and hydrilla after exposure to uniconazol for various time periods. Separation of means shown only for total stem length data. Note differences in concentration and in y-axis scales between the two plants

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SPECIAL SESSION - LAKE OKEECHOBEE

A History of Lake Okeechobee

by
Lloyd Mitchum*

Like all memorable heroines in the great romance novels, she has been described by many adjectives throughout the centuries. Her past is shrouded in myth and legend and has been for centuries, and men today are still attempting to uncover all her secrets. Early adventurers and explorers gave her many names, none of which really described the lady and all of her attributes.

She has often been described as beautiful, lonely, silent, mysterious, inspiring, exotic, treacherous and even, as she has exhibited in the past, deadly. To different men, and at different times in history, she has certainly been all of those things.

Today, as we near the beginning of a new century, this memorable lady has become somewhat aged, beset with ills resulting from years of high living, and while still proud, just a little battered, scarred, and worn from illness and ill use.

Laguna del Esperitu Santo (Lagoon of the Holy Spirit) was first mentioned in the annals of history in a story related during the early 16th century by Escalonte de Fontaneda. The Spaniard was captured by the Caloosa Indians and held as a slave. He escaped after 17 years and claimed the natives had told him of a great lake on whose shores were many towns containing 30 or 40 people.

In 1564, two Spaniards shipwrecked on the Gulf Coast of Florida were struggling through the uncharted wilderness of Florida's interior trying to make their way to St. Augustine. In their travels, they reported the sighting of a great lake of fresh water.

Lake Mayacco and Lake Macoco were other names applied over the years to the second largest freshwater lake in the United States. The "Big Water," which is the translation of the Indian word Okeechobee, remained as its final name.

The lady's existence remained shrouded in mystery until the mid-1830s when the US Army pursued Indians to its shores during the Seminole Wars.

With the explosion of agribusiness and development along nearly all 135 miles of her shoreline, it is somewhat difficult today to visualize how our lady appeared over 150 years ago when the white man first discovered her.

Mother Nature gifted this beautiful lady with an array of accessories to accentuate her pristine beauty. To summarize both historical and fictional accounts, let's try to imagine how she looked in all her splendor.

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*South Florida Water Management District, West Palm Beach, Florida.

Our lady stretches some 32 miles from east to west and about 37 miles north to south, and encompasses some 730 square miles and a half-million acres. There was quite a divergence of plant life, and an invisible dividing line marked the differences in growth on the shores around the lake.

The northern shoreline was ringed with dense stands of water oak, maple, cypress, pop ash, rubber, and palmetto trees and provided a lush green headpiece for our lady. For about 30 miles north of the shoreline, there were prairie lands and pine forests. The prairies contained wire grass and sedge, which became excellent grazing grounds for the hundreds of wild cattle which roamed the lands. The prairies were unbroken except for patches of the pines in flat woods, most of which were pitchy pines, some extending their branches skyward to a height of 75 to 100 ft.

The prairies were also dotted with numerous small pools that were named according to the type of growth found in them. For example, there were sawgrass ponds, flag ponds, and maiden cane ponds. In places, heads of cypress and small trees were growing in the shallow sloughs.

Meandering through these prairies and beyond, stretching northward for a distance of more than 100 miles was the scenic Kissimmee River.

The prairies, gifted with 55 to 60 in. of annual rainfall, had standing water to a depth of several inches for parts of the year. Today, this area is more familiarly known as the Kissimmee River valley and floodplain.

On the northwestern shoreline, Fisheating Creek, with its many marshes, drained into the lake.

On the south side of the lake, and stretching almost halfway up the east side of the lake, sawgrass in water reached in an unbroken sea almost to the southern tip of the state. This was called, and still is, the Everglades.

On the southwestern shore, trees became more sparse, and there were great stretches of willows and elder bushes. The lake bottom along this section of shoreline was so overgrown with flags, bonnets, and high grass that, when the lake rose in the summer rains, our lady would spread her shimmering skirts as far back as 6 miles. Her skirts in this area were dotted with blue blooming pickerelweed, which blended with the creamy white blossoms of the bottlebrush clumps.

On the south shore and halfway up the eastern shoreline, a solid belt of what was then known as custard apple trees decorated the eastern side of our lady's skirt. The strange-looking trees, completely blanketed with a moonvine cover, extended more than 2 miles in width at some locations. Beginning close to Clewiston, the forest extended all the way to Port Mayaca, about 32,000 acres of woods.

This now nearly extinct tree is a member of the pawpaw family. While the common name is custard apple, it is also called the pond apple and the alligator

apple. The branches were covered with bright, glossy green leaves, and the flower was creamy white, with a crimson center. Many feel it was a totally useless bit of vegetation, since its fruit, a pear-shaped yellow fruit with brown patches, was inedible and its wood would hardly burn; however, Mother Nature must have liked the custard apple to have provided so many of them.

Beneath its gnarled, tangled branches and twisted trunks, in a dark, shadowy domain, thanks to the moonvine cover, the ground was bare from lack of sunlight, but lacy ferns found a home among the tangle of the root system. Once in a while, a giant fern would unroll its brown-backed leaves. Gourd vines, with green pendant fruit, added another accessory to our lady's wardrobe. High in the tree branches, pineapple-like air plants perched like jewels strewn carelessly about the woods.

The bottom of the lake was very shallow, and was composed of hard muck, sand, and shell. The shoreline, for the most part, was black muck, with patches of sandy beaches scattered here and there along the wide expanse of shoreline.

Wildlife abounded in the area, adding further bright accessories to our lady's appearance. A wide array of birds and waterfowl dotted the landscape, which included roseate spoonbills, anhingas, whooping cranes, wood ibises, a large variety of ducks, coots, several species of egrets, blue and white herons, wild turkeys, water turkeys, finches, the Carolina parakeet, bald eagles, and storks. Today, many of them have become extinct. Thousands of the brightly colored parakeets perished in the freeze of 1895, although some old-time residents of Okeechobee claim to have seen some of them near the shoreline of the lake after the turn of the century.

The woods and waterways overflowed with otters, raccoons, opossums, squirrels, rabbits, turtles that literally paved the ground, deer, bears, panthers, alligators, snakes of nearly every species, and insects that could only be counted in the millions.

Fish of many species had heavy populations beneath the surface of our lady's bright shimmering skirts. Even today, she has the reputation of being an excellent fishing lake, and travelers come from all over the world to test their lines in her waters. Largemouth bass, several species of catfish, black crappie (speckled perch), bluegills, and redear perch continue to lure anglers to her shores.

Our lady's waters, when overflowing, continued southward across the sawgrass standing in continual water, and this "sea of grass" extended to the southern tip of the state.

The first change in the countenance of our mysterious beauty came in the late 1800s when Hamilton Disston dredged a navigable waterway from the town of Kissimmee to Lake Okeechobee. In the process, he also cut canals between the many lakes in central Florida, lowering their levels by as much as 9 to 11 ft. That water flowed southward through the Kissimmee River and into the lake.

Disston's company also began cutting a channel from the western shore of the lake and soon connected it with the Caloosahatchee River, creating an outlet to the Gulf of Mexico. With this outlet, commercial shipping was born within our lady's breast.

The company also began to cut canals southward from the southern tip of the lake, and completed the first 8 miles of the Miami Canal. Later, after the turn of the century, the Palm Beach Canal and the St. Lucie Canal were dug along the east side of the lake. Disston's financial collapse in 1896 brought an end to his cosmetic changes to the face of our lady.

Since the beginning of the 20th century, a series of storms over the lake earned the lady the reputation of being a killer. Her shallowness belies the danger she presents during sudden squalls and storms. Even today, she claims three or four lives in an average year, a warning to those who lose their fear and respect of her.

Hurricanes during 1926, and again in 1928, resulted in thousands of deaths and millions of dollars in damage to property. Following the second storm, there began a clamor from those who had settled near our lady's shores for help to protect them from her deadly waters.

A flood control district was formed in 1929, and in 1930, the US Army Corps of Engineers began construction of the Herbert Hoover Dike, which ringed the 135-mile shoreline. A combination of Federal and state financing provided work for many during the darkest days of the depression until the job was completed in 1937.

Further storms and excessive flooding in 1947, particularly along the Gold Coast sections of Palm Beach and Broward Counties, brought more cries of protest from residents. The result was the decision to channelize the meandering Kissimmee River, and to install flood control structures and manage the flow of water from the northern chain of lakes near Orlando. Structures similar to these were installed along the lakeshore across the various outlets from the lake, and in strategic locations throughout the southern part of the state. These actions, coupled with earlier man-made intrusions, completely changed forever the face of our beautiful, rapidly deteriorating lady.

Since 1970, the C-38 canal, which changed the meandering 100-mile-long river into a 50-mile straight channel, has brought unimpeded flow of nutrients, sediments, and floating vegetation into Lake Okeechobee. This, in turn, has caused a buildup of phosphorus and nitrogen, and our lady is currently exhibiting her state of ill health with excessively large blooms of algae on her once relatively clear shimmering waters.

The thousands of acres of floodplains and flow-through marshes, which filtered the waters from the northern chain of lakes as they passed through the many oxbows, were drained during the channelization.

Further deterioration of our lady's natural values occurred when some of her tributaries became dotted with the many dairies which moved into the area during the late 1950s and early 1960s, adding excessive amounts of phosphorus to the waters. On the southern and southwestern shores of the lake, a booming agricultural area, particularly the growth of the sugarcane industry, added large amounts of nitrogen to upset the natural balance of the waters.

Although concerns for the environment and for the health and well-being of our lady have been discussed for nearly 20 years now, it has only been in the past few years that positive steps have been taken to solve the problems.

In 1976, the first study group, the Coordinating Council on the Restoration of the Kissimmee River Valley, was formed. Since then, studies have been conducted by the Corps of Engineers, the South Florida Water Management District, the Kissimmee River Resource Planning and Management Committee, and the Lake Okeechobee Technical Action Committee.

A demonstration project on the Kissimmee River was begun 4 years ago with the installation of sheet metal weirs in Pool B at the upper end of the river. These devices are designed to force water back through the old twists, turns, and bends of the river, and to restore some of the nutrient-filtering properties that existed before channelization of the river.

Best Management Practices are being installed at the dairies in the area to stop the flow of phosphorus-laden waters into tributaries such as Taylor Creek and Nubbin Slough, and into Lake Okeechobee.

The demonstration project is producing results beyond the initial expectations of the water management officials. The quality of the waters flowing through the old oxbows is improving dramatically. Wildlife, particularly some of the waterfowl, is returning to the area. If the floodplains are restored, the sponge effect for filtering waters will be reinstated, cleansing the waters before they flow into the lake. Fishing in Lake Okeechobee is excellent, and currently the largemouth bass is providing anglers on the lake with daily thrills.

Though she is ailing, the lady still lures more and more to her shores. Marinas and fish camps dot the entire shoreline of the lake. Every year, more and more visitors become permanent residents who also want to enjoy all the beauty that Mother Nature has provided.

The lady will never be able to return to that quiet, mysterious body of water written about in years past. She may be ailing; her original cosmetic appearance has been forever altered by the encroachment of civilization. But man, who helped cause her illness, is now committed to keeping her illness from progressing further, and hopefully even to helping to improve the health of this second-largest body of fresh water in the United States. The lady is finding out that she has many friends concerned about her future and willing to help preserve her original features, which first lured men to her shores. These steps are being taken in the

hope that she can be restored to good health, so she may give pleasure to many generations to come.

Evaluation of Fluridone for Hydrilla Control in Lake Okeechobee and Effects on Other Aquatic Vegetation

by
Kenneth A. Langeland,* Daniel D. Thayer,**
and Francois B. Laroche*

INTRODUCTION

Hydrilla (*Hydrilla verticillata* (L.f.) Royle) is a submersed aquatic macrophyte in the family Hydrocharitaceae that probably originated in Asia (Cook and Luond 1982). Unique and highly specialized physiological and biological adaptations allow hydrilla to invade a wide variety of aquatic habitats and competitively exclude native aquatic plants (Haller and Sutton 1975; Van, Haller, and Bowes 1978). Because of the growth habit and prolific reproductive capabilities of hydrilla, it causes detrimental impacts on sport fish populations (Colle and Shireman 1980) and severely limits recreational, domestic, industrial, and agricultural water use (Shireman and Haller 1982). Expensive measures to manage the plant are therefore necessary. In Florida alone, up to $20 million is spent annually for hydrilla management.

The first discovery of hydrilla in the United States was two small infestations in Florida in 1959. By the late 1960s, hydrilla was causing severe problems in major water bodies of every watershed throughout Florida. Hydrilla was identified in Lake Okeechobee in 1972 along the shoreline north of Indian Prairie Canal. Although it was believed by some that hydrilla would not become a problem in Lake Okeechobee, the Florida Department of Natural Resources estimated 4,500 acres of hydrilla in the lake during a 1979 survey (Tarver et al. 1979) and, by 1984, this increased to over 20,000 acres (Schardt 1984). Hydrilla coverage in Lake Okeechobee has remained relatively constant to the present, but this level causes navigation problems and displaces native vegetation such as peppergrass (*Potamogeton illinoensis* Morong) and eelgrass (*Vallisneria americana* Michx.) (Schardt 1986).

Due in part to the size of the hydrilla infestation in Lake Okeechobee, management efforts have been restricted to maintenance of navigation trails and access areas with diquat and various formulations of endothall, and with cutterboats. Diquat and endothall are short-lived; contact herbicides and repeat applications are often necessary to maintain control during the growing season.

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During the period 1981 to 1985, fluridone was evaluated in several Florida lakes to determine the feasibility of registering it for aquatic use. During this testing it was determined that fluridone was very effective for controlling hydrilla and several other aquatic plants and that some native, beneficial aquatic plants were, to varying degrees, tolerant to it. After extensive testing under current US Environmental Protection Agency guidelines, fluridone was registered for aquatic use in 1985. Because hydrilla can be controlled for one or more years with fluridone, its use has greatly improved hydrilla management in several Florida lakes, such as Orange, Lochloosa, and Trafford.

Fluridone has not been used for hydrilla management on Lake Okeechobee because previously planned applications were halted by concerned citizens and state agencies. One of the major concerns has been the possible effect that fluridone could have on native plant communities in the lake, and the indirect effect that large-scale hydrilla management will have upon waterfowl visitation. Another uncertainty is the effectiveness of fluridone for hydrilla control in this large open-water system where water movement is subject to rapid wind-effected changes. The purpose of this study was to determine the effect of fluridone application on hydrilla and other aquatic plants in Lake Okeechobee.

MATERIALS AND METHODS

The 5-percent slow-release extruded clay pellet (SRP) (Sonar 5P) and 4-lb active ingredient (ai) per gallon aqueous suspension (Sonar 4 A.S.) fluridone formulations were applied, separately, to four (two per formulation) 25-acre rectangular plots (871 × 1,250 ft) in the Monkey Box area of Lake Okeechobee on March 3 and 4, 1987, at a rate of 2 lb ai/acre. Application was performed by personnel of the South Florida Water Management District (SFWMD), Okeechobee Field Station. Sonar 5P and Sonar 4 A.S. plots were alternated, and separated by an area approximately equivalent to two plots (Figure 1). This design was not intended to allow for comparison of the two formulations, but it had been suggested that this would result in maximum hydrilla control (personal communication, D. P. Tarver, Eli Lilly Company, Tallahassee, Florida).

Four sampling transects were established that originated from the navigation marker at the end of the Old Moore Haven Canal (commonly referred to as the "tripod") and terminated when they reached cattail (*Typha* sp.) or bulrush (*Scirpus validus* Vahl.). Compass bearings of these transects were as follows: transect 1 - 280 deg, transect 2 - 65 deg, transect 3 - 80 deg, transect 4 - 110 deg. A recording fathometer (Ratheon DE-719) was operated from an airboat along these transects, and buoys were dropped at intervals specified by markings on the fathometer strip chart (approximately 100 ft). Submersed vegetation was qualitatively sampled at each buoy by lowering and twisting a grapple at four geometrically opposed positions to the buoy. This type of sampler has been used successfully for aquatic vegetation by others (personal communication, D. P. Tarver). Although it may be biased toward sampling certain types of vegetation,

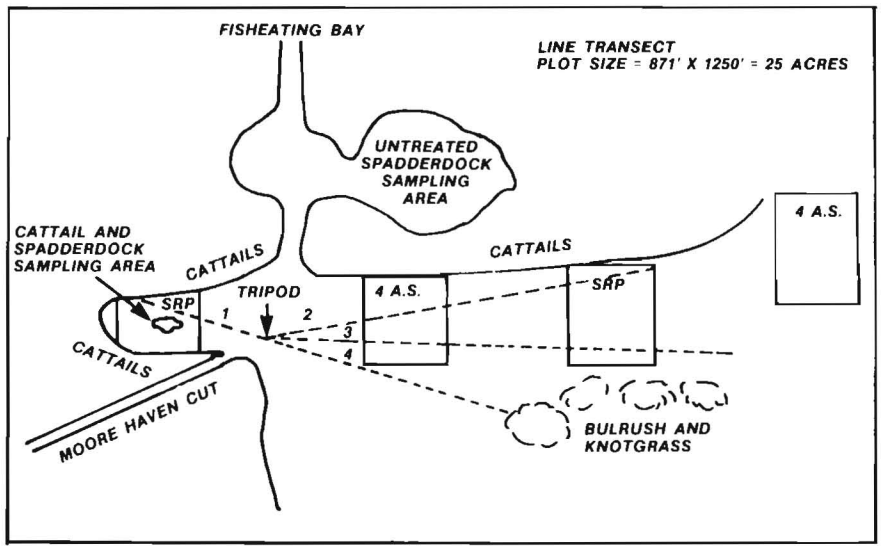


Figure 1. Location of fluridone applications, transects, and sampling areas in Lake Okeechobee (1987)

it will sample a particular species consistently through time. Comparisons in this study are not made among different species, but only within a species with respect to time. Vegetation encountered at each sample was recorded, and frequency of occurrence for each species encountered on each transect was calculated by dividing the number of times that a species was encountered by the total number of sampling points on the transect and expressed as a percent. Qualitative samples were located without regard to location of the sample in an area directly treated or not. In other words, the entire area encompassed by the treated plots was considered the treated area, because it was assumed, a priori, that the herbicide would move between plots. Aquatic macrophyte biomass was measured at buoys that fell directly in the treated areas with the Waterways Experiment Station Hydraulically Operated Submersed Aquatic Plant Sampler (Sabol 1984). Changes in cattail and spatterdock (*Nuphar luteum* (L.) Sibth. and Smith) density were measured directly in plot 1 and in an area remote from the sampling area (check) by counting stems and leaves inside 0.25-sq m quadrats. Visual observations were made of bulrush in proximity to the treated area. Sampling was conducted monthly from March through October 1987.

RESULTS AND DISCUSSION

Sustained northeast winds of 22 mph occurred on the day following herbicide application. We were concerned that these winds could cause sufficient water movement to flush the herbicide out of the treatment area and cause dilution that would reduce effectiveness. Three months after application, our concern increased because little decrease in hydrilla frequency was observed (Figures 2-5). However, fluridone symptoms (etiolation of actively growing plant parts and leaves) were observed on most hydrilla shoot tips and, in some areas, advanced fluridone

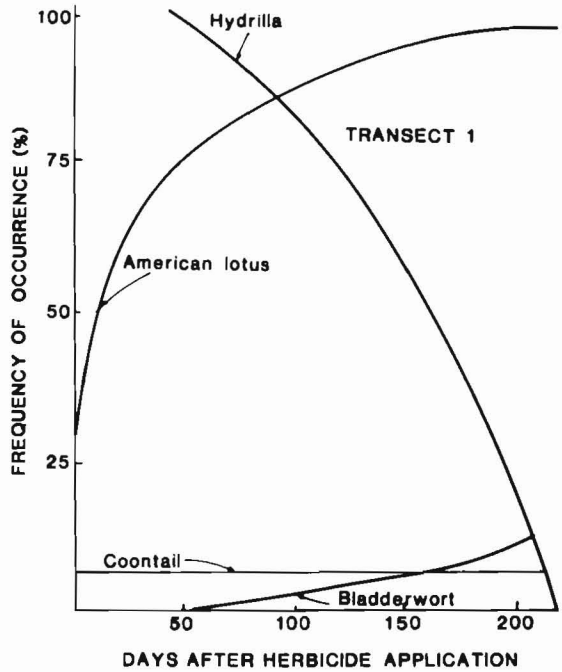


Figure 2. Responses of hydrilla ($y = 105 - 0.002\ x^2$, $r^2 = 0.99$), American lotus ($y = 16 \ln x$, $r^2 = 0.95$), bladderwort ($y = 0.0003\ x^2$, $r^2 = 0.71$), and coontail (no significant change) to fluridone applications on Lake Okeechobee. All slope coefficients (except coontail) are significantly greater than zero at the 0.05 level of significance according to Student's t

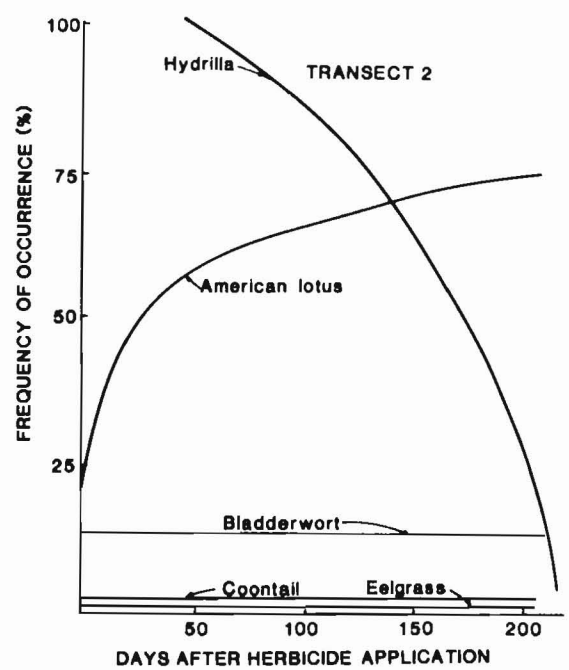
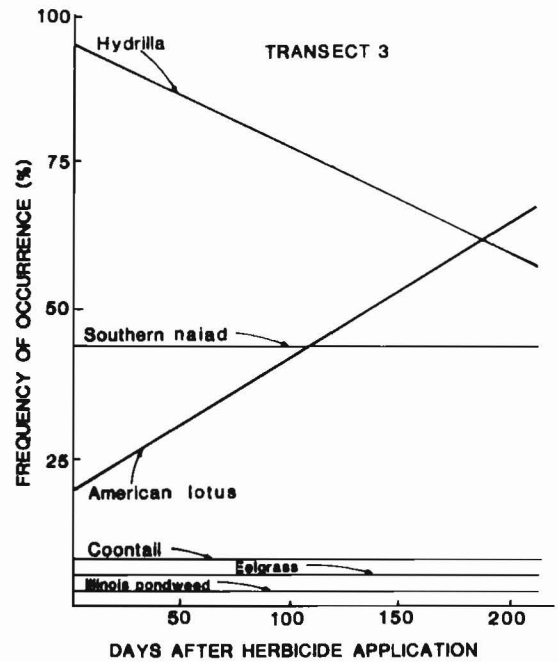


Figure 3. Responses of hydrilla ($y = 106 - 0.002\ x^2$, $r^2 = 0.88$), American lotus ($y = 12.47 \ln x$, $r^2 = 0.77$), bladderwort (no significant change), coontail (no significant change), and eelgrass (no significant change) to fluridone applications on Lake Okeechobee. Slope coefficients for hydrilla and American lotus response curves are significantly greater than zero at the 0.05 level of significance according to Student's t

Figure 4. Responses of hydrilla ($y = 94.54 - 0.18\ x$, $r^2 = 0.76$), American lotus ($y = 18.99 + 0.23\ x$, $r^2 = 0.72$), southern naiad (no significant change), coontail (no significant change), eelgrass (no significant change), and Illinois pondweed (no significant change) to fluridone applications on Lake Okeechobee. Slope coefficients for hydrilla and American lotus response curves are significantly greater than zero at the 0.05 level of significance according to Student's t



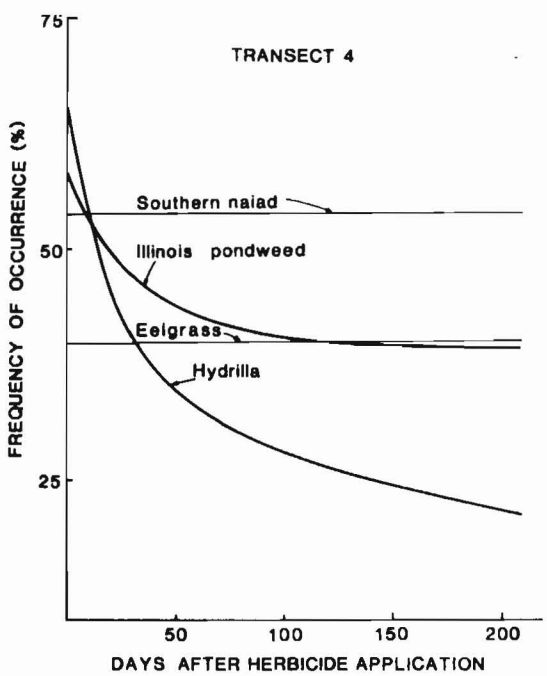


Figure 5. Responses of hydrilla ($y = 64.69 - 7.72 \ln x$, $r^2 = 0.66$), Illinois pondweed ($y = 58 - 3.68 \ln x$, $r^2 = 0.68$), southern naiad (no significant change), and eelgrass (no significant change) to fluridone applications on Lake Okeechobee. Slope coefficients for hydrilla and Illinois pondweed response curves are significantly greater than zero at the 0.05 level of significance according to Student's t

symptoms of fragmentation were observed. Four months after application, decreases in hydrilla frequency were evident along all transects and, 7 months after application, hydrilla frequency approached zero on transects 1 and 2 and decreased by about 50 and 75 percent on transects 3 and 4, respectively (Figures 2-5). The slower (or reduced) control on transects 3 and 4 may be explained by their location on the open-water side of the treatment area.

Fluridone is a slow-acting herbicide relative to diquat and endothall. Its primary action is on new and actively growing plant parts; older mature plant tissue disappears slowly either as a secondary response or by natural senescence. This explains why the decrease in hydrilla frequency is very gradual. This gradual decline is probably advantageous because it allows a maximum amount of herbicide to be absorbed before the vegetation becomes stressed, and because it minimizes stresses on the ecosystem, such as the increased biological oxygen demand caused by decomposition of a large amount of vegetation in a short time.

Illinois pondweed was the only submersed plant, other than hydrilla, that significantly decreased in frequency following fluridone application (Figure 5). This decrease was slight and was observed only on transect 4. Illinois pondweed is reported as intermediate-sensitive (Elanco Products Company 1985) or tolerant (personal communication, W. T. Haller, Gainesville, Florida) to fluridone, and it is therefore doubtful that it declined on the periphery of the treated area as a result of the fluridone application. This conclusion is supported by the observation that, although occurring at low frequencies, Illinois pondweed did not decrease on transect 3, which traverses the actual treated areas. It is more likely that decrease of Illinois pondweed on transect 4 was a result of competition from southern naiad (*Najas guadalupensis* (Spreng.) Magnus), which was abundant on this transect. Southern naiad did not significantly change in frequency on either transect 3 or 4. Coontail (*Ceratophyllum demersum* L.) occurred at low frequencies on transects 1, 2, and 3 and did not significantly change during the study. This again is surprising because coontail is reported as being sensitive to fluridone. However, this difference may be explained by differences in timing or environmental conditions of this application versus conditions under which previous observations were made. Bladderwort, which has also been reported as sensitive to fluridone, either increased in frequency (Figure 2) or remained unchanged (Figure 3). Eelgrass, which is a native aquatic plant of major importance in Lake Okeechobee and can be replaced by hydrilla, did not significantly change following fluridone application (Figures 3-5). This is consistent with previous observations that eelgrass is intermediate-tolerant to fluridone.

Significant decreases of nontarget emergent aquatic vegetation that could be attributed to the fluridone application were not observed. American lotus significantly increased (slope coefficient significantly greater than zero at the 0.05 level of significance when regression analysis was performed using days after fluridone application as the independent variable) in frequency during the growing season on all three transects where it occurred (Figures 2-4). Although we do not

have data from previous growing seasons or from untreated areas for comparison, we suggest that the increases observed indicate that the fluridone application did not cause a substantial detrimental impact to American lotus. Likewise, cattail populations increased significantly as measured by leaf counts in the Sonar 5P-treated area (Figure 6). Spatterdock is reported as sensitive to fluridone, and spatterdock leaf density decreased by greater than 50 percent in the sampling area (Figure 7). However, spatterdock decrease in this study cannot be attributed only

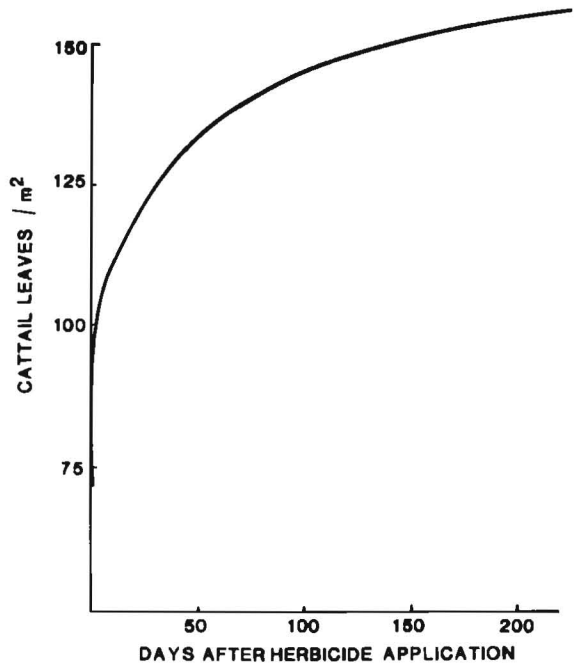


Figure 6. Increase in cattail abundance, as measured by leaf numbers, after fluridone applications in Lake Okeechobee ($y = 71 + 14.89 \ln x$, $r^2 = 0.28$). Slope coefficient is significant at the 0.05 level of significance according to Student's t

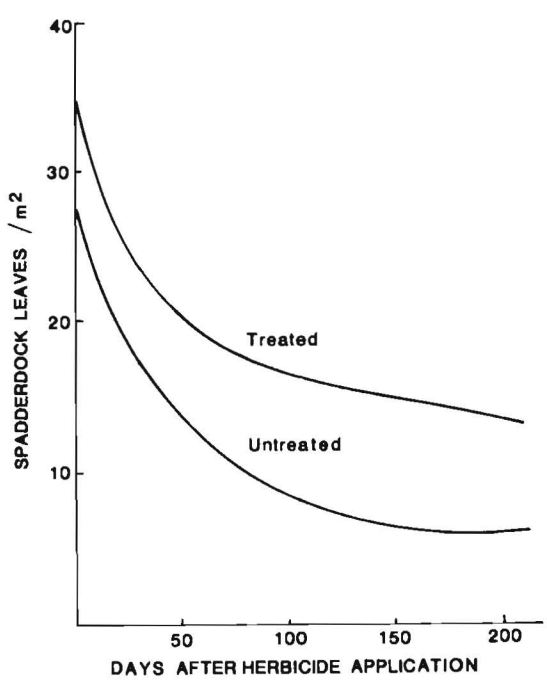


Figure 7. Changes in spatterdock leaf density after fluridone applications in Lake Okeechobee (treated, $y = 35.44 - 4.01 \ln x$, $r^2 = 0.37$; slope coefficient is significantly greater than zero at the 0.05 level according to Student's t) and in an untreated area of Lake Okeechobee ($y = 28.36 - 4.17 \ln x$, $r^2 = 0.43$; slope coefficient is significantly greater than zero at the 0.05 level according to Student's t)

to the herbicide treatment because spatterdock also decreased in the untreated area (Figure 7). Both of these decreases could have been a result of competition from cattail, American lotus, and waterhyacinth (*Eichhornia crassipes* (Mart.) Solms) or other factors. Bulrush did not occur within the actual fluridone-treated area. Visual observations were made to bulrush communities in proximity to the area, and no reductions or fluridone symptoms were observed during the study.

Aquatic macrophyte biomass (composite of all submersed species and American lotus) decreased in both Sonar 5P-treated plots (Figure 8) but did not significantly

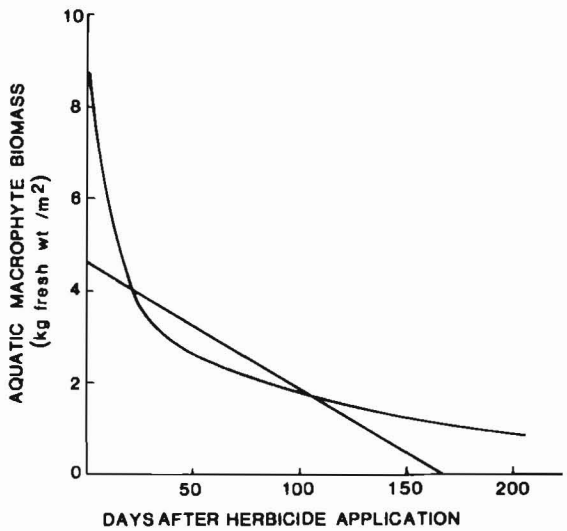


Figure 8. Response of aquatic plant biomass to applications of fluridone (Sonar 5P) in Lake Okeechobee ($y = 4.52 - 0.02\,x$, $r^2 = 0.84$; $y = 8.67 - 1.46 \ln x$, $r^2 = 0.81$). Slope coefficients are significantly greater than zero at the 0.001 level according to Student's t

change in the Sonar 4 A.S.-treated plot. The decrease in the Sonar 4 A.S.-treated plots is most likely due to the decrease in hydrilla as a result of the fluridone application, as is reflected by the decrease in hydrilla frequency on all transects. Although American lotus increased throughout the growing season, this was not enough to compensate in fresh weight biomass for the decrease in hydrilla. While naiad frequency remained unchanged, it was observed to increase in dominance with respect to hydrilla in biomass samples from the Sonar 4 A.S.-treated plot. Replacement of hydrilla biomass with naiad biomass probably explains the lack of change in total aquatic plant biomass in this plot. As previously mentioned, this application was not designed to compare effects of the different formulations of fluridone. However, there may be an indication that the Sonar 5P formulation had a greater impact on southern naiad than the Sonar 4 A.S., and this should be investigated further in the future.

Nutrient release as a result of aquatic weed control operations and the potential effect on algal blooms has become a topic of concern with respect to Lake Okeechobee. Preliminary evaluation of data (no statistical analysis) collected by the SFWMD indicates that nutrient and phytoplankton concentrations were not affected by the herbicide application. This is probably a result, at least in part, of the slow activity of fluridone and assimilation of nutrients released by hydrilla by

other components of the ecosystem such as American lotus and southern naiad.

A concern at the beginning of this study was that the effects of fluridone on hydrilla would be masked by natural suppression by American lotus. Sufficient areas devoid of American lotus were probably traversed by transects to overcome this potential effect. However, to further study the potential suppression of hydrilla by American lotus and the effect that this might have on interpreting the effect of the fluridone application on hydrilla biomass, we compared hydrilla biomass in an untreated hydrilla monoculture, untreated hydrilla in association with American lotus, fluridone-treated hydrilla in association with American lotus, and the fluridone-treated area prior to treatment which had previously been a mature American lotus community (Table 1). A trend toward lower hydrilla biomass was observed in the untreated hydrilla growing in association with American lotus as compared with the pretreatment biomass and the hydrilla monoculture. However, this difference was not statistically significant. Therefore, we are confident that any decreases observed in hydrilla after the fluridone application are in addition to effects of American lotus.

Table 1
Hydrilla Biomass in Various Areas of Lake Okeechobee

	Biomass, kg fresh weight/m^2		
Area	*Lower 90% Confidence Limit*	*Average*	*Upper 90% Confidence Limit*
Fluridone-treated area before herbicide application (March 1987)	4.30	5.9	7.50
Untreated hydrilla monoculture (October 1987)	4.30	5.9	7.40
Untreated hydrilla in association with American lotus (October 1987)	2.10	3.7	5.30
Fluridone-treated hydrilla in association with American lotus (October 1987)	0.00	0.02	1.60

CONCLUSIONS

Data from this study demonstrate that hydrilla can be managed with fluridone in Lake Okeechobee while having minimum reduction of native plant populations. In this study, southern naiad, at least in the Sonar A.S.-treated plot, and American lotus proliferated to the point of having as much detrimental impact on navigation

as hydrilla. However, these plants may have greater redeeming qualities such as preferred wildlife utilization. Future research should therefore determine management strategies for these plants which consider their relative importance and detrimental impacts to water use.

This study provides sufficient data to justify further large-scale hydrilla management on Lake Okeechobee. However, this 1 year can only be considered as preliminary, and monitoring should be continued to determine the long-term effects of fluridone applications to Lake Okeechobee plant communities. Areas for herbicide applications should be chosen where a maximum of public benefit will occur, and where additional information can be collected on the long-term beneficial and detrimental impacts of herbicide applications on the Lake Okeechobee ecosystem. The effectiveness for controlling hydrilla and the impacts to nontarget aquatic plants should be compared between fluridone formulations and other registered aquatic herbicides such as endothall.

ACKNOWLEDGMENTS

This study was funded, in part, by a grant from the South Florida Water Management District. We wish to thank personnel from the SFWMD Okeechobee Field Station for their assistance in herbicide application and for responding to calls for help throughout the study. The US Army Engineer District, Jacksonville, generously made their biomass sampler available to us, and personnel at their Clewiston Field Office generously assisted in repairs.

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ECOLOGY OF SUBMERSED AQUATIC PLANT SPECIES

Interactive Effects of Light, Sediment Fertility, and Inorganic Carbon Supply on the Growth of Submersed Aquatic Macrophytes

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

INTRODUCTION

A variety of environmental variables have been shown to be important in affecting the growth of submersed aquatic macrophytes (Barko, Adams, and Clesceri 1986). Studies of the physiological ecology of these plants conducted in the Environmental Laboratory, WES, have focused principally on light, sediment fertility, and inorganic carbon availability as key environmental variables. The availability of light is of unique importance in aquatic systems, due to marked attenuation of light with depth and turbidity. As in terrestrial systems, sediment (soil) composition largely determines nutrient availability. Key elements, including nitrogen, phosphorus, and various micronutrients, are obtained by submersed macrophytes by root uptake directly from sediment. These elements, nitrogen in particular, can become growth-limiting when depleted from sediment (Barko et al. 1988). Due to slow CO_2 diffusion rates in water and boundary layer resistance to uptake at leaf surfaces, the availability of inorganic carbon in freshwater systems is another potentially important limiting factor.

Past efforts to improve our understanding of the physiological ecology of submersed aquatic macrophytes have been directed toward the evaluation of independent effects of environmental factors under otherwise uniform environmental conditions. These studies have clearly advanced our understanding of environmental effects at the physiological level, but by design (i.e., lack of attention to interactions) have been somewhat deficient at the ecological level. Most recently, we have initiated efforts to better define, in ecological terms, interactions affecting macrophyte growth in nature.

Our objective in this study was to evaluate individual responses of *Hydrilla verticillata* and *Vallisneria americana* to a factorial array of light, sediment fertility, and inorganic carbon availability. Results are intended to augment the current understanding of effects of these variables on submersed macrophyte growth and, in combination with results from the related study of Smart and Barko (1989), to provide a basis for investigating mechanisms influencing the outcome of competition among submersed macrophyte species.

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METHODS AND MATERIALS

Growth responses of *Hydrilla* and *Vallisneria* were examined individually in the Environmental Laboratory greenhouse facility in large (1,200-ℓ) fiberglass tanks under factorial (2 × 2 × 2) conditions of light, sediment fertility, and inorganic carbon availability. Variables were assigned at two levels, low and high, with the low level of all variables ("baseline") roughly representative of minimal requirements for macrophyte growth.

Experimental light levels were controlled with neutral-density shade cloth. Different sediment fertilities were achieved by incorporating in the design both "used" and "fresh" sediment obtained by dredging from nearby Brown's Lake (refer to Barko et al. 1988 for details). In effect, used sediment contained critical nutrients (nitrogen and phosphorus) at substantially lower levels than fresh sediment. Air compressors facilitated CO_2 supply and mixing. Air to half of the tanks was augmented by addition of compressed CO_2 from cylinders to achieve a 10× increase in inorganic carbon supply (high-carbon treatments). Species and treatment combinations per species were assigned to separate tanks. For each species, treatment combinations were replicated 10 times. Replicates consisted of 1-ℓ sediment containers, planted and harvested from each tank.

The solution in which the macrophytes were grown was identical to that described in Table 1 of Smart and Barko (1985). In summary, it contained major elements except N and P, which were excluded to minimize confounding effects of algae growth. Solution temperature was maintained throughout the study at 25° C (±1° C) with liquid circulator/chillers. The duration of the experiment was 10 weeks.

Evaluation of macrophyte growth was based on changes in shoot length, shoot density (number), and total biomass. Shoot density in *Vallisneria* actually represents the number of individual plants, rather than true shoots as found in *Hydrilla*. Specific methods of macrophyte harvesting, enumeration, and measurement were as described in Barko and Smart (1981). Root and shoot biomass were determined separately, then summed to calculate total biomass. Statistical analyses of data were performed using the Statistical Analysis System. Results reported here as statistically significant were examined at the 5-percent probability level.

RESULTS

With both *Hydrilla* and *Vallisneria*, the availability of light had the greatest overall effect on total biomass production (Figures 1 and 2). Under the low light condition (<125 $\mu E/m^2/sec$), levels of CO_2 and sediment fertility had essentially no effect on biomass production in *Hydrilla* (Figure 1). In contrast, under the high light condition (ca. 550 $\mu E/m^2/sec$ at midday), biomass production in this species increased significantly at high levels of CO_2, fertility, and their combination.

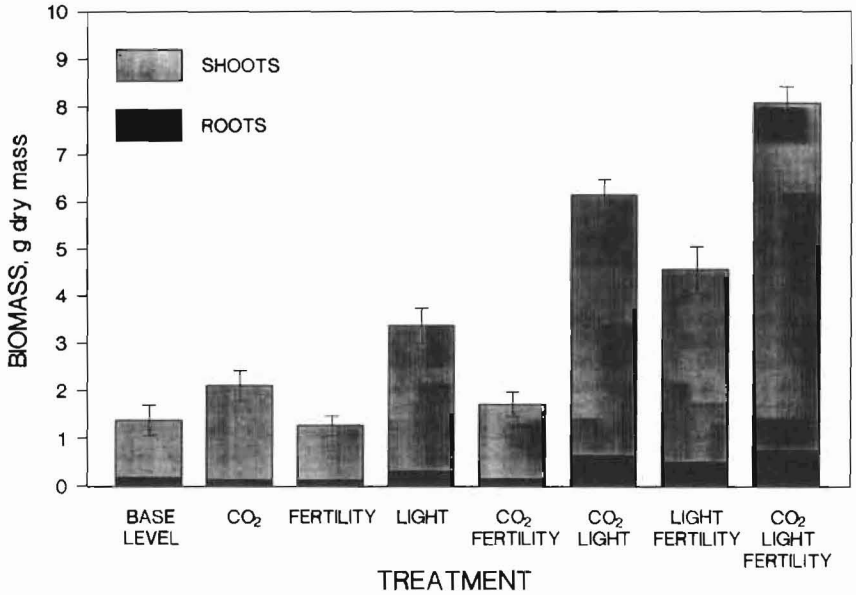

Figure 1. Effects of treatment combinations on biomass production in *Hydrilla*. Base-level treatment represents low levels of all variables. Values are means (n = 10) with associated standard deviation bars

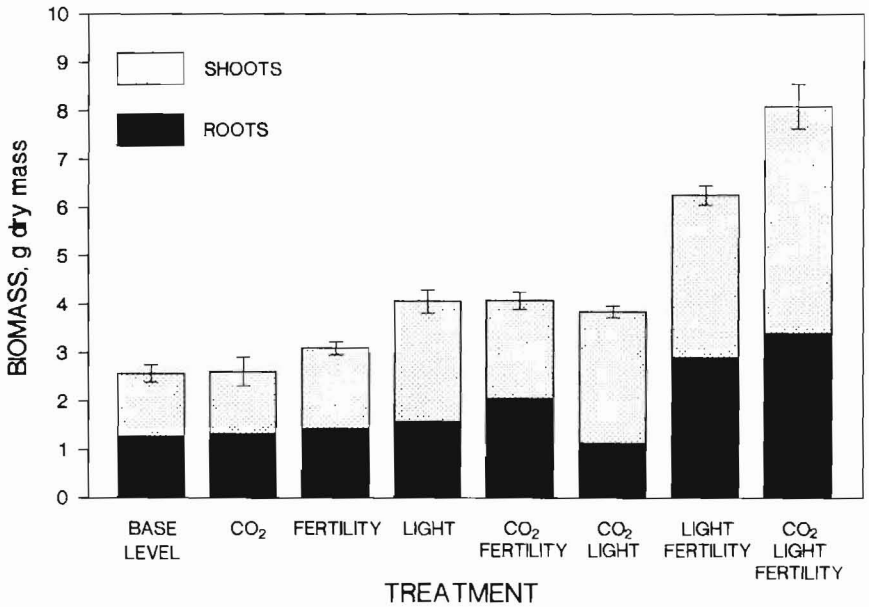

Figure 2. Effects of treatment combinations on biomass production in *Vallisneria*. Base-level treatment represents low levels of all variables. Values are means (n = 10) with associated standard deviation bars

Notably, CO_2 had a greater effect on biomass production in *Hydrilla* than did sediment fertility.

Under the low light condition, levels of CO_2 and sediment fertility had no effect on biomass production in *Vallisneria* (Figure 2). However, the combination of these factors significantly increased biomass production in this species to about the

same level as that achieved with high light alone. Under the high light condition, levels of CO_2 had no effect on biomass production in *Vallisneria*. However, high sediment fertility, alone and in combination with CO_2, resulted in major increases in biomass production in this species under the condition of high light.

Root-to-shoot biomass ratios in *Vallisneria* (ca. 0.5 to 1.0) greatly exceeded those in *Hydrilla* (<0.15) across all treatments. This ratio for both species was essentially unaffected by treatments in the investigation (Figures 1 and 2).

In *Hydrilla*, and to a lesser extent in *Vallisneria*, shoot length was greatest under the condition of low light (Figures 3 and 4). Addition of CO_2 alone and in

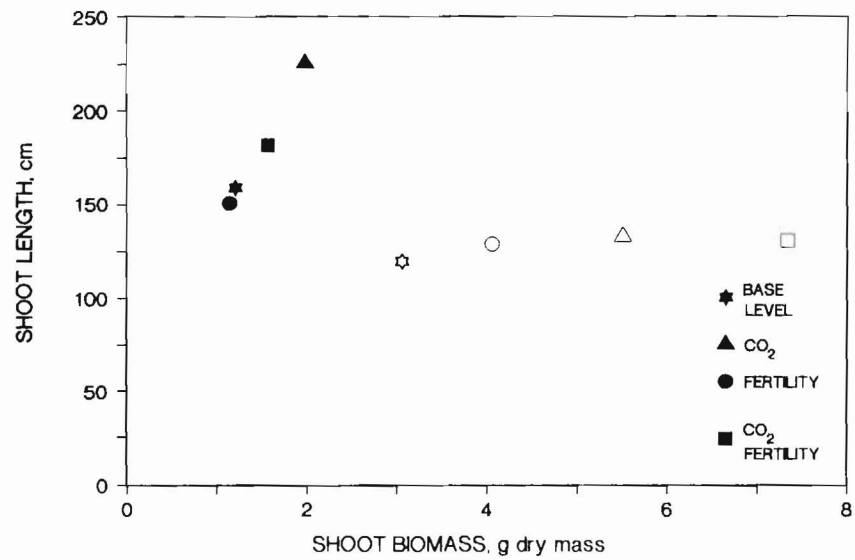

Figure 3. Relationship between shoot length and biomass production in *Hydrilla*. Closed and open symbols represent low and high light levels, respectively. Base-level treatments (low CO_2 and fertility) are indicated within each light level. Values are means (n = 10)

combination with high sediment fertility at low light increased shoot length in *Hydrilla*, while fertility alone had no effect on shoot length in this species. At high light, shoot length in both species was essentially unaffected by any of the treatments.

In both species, shoot density was greatest under the condition of high light (Figures 5 and 6). At the high light level, shoot density in *Hydrilla* increased with increased biomass production. However, in marked contrast to the response of *Hydrilla*, shoot density in *Vallisneria* at the high light level decreased somewhat with increased biomass production. At the low light level, shoot density in both of these species was statistically unaffected by treatments, as biomass production remained essentially unchanged.

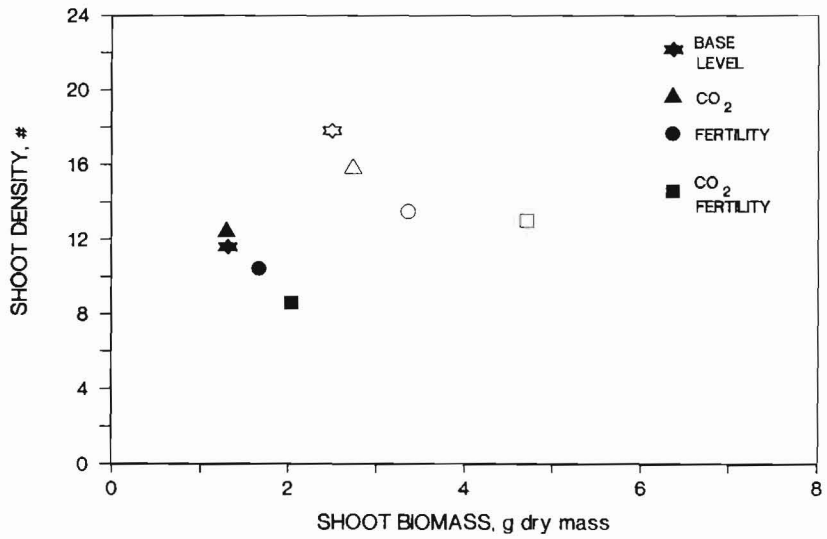

Figure 4. Relationship between shoot length and biomass production in *Vallisneria*. Closed and open symbols represent low and high light levels, respectively. Base-level treatments (low CO_2 and fertility) are indicated within each light level. Values are means (n = 10)

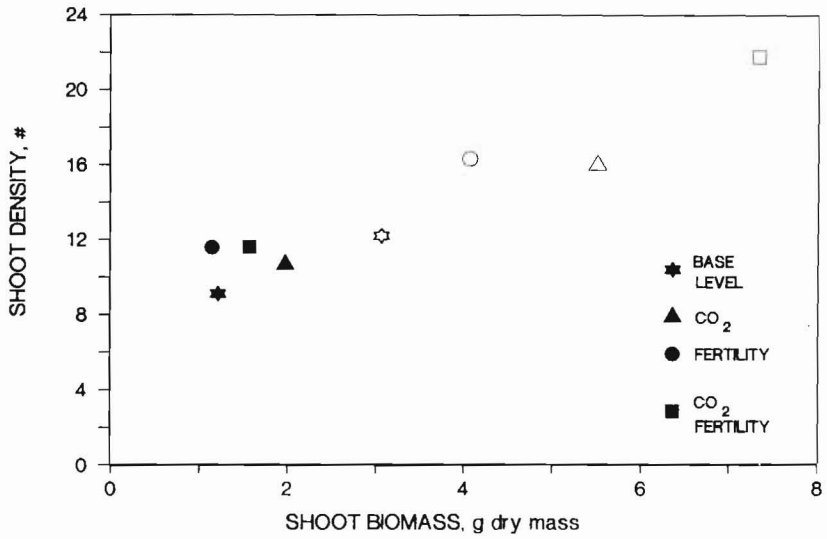

Figure 5. Relationship between shoot density and biomass production in *Hydrilla*. In this species, shoot density represents number of individual shoots. Closed and open symbols represent low and high light levels, respectively. Base-level treatments (low CO_2 and fertility) are indicated within each light level. Values are means (n = 10)

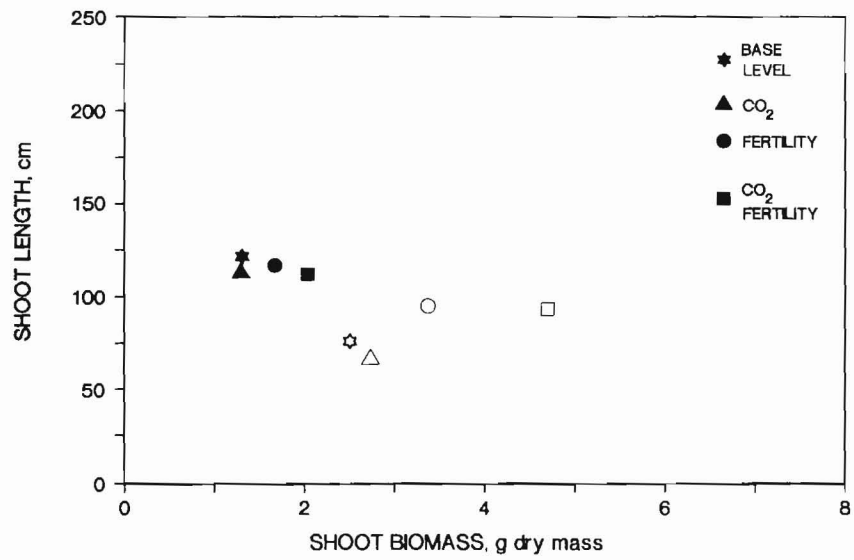

Figure 6. Relationship between shoot density and biomass production in *Vallisneria*. In this species, shoot density represents number of individual plants. Closed and open symbols represent low and high light levels, respectively. Base-level treatments (low CO_2 and fertility) are indicated within each light level. Values are means (n = 10)

DISCUSSION

Biomass production and shoot morphology (number and density) in *Hydrilla* and *Vallisneria* were clearly affected interactively by the three variables considered in this investigation. Relative lack of biomass responsiveness to treatments under the low light condition suggests that interactions with other variables affecting macrophyte growth are likely to be significant only under conditions of adequate light. These results attest once again to the prime importance of light availability to submersed macrophytes in aquatic systems (Spence 1972).

Under the condition of high light, biomass production in *Hydrilla* and in *Vallisneria* was most sensitive to levels of CO_2 and fertility, respectively. These results suggest that the two species may differ somewhat in their environmental requirements. *Hydrilla* in this investigation appeared to be more efficient than *Vallisneria* in coupling carbon fixation in photosynthesis with nutrient uptake from sediment. This apparent difference in efficiency of nutrient use by these species may result in part from differences in their allocation of nutrients and biomass to shoots versus belowground structures. *Vallisneria*, with a much greater root-to-shoot ratio than *Hydrilla*, may have a greater requirement for nutrients than *Hydrilla* during initial colonization of sediment.

Under the condition of low light, shoot length in *Hydrilla* was appreciably greater overall than under the condition of high light. As noted in previous studies (Barko and Smart 1981 and literature cited therein), *Hydrilla* is very

effective at forming a foliar canopy under low light conditions. Canopy development appears to preclude increased shoot density. Thus, under conditions of low light, *Hydrilla* maximizes foliar exposure to irradiance at the water surface. This provides a particular advantage in turbid aquatic systems, such as the Potomac River, where *Hydrilla* grows nearly monotypically (Hammerschlag 1988). Under the high light condition in the current investigation, *Hydrilla* grew robustly by maximizing shoot development at the expense of shoot elongation. This species is clearly quite adaptable to the broad range in light climates occurring in aquatic systems. Based on results of this investigation and others (Haller and Sutton 1975), *Vallisneria* does not possess the canopy-forming ability of *Hydrilla*. Thus, under low light conditions it appears to be disadvantaged relative to *Hydrilla*, particularly in deep waters.

The decrease in density of *Vallisneria* plants observed here in response to treatments under high light conditions suggests a different form of adaptation to reduced light. With increased biomass in this investigation, *Vallisneria* produced larger but fewer plants, with a presumably greater share of light available to each. We view this as a response to self-shading and suggest that regulation of plant density in *Vallisneria* may parallel (in an adaptive sense) canopy formation on the part of *Hydrilla*.

Whereas decreased plant density on the part of *Vallisneria* under high light may diminish somewhat the effects of self-shading on total biomass production, it is unclear from results of this study whether or not this response confers any advantage under low light. Nevertheless, we feel that *Vallisneria* would be an inferior competitor to *Hydrilla* under low light conditions, due to the canopy-forming ability of the latter. Thus, we would predict displacement of *Vallisneria* by *Hydrilla* in low light environments. With adequate light, however, the outcome of competition between these species is more difficult to predict, since it will undoubtedly vary dependent upon inorganic carbon availability and sediment fertility.

The predictions elaborated above are currently being evaluated in studies of submersed macrophyte competition. Preliminary results of these studies, designed to examine interactive responses to environment of *Hydrilla* and *Vallisneria* grown together, are reported in Smart and Barko (1989). We suggest that studies of aquatic macrophyte competition, designed with specific knowledge of interactions among environmental variables affecting macrophyte growth, will lead to the development of innovative management methodologies.

ACKNOWLEDGMENTS

The authors extend appreciation to D. G. McFarland, H. L. Eakin, G. A. Bird, B. Ashmore, and B. F. Henderson of the US Army Engineer Waterways Experiment Station (WES) for their many efforts in the performance of greenhouse experiments and laboratory analyses. W. D. Taylor of the WES provided technical

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Competitive Interactions of Submersed Aquatic Macrophytes in Relation to Water Chemistry and Other Environmental Conditions

by
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INTRODUCTION

Three aspects of an aquatic plant community largely determine whether or not an aquatic weed problem exists. These include the species composition, areal extent, and the level of biomass production of the community. Species composition seems to be the most important aspect of aquatic plant communities with regard to their potential for causing water use problems. Certain species are generally regarded as weeds and are considered to cause problems wherever they occur. It is therefore very important to determine the factors controlling the distribution of these weedy species.

Since species composition of plant communities results from the interplay between environmental conditions and the biological characteristics of the plants, we need to understand the relationships between individual plant species and their environment. We have recently given considerable attention to the physiological responses of many plant species to characteristics of their abiotic (nonliving) environment. These conditions have included light, temperature, inorganic carbon supply, and sediment fertility (Barko 1986, Smart 1988).

We have found similarities in the environmental requirements of many species of aquatic plants, both introduced and native. Weedy species such as *Hydrilla* do not differ appreciably from nonweedy species in their gross environmental requirements. In fact, most common species exhibit quite similar requirements for light, temperature, carbon, and nutrients (Smart and Barko 1984). Differences in individual species' preferences for particular abiotic environmental conditions thus cannot always account for differences in species composition of submersed plant communities. The species composition of aquatic plant communities thus cannot often be directly ascribed to particular characteristics of the abiotic environment. While species composition may not be directly determined by individual species' responses to the abiotic environment, it may be affected by biotic (competitive) interactions among species. Thus, species composition of the community may be determined by the relative competitive abilities of the individual component species. To test this hypothesis, we have initiated studies of interactions between introduced, weedy species and native, nonweedy species.

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OBJECTIVE AND APPROACH

The objective of the research was to identify potentially important factors and mechanisms involved in competition between introduced, weedy species and native, nonweedy species. We chose *Hydrilla verticillata* and *Vallisneria americana* as examples of these respective types. To determine the factors involved in competition between these species, we studied competitive interactions under several environmental conditions. We varied light, carbon availability, and sediment fertility. We selected these environmental conditions because they greatly affect submersed aquatic plant growth, and are thus most likely to affect competition.

METHODS

The experiment was conducted in 1,200-ℓ fiberglass tanks housed in a greenhouse facility at the Waterways Experiment Station. Different grades of neutral-density shade fabric provided the different light levels used. Maximal midday photosynthetically active radiation levels were 125 and 550 $\mu E\ m^{-2}\ sec^{-1}$. The solution used in the experiments contained major cations and anions, including HCO_3^-, but did not contain either nitrogen or phosphorus. Plant growth was thus dependent on the sediment as the source of these elements. We used two sediment nutrient levels: fresh Brown's Lake sediment and the same sediment after a period of submersed plant growth. Previous studies in our laboratory showed that nitrogen availability limits the growth of *Hydrilla* on "used" sediment (Barko et al. 1988). We provided two levels of carbon supply by varying the concentration of CO_2 in the aerating gas supplied to twin airlifts in each tank. The levels used were ambient CO_2 (350 $\ell\ \ell^{-1}$) and 10× ambient. Prior studies in our laboratory showed that plant photosynthesis rapidly depletes inorganic carbon from solution unless carbon is replenished by aeration with CO_2-enriched air (Smart 1988).

Each tank contained forty 1-ℓ containers planted with four *Hydrilla* plants, four *Vallisneria* plants, or a mixture of two plants of each species. After 8 weeks, we harvested 10 replicate containers from the center of each tank. We sampled plants from the center of the tanks to maximize plant interactions. We harvested and determined dry weight biomass of both shoots and roots.

RESULTS

We consider the results of the species' individual responses (in the absence of competition) in detail elsewhere in this volume (Barko and Smart 1989). These results are summarized briefly as follows. Light limited the growth of both species under the low light conditions. Once light limitation was overcome, *Hydrilla* responded to an increase in carbon availability but not to an increase in nutrient supply. *Vallisneria* responded to an increase in sediment fertility but not to an

increase in carbon availability. Both species produced maximal biomass when all environmental factors were provided at the high level.

The ratio of total biomass accumulation by the individual species is an indicator of their relative competitiveness (Figure 1). We present the ratios of biomass

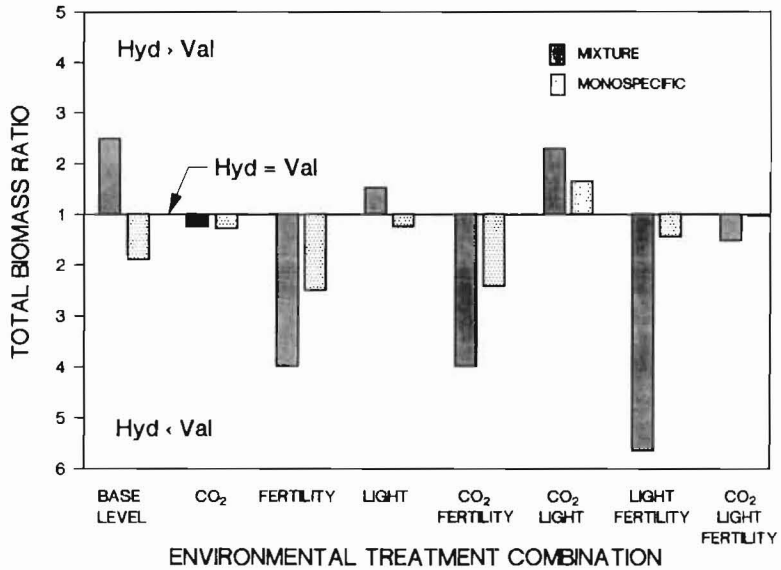

Figure 1. Ratio of total biomass accumulated by the competitive winner to that of the competitive loser. Values plotted above the horizontal equivalence line indicate conditions in which *Hydrilla* was the competitive winner (i.e., *Hydrilla* biomass accumulation exceeded that of *Vallisneria*), while values below the equivalence line indicate conditions in which *Vallisneria* biomass exceeded that of *Hydrilla*. For comparative purposes, we present data for conditions where the two species were growing alone (monospecific, light bars) and where they were growing together in competition (mixture, dark bars). Ratios are determined from means of 10 replicates

production for plants growing alone and also for those growing in mixture. Differences between the ratios calculated for the monospecific and mixture conditions indicate competitive interactions. *Vallisneria* is an effective competitor with *Hydrilla*, particularly when the two species grow in mixture under the higher fertility conditions.

In a preliminary attempt to identify potential factors and mechanisms involved in competition between these two species, we briefly examine some of the results obtained in this study. Under the high light-high CO_2 conditions, *Hydrilla* was the more successful competitor, while *Vallisneria* was more successful under the high light-high fertility conditions (Figure 1). These different outcomes can be explained by examination of the physiological and morphological responses of the two species.

Nitrogen limited the growth of both species grown individually under the high

light-high CO_2 conditions. Since sediment fertility (nitrogen availability) affected the outcome of competition between these species, nitrogen accumulation under nitrogen-limiting conditions should be a useful indicator of the potential competitive ability of these species. Nitrogen accumulation by the two species was similar (Figure 2) when the plants were growing separately under nitrogen-limiting

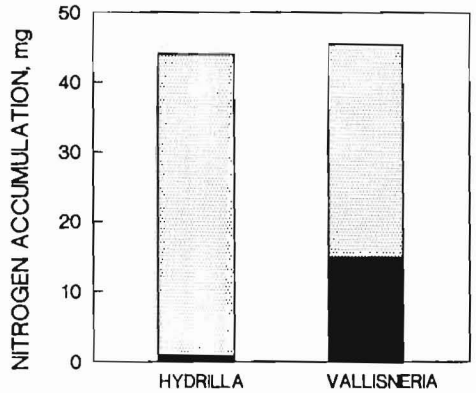

Figure 2. Nitrogen accumulation by *Hydrilla* and *Vallisneria* growing separately (no competition) under nitrogen-limiting growth conditions (i.e., the high light-high CO_2 condition). Dark-shaded portions of the bars denote nitrogen contained in roots, while light-shaded portions denote nitrogen contained in shoots. Values are means of two replicate composite samples

conditions (high light-high CO_2). This similarity in nitrogen accumulation indicates that neither species had a competitive advantage with regard to nitrogen uptake. However, while *Hydrilla* allocated much of its nitrogen to shoots, *Vallisneria* allocated a substantial portion of nitrogen to roots.

From the previous data, we can calculate the quantity of nitrogen required to produce a given mass of shoots. While *Hydrilla* requires only 8 mg of nitrogen to produce a gram of shoot biomass, *Vallisneria* requires over 16 mg of nitrogen. Thus, given access to the same low level of sediment nitrogen, we would expect that *Hydrilla* would exhibit about twofold greater shoot biomass production. This greater production of shoots might make *Hydrilla* a more effective competitor than *Vallisneria* under nitrogen-limiting conditions.

Examination of plant morphology confirms the importance of nitrogen allocation in affecting the outcome of competition between these two species. Under the high light-high CO_2 condition, nitrogen limited the growth of each of the species growing individually, and potentially limited total plant growth when the two species were grown in mixture. Under these conditions, *Vallisneria* was unable to produce sufficient shoot biomass to reach the water surface and form a canopy, either when growing alone or with *Hydrilla* (Figure 3). On the other hand, *Hydrilla* was able to produce sufficient shoot biomass to overtop *Vallisneria*, giving it access to a greater share of solar irradiance and CO_2 derived from the atmosphere. These benefits enabled *Hydrilla* to easily win the competitive struggle.

Under the high light-high fertility condition, the availability of CO_2 limited growth of each of the species growing individually (Barko and Smart 1989), and potentially limited biomass production of the mixture. *Vallisneria*, which is more affected by nitrogen availability than by CO_2 availability, produced sufficient shoot biomass to reach the water surface and form a canopy, both when growing alone and in mixture with *Hydrilla* (Figure 4). While *Hydrilla* was able to form a dense

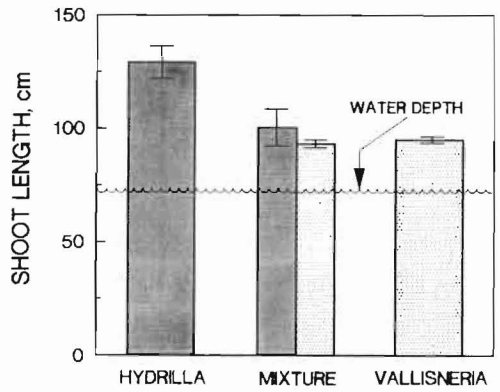

Figure 3. Mean maximum shoot length of *Hydrilla* and *Vallisneria* grown separately (no competition) and in mixture under the high light-high CO_2 condition. The line for water depth indicates potential canopy formation. Values are means of 10 replicates with associated standard error bars

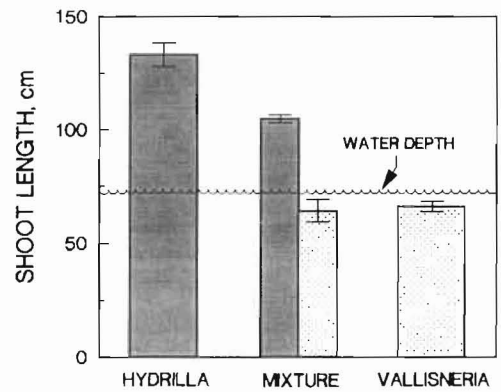

Figure 4. Mean maximum shoot length of *Hydrilla* and *Vallisneria* grown separately (no competition) and in mixture under the high light-high fertility condition. The line for water depth indicates potential canopy formation. Values are means of 10 replicates with associated standard error bars

canopy when growing alone, it was unable to overtop *Vallisneria* in the mixture. Since the growth of *Hydrilla* is greatly diminished under conditions of low CO_2 availability (Barko and Smart 1989), the growth of this species would be impaired by competition with the more efficient *Vallisneria* for inorganic carbon. Competition for inorganic carbon hindered shoot biomass production by *Hydrilla* and precluded the development of a canopy by this species. Prevented from overtopping the competing *Vallisneria* shoots, *Hydrilla* was unable to obtain additional access to solar irradiance and CO_2 diffusing from the atmosphere. In this case, *Vallisneria* won the competitive struggle.

DISCUSSION

These results indicate that, under certain conditions, *Vallisneria* can be an effective competitor with *Hydrilla*. Increased sediment fertility in this experiment allowed the slower growing *Vallisneria* to become established even in the presence of competing *Hydrilla*. Once *Vallisneria* becomes well established, it may be able to persist and even suppress the growth of *Hydrilla*, at least over the short term.

From the results obtained in this study, *Hydrilla* does not seem to be physiologically more competitive than *Vallisneria*. However, *Hydrilla* is rapidly expanding its distribution and, in some cases, is displacing native species such as *Vallisneria*. If this range extension by *Hydrilla* is not due to a more efficient physiology, it may be attributed to its more effective reproductive and dispersal mechanisms. *Vallisneria* populations expand primarily by relatively slow vegetative growth or, perhaps less frequently, by seeds. *Hydrilla* populations, however, can expand by vegetative growth, fragmentation, and production and dispersal of turions and

tubers. Fragmentation, dispersal, and vegetative growth provide for a very rapid and effective means of spread in the aquatic environment. It may be significant that the worst aquatic weeds all reproduce by fragmentation and vegetative growth.

The primary competitive advantage held by weedy species is their ability to rapidly colonize available habitats through fragmentation and dispersal of propagules. In examining competitive interactions, we must broaden our consideration of environmental conditions to include availability of habitat and the frequency of disturbance. Rapid reproduction rates, wide dispersal patterns, and regrowth from perennating organs such as tubers are biological characteristics which convey competitive advantages under conditions of frequent disturbance.

FUTURE RESEARCH

In our future research we will focus on determining the relative competitive abilities of several introduced and native species. We will also determine the important environmental factors that affect the outcome of competition. We hope to identify potential means for promoting the reestablishment and/or persistence of native, nonweedy vegetation. If we can successfully establish nonproblem species such as *Vallisneria*, we may be able to slow the spread of weedy species such as *Hydrilla*.

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Influences of Environmental Factors and Their Interactions on Growth and Tuber Formation in *Hydrilla*

by
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INTRODUCTION

Hydrilla verticillata (L.f.) Royle is a highly prolific, rooted, submersed macrophyte (Haller 1976; Van, Haller, and Bowes 1978; Cook and Luond 1982; Yeo, Falk, and Thurston 1984). For three decades since its introduction to Florida (Blackburn et al. 1969), *Hydrilla* has extended its invasion of freshwater systems mainly throughout southern regions of the country (Steward et al. 1984, Spencer and Anderson 1986) and has become a persistent menace to aquatic resources. The ability of *Hydrilla* to displace favorable native submersed vegetation is attributed to several photosynthetic and reproductive characteristics (Haller 1976; Van, Haller, and Bowes 1978). These include a minimal light requirement for photosynthesis (Van, Haller, and Bowes 1976; Bowes et al. 1977), a high rate of dry matter production (Singh and Sahai 1977), and diverse and effective means of asexual reproduction (Haller and Sutton 1975, Pieterse 1981).

Dispersal and perennation of *Hydrilla* are facilitated by a variety of vegetative propagules, i.e., regenerative fragments, rhizomes, stolons, tubers, and turions (Pieterse 1981). Among these propagules, tubers (or subterranean turions) appear to be most important in reestablishing *Hydrilla* populations following adverse climatic conditions or application of control operations (Weber 1973; Basiouny, Haller, and Garrard 1978). Tubers form on stolon apices embedded in the sediment, and thus are protected from most chemical treatments of aboveground plant mass (Steward 1969; Basiouny, Haller, and Garrard 1978). Tubers are also structurally sound, affording greater resistance to mechanical disturbance, heat loss, and desiccation of stolon meristematic tissues (Steward and Van 1985, Salisbury and Ross 1985).

Ecological studies of growth and tuber formation in *Hydrilla* have focused primarily on response to specific environmental variables. Major consideration has been given to effects of day length, temperature, and sediment fertility. Research to date indicates that short photoperiods induce tuber formation in *Hydrilla* (Haller 1976; Haller, Miller, and Garrard 1976; Van, Haller, and Bowes 1978; Van, Haller, and Garrard 1978; Bowes, Holaday, and Haller 1979), and that under short-day conditions, tuber formation increases with increased biomass and water temperatures up to about 33° C (Van, Haller, and Garrard 1978). Reductions in

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both growth and tuberization due to inadequate sediment fertility have been demonstrated in studies of Van and Haller (1979), Bruner and Batterson (1984), Barko and Smart (1986), and McFarland and Barko (1987). Whereas day length, temperature, and sediment fertility have all been shown to have strong independent effects on growth and tuber formation in *Hydrilla*, it is possible that, in nature, interactions among these variables may significantly modify plant response.

Based on investigations conducted under controlled greenhouse conditions, this article examines effects of temperature, day length, and their interaction on growth and tuber formation in dioecious *Hydrilla*. Responses are contrasted across two sediments differing in texture and nutrient availability. Assessments here are intended to further the knowledge of processes regulating growth and perennation of *Hydrilla* in different habitats and, ultimately, to contribute to the advancement of practices in aquatic plant management.

METHODS AND MATERIALS

The research was conducted in two 10-week phases in a greenhouse facility at the Waterways Experiment Station (WES), Vicksburg, Mississippi (Barko and Smart 1981). At this location, 32°23′ N, 90°52′ W, the initial short-day phase (November-January) provided an approximate 10-hr daylight exposure; the subsequent long-day phase (May-July) allowed a daylight exposure of about 14 hr (List 1951).

In both phases, three separate 1,200-ℓ white fiberglass tanks were used to provide incremental (5°) differences in experimental temperatures between 20° and 30° C. The tanks were filled 83 cm deep with the culture solution described in Smart and Barko (1985). The solution was continuously circulated and thermally controlled (±1° C) by liquid circulators connected independently to each tank.

The sediment used in this study was collected from Brown's Lake, WES; it was a fine-textured, inorganic substrate (characterized in Barko and Smart 1986) with particle size fractions of 5 percent coarse (>50-μ diam) and 95 percent fine (≤50-μ diam) by dry mass. At the beginning of each phase, the sediment was mixed thoroughly and divided into two portions. One of these was amended with washed builders' sand, an infertile addition that resulted in a 22 percent coarse, 78 percent fine particle size distribution. The other was amended with ammonium chloride (30.8 mg N ℓ^{-1}) to ensure sufficient nitrogen availability (Barko, unpublished data) to support unlimited *Hydrilla* growth during the 10-week study period. Sediment treatments were replicated in 2-ℓ polyethylene containers, six times per tank; each container provided a sediment depth of 15 cm, a surface area of 145 cm^2, and a sediment volume of 1,700 ml.

Dioecious *Hydrilla* used in the study was obtained from the WES laboratory stock established from earlier collections made in Lake Seminole, Florida. Four

12-cm-long apical cuttings were planted to a depth of 5 cm per container. A thin layer of clean sand was placed on the sediment surface to minimize particle disturbance and nutrient diffusion into the water column (Smart and Barko 1985). When planting was completed, neutral-density shade fabric was positioned over the tanks, reducing ambient irradiance levels by 33 percent. Midday photosynthetically active radiation inside the tanks measured about 1,000 and 600 $\mu E\ m^{-2}\ sec^{-1}$ during long and short days, respectively.

After 10 weeks of growth in each study phase, aboveground and belowground plant structures were harvested, oven-dried (at 80° C), and weighed. Evaluations of *Hydrilla* growth were based on measurements of total biomass (roots and shoots), with differentiation of tuber contributions to root mass. Effects of treatments on tuber number were evaluated by direct counting. All data were analyzed statistically using analysis of variance (ANOVA) procedures of the Statistical Analysis System (Raleigh, North Carolina). Hereafter, statements of statistical significance refer to probability levels of 5 percent or less.

RESULTS

The significance of temperature and day length effects on *Hydrilla* growth and tuber formation is examined statistically across sediments in Table 1. From these

Table 1
Two-Way ANOVA of Growth and Tuber Formation Responses in *Hydrilla* Relative to Temperature and Day Length on Different Sediments

		Sediment			
Plant	*Environmental*	*N-Amended*		*Sand-Amended*	
Response	*Variable*	*P*	*F Value*	*P*	*F Value*
Total biomass	Temperature	<0.001	21	<0.001	24
	Day length	<0.001	202	<0.01	11
	T × DL*	NS**	3	NS	2
Root:shoot	Temperature	<0.05	4	<0.001	11
	Day length	NS	<1	<0.05	5
	T × DL	NS	2	NS	<1
Tuber number	Temperature	<0.05	5	NS	2
	Day length	<0.05	6	<0.01	11
	T × DL	<0.001	11	<0.01	7
Tuber mass	Temperature	NS	<1	NS	<1
	Day length	NS	1	NS	3
	T × DL	<0.05	4	<0.01	6

*Interaction of temperature and day length.
**Not significant at probability level of 5 percent.

data, it is apparent that independent variables generally affected growth responses more than did their interactions. However, tuber formation (mass and number) was significantly influenced by the interaction of temperature and day length. Sediment type, while influencing biomass production, had no significant effects on

tuber formation in this study. Inclusion of sediment effects in a three-way ANOVA did not alter these results.

Total biomass and root-to-shoot ratio

Total biomass in *Hydrilla* was greater under long-day than short-day conditions (Figure 1). The effect of day length was pronounced on the fertile N-amended sediment where, under short days, growth was apparently limited by reduced (10-hr) intervals of daylight exposure. Patterns of biomass production in relation to temperature were similar under both conditions of photoperiod. Biomass increased with increasing temperature to at least 25° C, with maximum production occurring at 30° C. Under both day lengths, root-to-shoot ratios declined somewhat with increasing temperature. Although these ratios were generally higher on the sand-amended sediment, significant differences due to sediment type occurred under long days at 20° C.

Tuber number and tuber mass

Interactive effects of temperature and day length elicited opposing responses in *Hydrilla* tuber production (Figure 2). Under short days, tuber formation was inhibited at 20° C. Above 20° C, tuber mass and number increased with increasing temperature up to 30° C. Conversely, no tubers were formed at 30° C under long days, but tuber production increased with decreasing temperature down to 20° C. The effect of day length on tuber number was marked under short days, where, over all temperature and sediment conditions, short-day tuber number was about 4 times greater than long-day tuber number. Although day length effected only minor differences in tuber mass (i.e., total tuber mass), individual tuber mass was substantially reduced under short days. Based on means pooled for tuber-producing replicates, the mean mass per tuber formed during long days was 147.9 ± 42.0 mg (mean and standard error, n = 8); the comparable short-day mean was 24.9 ± 0.3 mg per propagule (mean and standard error, n = 17), reflecting a sixfold difference in mass between day lengths.

DISCUSSION

Results of this study indicate that temperature and day length strongly influence growth and tuber formation in *Hydrilla*. Observations here support results of previous studies that have examined independent effects of these variables on submersed macrophytes (Van, Haller, and Garrard 1978; Barko and Smart 1981; Barko, Hardin, and Matthews 1982; Spencer and Anderson 1986; McFarland and Barko 1987; Steward and Van 1987). Unique to this study, however, is the finding that under short versus long days, tuber formation was stimulated mostly at high and moderately low temperatures, respectively. These differences in response represent a significant interaction between environmental variables.

Although the numbers of tubers produced during long days were fewer, the masses of individual tubers were greater than those produced under short days.

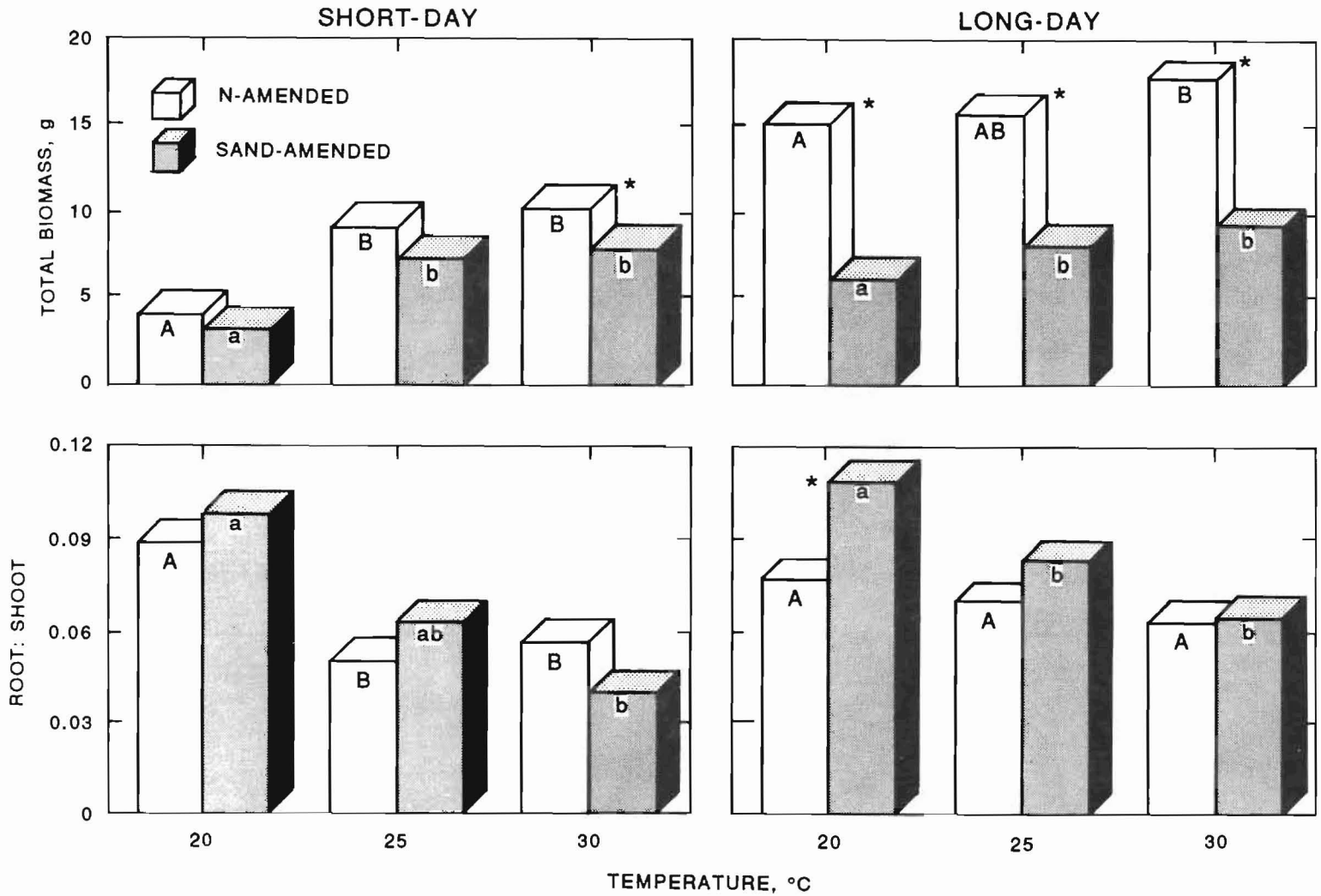

Figure 1. Effects of temperature and sediment type on growth of *Hydrilla* under long- and short-day conditions. Within each subfigure, biomass values or root-to-shoot ratios sharing the same letter (upper case for N-amended sediment and lower case for sand-amended sediment) do not differ significantly from each other. Asterisks denote significant effects of sediment type on growth. Duncan's Multiple Range Test was used to determine statistical significance at $P < 0.05$

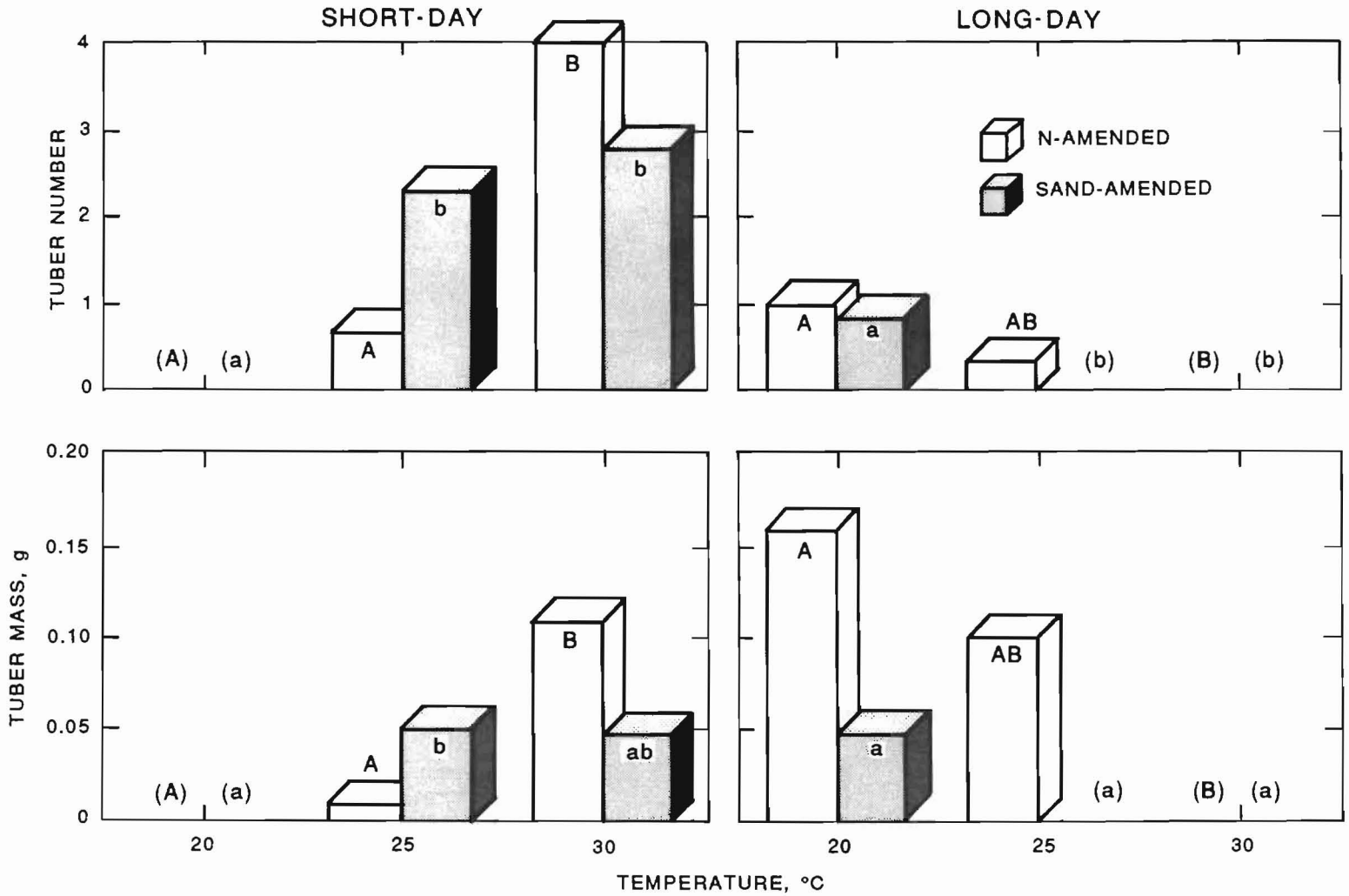

Figure 2. Effects of temperature and sediment type on tuber formation in *Hydrilla*. Within each subfigure, tuber numbers or tuber masses sharing the same letter (upper case for N-amended sediment and lower case for sand-amended sediment) do not differ significantly from each other. Asterisks denote significant effects of sediment type on tuber formation. Duncan's Multiple Range Test was used to determine statistical significance at $P < 0.05$

Studies of Haller, Miller, and Garrard (1976), Schaffer and Gadgil (1980), and Spencer (1986) report data indicating the importance of propagule size on germination, competitive success, and growth of several plant species, including *Hydrilla*. In view of their findings, the increased mass of long-day tubers documented in our study may contribute to the longevity and postgermination vigor of these propagules.

Tuber formation in *Hydrilla* generally occurs under conditions of short photoperiod (fall through early spring) (Haller 1976; Haller, Miller, and Garrard 1976). However, due to the influence of temperature on response to day length, tuber production may extend into the summer in cool-water systems. This may explain in part the year-round growth of *Hydrilla* in some Florida springs (Haller, Miller, and Garrard 1976). Further research of the occurrence and mass-related vigor of long-day propagules would be beneficial in assessing survival strategies and in evaluating control methodologies for specific habitats as well.

As in past investigations (Barko and Smart 1985, 1986), total biomass was relatively diminished due to nutrient limitation of the sandy sediment. Yet, despite sediment conditions causing significant decreases in biomass production, tuber production was not significantly affected. Because tuber formation appears to be less sensitive to sediment fertility than biomass responses, sediment composition may be a better indicator of *Hydrilla* production potential than tuber formation.

ACKNOWLEDGMENTS

The technical assistance of M. S. Hennington and R. Warren are gratefully acknowledged. Appreciation is also extended to R. M. Smart, M. C. Bruner, and T. R. Batterson for their contributions to experimental design. Financial support was provided by the US Army Corps of Engineers Aquatic Plant Control Research Program.

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Effects of Benthic Barriers on Substratum Conditions: An Initial Report

by
Douglas Gunnison* and John W. Barko*

INTRODUCTION

Benthic barrier mats provide a means to physically limit the occurrence of nuisance growths of aquatic plants.** By shutting off light to the substrate surface, benthic barriers shade the plant from incoming solar radiation and thereby exert control. Benthic barriers are attractive for use since they can be deployed once and left for the entire growing season, without need for repetitive efforts. However, before advocating widespread use of benthic barriers for Corps water resources projects, it is important to evaluate the effectiveness and environmental consequences of barrier placement. While the positive and negative aspects of barrier use are generally understood,**,† the impact of barrier use on the aquatic environment is essentially unknown.

Benthic barriers are purported to be gas permeable; that is, some degree of diffusion of gases through the barrier fabric is possible. This property is desirable to (a) allow gases released during plant decomposition and sediment metabolism to escape from beneath the fabric and (b) permit continuous renewal of dissolved oxygen levels at the sediment surface. The latter process is essential to permit survival of benthic fauna beneath the barriers.

This work was initiated in July 1988 to provide quantitative information on the effectiveness and environmental effects of benthic barriers. The specific objective of the study reported here was to characterize the effects of barrier placement on the volume and composition of gases evolved from sediment, the interstitial water chemistry profiles in sediment, and the population density and species composition of benthic fauna.

METHODS AND MATERIALS

The Eau Galle Reservoir field site (Spring Valley, Wisconsin) was selected for the 1988 work for two reasons. First, to maximize the work that could be accomplished in fiscal year (FY) 1988 while allowing for the late start of the

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

**G. D. Cooke. 1986. "Sediment Surface Covers for Macrophyte Control," *Lake and Reservoir Restoration*, G. D. Cooke et al., eds., Butterworth Publishers, Stoneham, Massachusetts, pp 349-360.

†S. Engel. 1984. "Evaluation Stationary Blankets and Removable Screens for Macrophyte Control in Lakes," *Journal of Aquatic Plant Management*, Vol 22, pp 43-48.

project (June 1988), it was important to initiate work in an area having an ongoing research program. Second, Eau Galle Reservoir is typical of lakes in the region in terms of sediment type, trophic status, and nuisance growths of submersed aquatic plants.

Four benthic barrier mats, each having dimensions of 7 × 10 ft, were equipped with three funnels evenly spaced along the center of the mat. Two of the mats were placed in parallel in vegetated areas of the reservoir dominated by *Ceratophyllum*. The depth of the overlying water in this area was approximately 1 m. The remaining two mats were deployed in parallel in unvegetated areas at a depth of approximately 2 m. Barriers were held down by rebars along the longer (10-ft) sides and weighted with bricks placed at intervals over the barrier surfaces.

The funnels on each mat were attached to a gas collection system that terminated with a removable gas sampling bottle. Gas collection bottles were removed as required, upon becoming filled with gas, and were replaced with fresh units. The bottles were immediately returned to the lab for analysis of gas composition.

Plexiglas sediment interstitial water samplers were deployed in the area of each mat to provide data on chemical profiles in sediment interstitial water at the initiation of the study. Interstitial water samplers were removed 10 days following study initiation, and new interstitial water samplers were deployed at 2-week intervals thereafter. Toward the end of the study (after 2 months), interstitial water samplers were placed into sediment directly beneath each mat.

To determine the effect of barrier mats upon benthic fauna, surveys were made of the species composition and density of the benthic animal community occurring around and under each mat (benthic invertebrate data are not available for reporting in this paper).

RESULTS AND DISCUSSION

Barrier placement at the vegetated site was followed almost immediately by release of large quantities of gases; these caused the barriers to billow up noticeably within the first 3 days and resulted in a significant, but partial, movement of one of the two barriers from its original location. Initial volumes of gases released at the vegetated sites were substantially larger than could be contained within the sampling bottles. Based on observations of volumes of gas trapped under the mats, the gas volumes lost were roughly triple those actually collected. Following the first week of barrier placement, the rate of gas production at the vegetated sites diminished sharply. Gas collection systems at the unvegetated sites contained no visible gas after 3 days of placement and only minor amounts of gas when finally removed at 8 weeks. Placement of barriers over existing plant beds during warm summer conditions at Eau Galle Lake clearly

resulted in the formation of large volumes of gas as a result of plant decomposition. These large gas volumes were not produced in unvegetated areas.

Initial gas samples from vegetated sites contained oxygen, nitrogen, and trace levels of carbon dioxide and methane. Samples taken from vegetated sites after 8 weeks contained primarily methane and carbon dioxide, with small amounts of nitrogen and no oxygen. The gases trapped initially indicated decomposition occurring under aerobic conditions, while the gases trapped finally were those anticipated during anaerobic decomposition. In contrast with samples from vegetated sites, gas samples from unvegetated sites were released very slowly over the 8-week study period and contained methane, carbon dioxide, nitrogen, and trace levels of oxygen.

Analyses of sediment interstitial water data revealed no effects of barrier placement on chemistry of the interstitial water at either the vegetated or unvegetated sites. However, there were differences between vegetated and unvegetated sites. These included elevated levels of ammonium-nitrogen, orthophosphate phosphorus, ferrous iron, and manganous manganese in interstitial waters of vegetated sites relative to levels of these constituents in unvegetated sites.

The FY 1988 study revealed definite differences in the volumes of gases evolved beneath benthic barriers placed at vegetated and unvegetated locations. The volume of gases produced at vegetated sites was large enough to lift the barriers away from the substrate. Buoyancy resulting from gases produced during decomposition appears to have negative consequences with respect to barrier effectiveness over the short to medium term (1 week to 2 months). The long-term importance of this process is unknown, but will be addressed in future investigations in the field.

FUTURE STUDY

Several facets of the influence of benthic barriers on gas production will be examined during FY 1989 under controlled conditions in the laboratory. These include examination of (a) the rates of gas evolution resulting from decomposition of various types of organic matter, (b) the influence of changes in types of plant material and sediment on the rate and extent of decomposition and the nature of gases produced, (c) the effects of temperature on the rate and extent of decomposition and the nature of gases produced (we know increased temperatures increase rates, but by how much?), (d) the effect of biofouling of barrier surfaces on permeability of barriers to gases and barrier effectiveness, and (e) the effects of gas production on benthic fauna. All of these areas will be examined prior to undertaking additional fieldwork.

Glucosinolates and Phenolics in Aquatic Macrophytes: Implications for Allelopathy Studies and Suggested Practical Uses for Metabolic Blocking Agents

by
W. Charles Kerfoot*

INTRODUCTION

Survey investigations in Michigan have revealed (a) that noxious or unpalatable compounds are widespread and diverse among shoreline emergents in families known for their terrestrial anti-insect compounds, (b) that rooted, floating macrophytes, especially several genera of lily pads (e.g., *Nymphaea*, *Brasenia*, *Nuphar*), contain high concentrations of phenolics in addition to alkaloids, and (c) that submersed macrophytes have greatly reduced levels of both phenolics and alkaloids, although certain compounds (e.g., ferulic acid, anthocyanins) are retained in modest concentrations. This past summer, we investigated whether anti-insect compounds were effective against aquatic invertebrates, and the relationship between phenolic and "allelopathy" bioassay results. Additionally, we explored the use of metabolic blockage techniques. The latter protocol has both basic and applied promise.

EMERGENTS: INSECT PROTECTION EXTENDED TO CRUSTACEANS

A clear example of noxious substances released upon tissue damage is illustrated by a member of the Cruciferae, the common watercress (Figure 1) (Newman and Kerfoot 1988; Newman, Kerfoot, and Hanscom, in press). Like other crucifers, watercress (*Nasturtium aquaticum*) contains the glucosinolate gluconasturtin, a glucoside of a phenyl-ethyl mustard oil (Schultz and Gmelin 1952). When tissue is damaged by insects, the glucosinolate of watercress will undergo hydrolysis, mediated by myrosinases, to form two principal components: a phenylethylisothiocyanate (2-phenylethyl isothiocyanate) and a hydrocinnamonitrile (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). The 2-phenylethyl isothiocyanate is considered the predominant flavor component and imparts the characteristic "hot" taste of watercress (Freeman and Mossadeghi 1972).

Glucosinolides are recognized to protect mustards against insect damage (Louda and Rodman 1983). We investigated whether the same protective function carries

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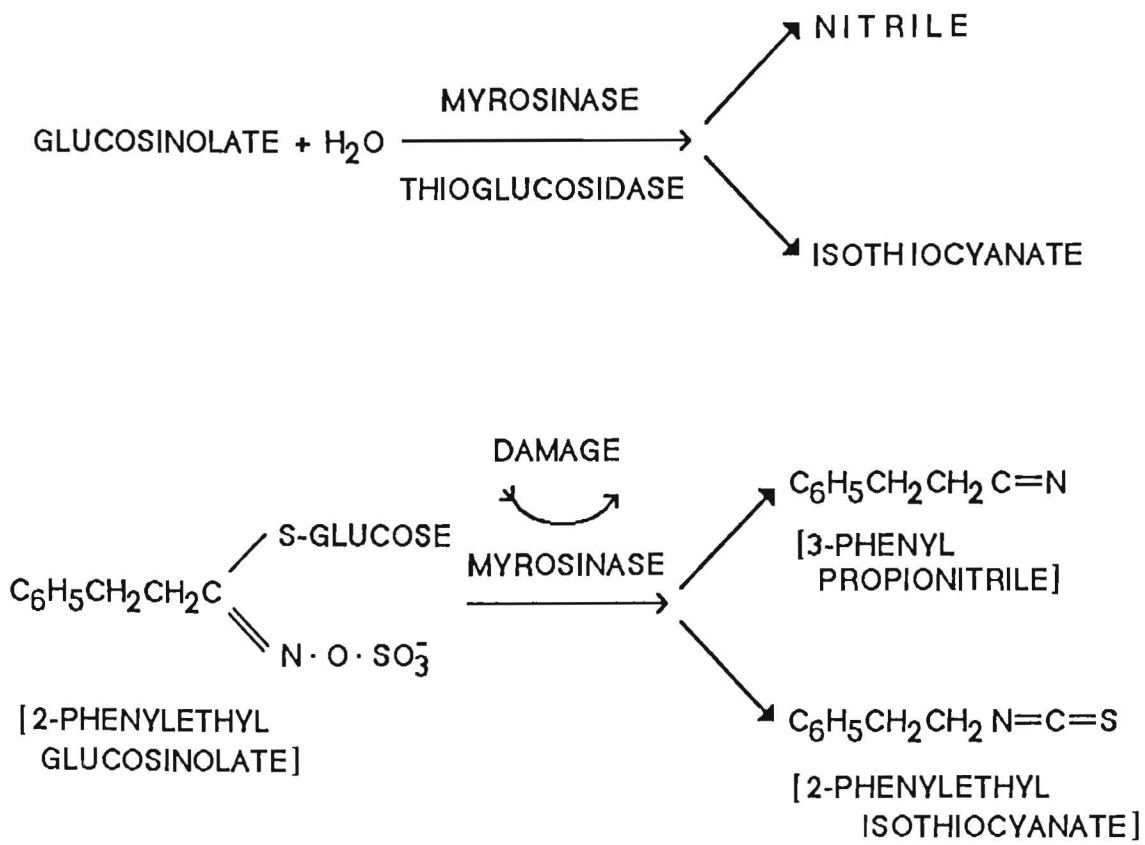


Figure 1. Glucosinolate reactions in watercress (*Nasturtium officinale* R.Br.) (Synonym: *Rorippa nasturtium-aquaticum* (L.) Hayek)

over to arthropods in general, more specifically to *Gammarus pseudolimnaeus*, an amphipod that frequently lives among the plant's roots. *Gammarus* is an important stream detritivore that consumes senescing or decomposing leaves. Tests involved determination of tissue food quality (Perkin-Elmer 24000 CHN Total Elemental Analyzer) for green and yellow leaves; feeding choice tests (standard leaf disk assay, 105-mm^2 plug, 100-mm petri dish); measurement of glucosinolide concentration in leaf tissue (Blua and Hanscom 1986); and determination of compound toxicity (LC_{50} values, using authentic compounds).

Tests conducted at both Michigan and Connecticut sites showed that *Gammarus* strongly preferred yellowed over green leaves (ca. 5× more consumption), despite the higher nitrogen content of the latter (2.7 versus 5.4%), and that green leaves contained enough glucosinolate that crushed or frozen fresh leaves would kill amphipods. *Gammarus* was found sensitive to both hydrocinnamonitrile and phenylethylisothiocyanate, especially to the latter (e.g., 48 hr LC_{50} of 3.6 mg/ℓ) (Newman, Kerfoot, and Hanscom, in press). Senescing and decomposed leaves contained much lower levels of glucosinolides, explaining the higher palatability of these leaves.

PHENOLICS: CORRESPONDENCE BETWEEN SURVEY VALUES

Surveys of total soluble phenolics in macrophytes, based on Folin-Denis assays, emphasize the J-shaped nature of incidence (Figure 2). That is, only a relatively

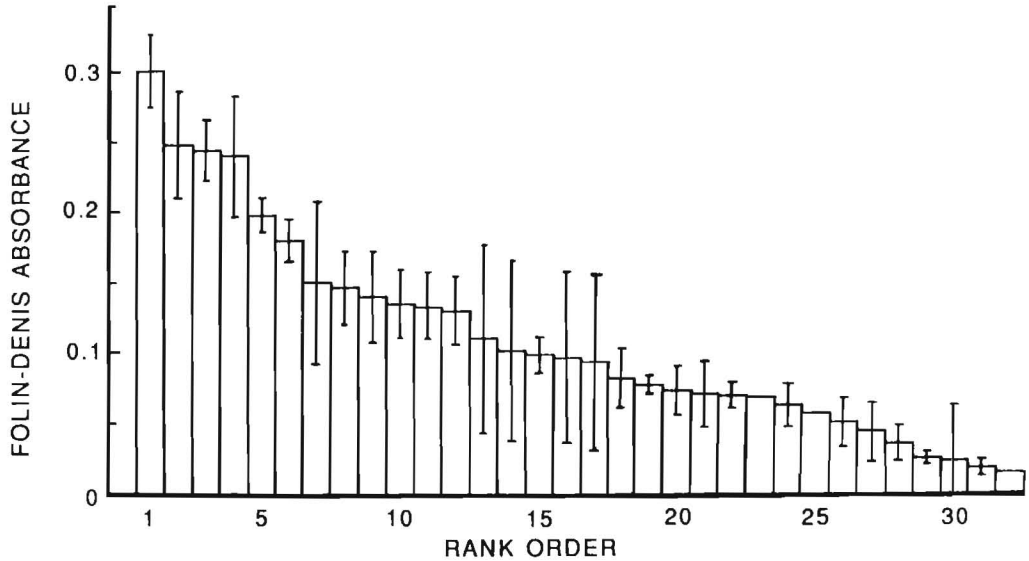


Figure 2. Distribution of total phenolics (aqueous extract). Folin-Denis absorbance values of surveyed Michigan macrophytes arranged by decreasing value. Vertical lines mark 95-percent confidence intervals. (Numbers correspond to taxa listed in Table 1)

few taxa have highly elevated concentrations, whereas a great many have modest concentrations. The highest phenolic values among profiles coincided with the presence of tannins, especially hydrolyzable tannins. Highest yields were for floating, rooted macrophytes, especially species of lily pads (e.g., *Nymphaeae*, *Brasenia*, *Nuphar*), although other high-scoring dicot genera (*Ludwigia*, certain *Myriophyllum*) also gave positive indications of hydrolyzable tannins. In contrast, lowest values were found in fully submersed genera and species (e.g., *Najas*, *Elodea*, *Ceratophyllum*, *Hydrilla*, *Egeria*, *Vallisneria*). Even the exceptions among submersed and marginal emergent categories reinforced 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).

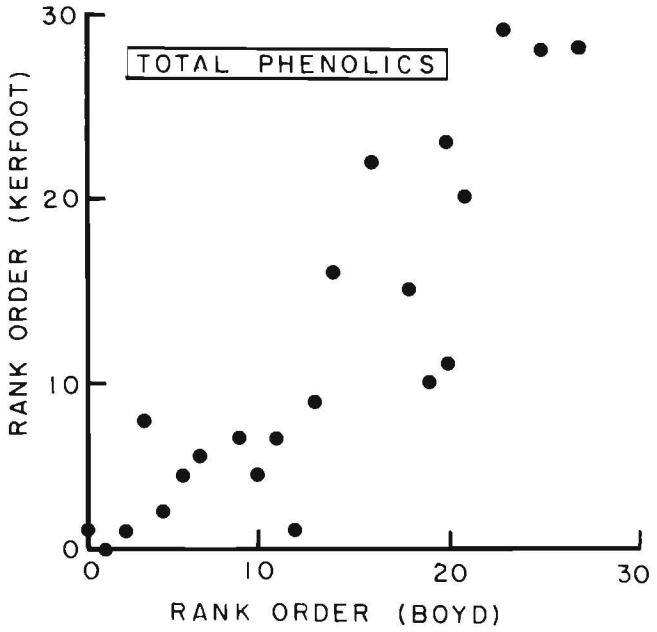
Correlation between the Michigan survey and the only previously available survey, i.e., the southeastern USA survey of Boyd (1968) was high, when the rank order of comparable species was plotted (Figure 3a, Table 2). Thus, the two surveys were in general agreement.

Table 1
Rank Order for Total Phenolics (Folin-Denis Assay). Number of Different Plants (N) and Standard Deviations (SD) Are Given

Rank	*Species*	*N*	*Folin-Denis Absorbance (SD)*
1	*Nymphaea odorata*	44	0.302 (0.087)
2	*Ludwigia palustris*	13	0.249 (0.065)
3	*Brasenia schreberi*	33	0.245 (0.061)
4	*Decodon verticillatus*	10	0.241 (0.061)
5	*Nuphar variegata* (fl)	56	0.198 (0.048)
6	*Potamogeton natans*	14	0.180 (0.026)
7	*Alisma* sp.	5	0.150 (0.050)
8	*Typha latifolia*	7	0.147 (0.047)
9	*Polygonum hydropiperoides*	9	0.140 (0.041)
10	*Typha angustifolia*	8	0.135 (0.028)
11	*Sparganium angustifolium*	14	0.133 (0.041)
12	*Eleocharis smallii*	13	0.130 (0.061)
13	*Carex* sp.	4	0.111 (0.041)
14	*Iris* sp.	2	0.102 (0.007)
15	*Potamogeton nodosus* (fl)	6	0.099 (0.013)
16	*Carex* sp.	4	0.097 (0.037)
17	*Myriophyllum verticillatum*	6	0.094 (0.060)
18	*Potamogeton filiformis*	6	0.083 (0.020)
19	*Eichhornia crassipes*	5	0.078 (0.004)
20	*Ceratophyllum demersum*	7	0.073 (0.020)
21	*Lemna* sp.	6	0.072 (0.023)
22	*Sagittaria latifolia*	10	0.071 (0.014)
23	*Pontedaria*	1	--
24	*Carex* sp.	7	0.064 (0.017)
24	*Nasturtium aquaticum*	2	0.058 (0.011)
25	*Potamogeton richardsoni*	1	0.058
26	*Carex* sp.	5	0.052 (0.013)
27	*Equisetum* sp.	7	0.046 (0.020)
28	*Scirpus* sp.	4	0.037 (0.007)
29	*Elodea canadensis*	10	0.027 (0.005)
30	*Utricularia* sp.	2	0.025 (0.004)
31	*Chara* sp.	5	0.020 (0.004)
31	*Hippurus*	7	0.020 (0.010)
32	*Najas*	1	0.017

Note: Samples run on leaves only.

The presence of hydrolyzable tannins in *Nymphaea*, *Nuphar*, and certain *Myriophyllum* is consistent with earlier, scattered reports. Bate-Smith (1962, 1968, 1974) and Bate-Smith and Metcalfe (1957) reported tannic substances from the leaves and roots of *Nymphaea* and *Nuphar* and noted that tannins were abundant enough in rhizomes of *Nymphaea* for local use as tanning sources (Howes 1953). In their preliminary surveys, Su, Abul-Hajj, and Staba (1973) also reported evidence for tannins in *Nymphaea* and *Nuphar* tissues and identified three compounds in hydrolyzed tissue: tannic acid, gallic acid, and ethyl gallate. The first two probably represent intermediate and final breakdown products of gallotannins. Planas et al. (1981) reported ellagic, gallic, and tannic acids as among the most abundant of 18 identified phenolic compounds from *Myriophyllum spicatum*, suggesting that this macrophyte was rich in hydrolyzable tannins. Again, the positive tests for tannic acid probably record intermediate breakdown products of gallotannins during hydrolysis.



a. Michigan survey and Boyd's (1968) southeastern macrophyte survey

b. Boyd's (1968) survey of total phenolics against Elakovich and Wooten's (in preparation) "allelopathy" bioassay scores (*Lemna* target, see Table 2)

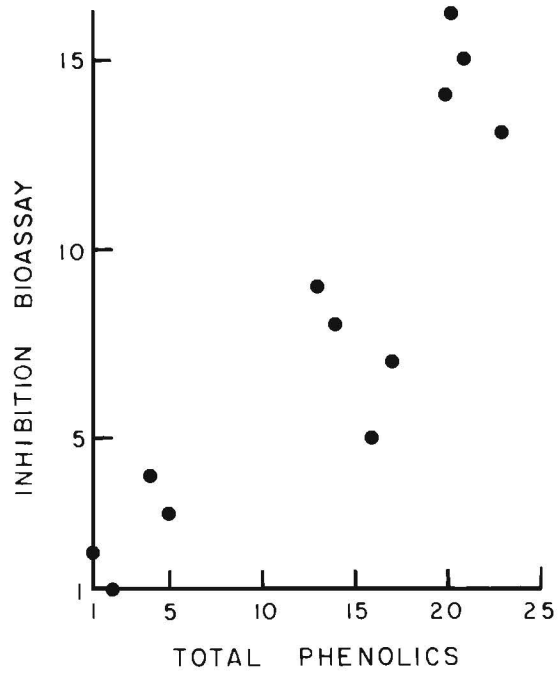


Figure 3. Rank order correlations between surveys

Table 2
Spearman Rank Order Correlation Coefficients Between Boyd (1968) and Elakovich-Wooten (E-W) Assays. (The Spearman rank order between the Boyd (1968) and Kerfoot phenolic assays is 0.892)

Survey	*Boyd*	*E-W (Set 1)*	*E-W (Set 2)*
Boyd	1.000		
E-W (Set 1)	-0.898	1.000	
E-W (Set 2)	-0.731	0.928	1.000

Note: Boyd measured total phenolics as percent ash-free dry weight, whereas Elakovich-Wooten recorded suppression of *Lemna* by crude extracts (set 1, chlorophyll-a reduction; set 2, frond growth inhibition) (mean of two values used; data from Elakovich and Wooten, in preparation).

At least three possible contributing factors could explain the elevation of phenolics in floating macrophytes and the observed major decline in submersed species: (a) higher food value and exposure of floating macrophytes to insect herbivory versus escape of submersed taxa, (b) reduction of deleterious ultraviolet (UV) radiation levels for submersed taxa, or (c) progressive elimination of phenolic pathways associated with loss of lignocellulose (Kerfoot 1988a,b). Phenolics may be retained in certain submersed species because of advantageous antipathogen/herbivore or "allelopathic" properties.

CORRELATION BETWEEN TOTAL PHENOLIC AND "ALLELOPATHY" BIOASSAYS

Recently, Elakovich and Wooten (1987; in preparation) exposed two types of target plants, terrestrial lettuce seedlings and aquatic *Lemna minor* (duckweed), to aqueous extracts from a variety of southeastern US macrophytes. The *Lemna* experiments considered three measures of inhibition (frond number, dry weight gain, and chlorophyll-a increase) over a period of 7 to 11 days. In these tests, results varied somewhat between variables, yet the five macrophyte extracts that consistently inhibited target growth by at least 60 percent were *Nymphaea odorata* (roots, leaves, and stems), *Myriophyllum aquaticum*, *Brasenia schreberi*, and *Cabomba caroliniana*. These inhibition scores are strongly correlated (rank-order) with total phenolic concentrations reported from either the southeastern survey of Boyd (Figure 3b) or the Michigan survey (Table 2).

Inhibition of plant growth by soluble phenolics is well known and a standard result of Muller type bioassays (e.g., Guenzi and McCalla 1966). However, the relevance of these results to field situations is not clear. Phenolics could function in an inhibitory way only if compounds "leak" from epidermal tissues, possibly suppressing periphyton growth or through root "competition." Otherwise, the

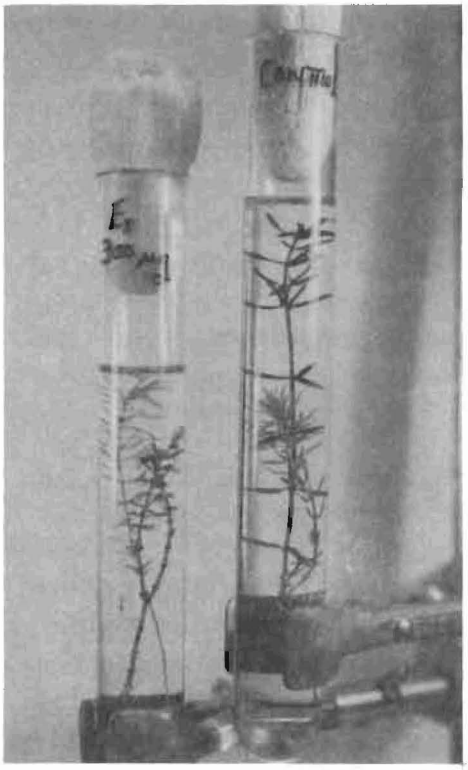
bioassay is only a crude assay for chemical substances held tightly within plant tissues, yet liberated during damage.

METABOLIC BLOCKAGE TECHNIQUE

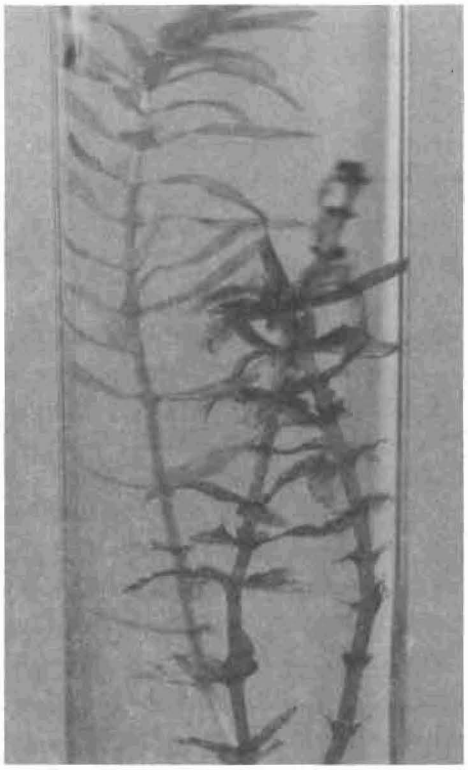
To experimentally evaluate the "macrophyte protection" hypotheses, we initiated preliminary metabolic blockage experiments. These experiments attempted to alter the secondary compounds in macrophyte tissue (*Nasturtium*, *Elodea*, *Hydrilla*) by biochemically blocking the shikimic acid cycle. The resulting tissues and plants could then be used to evaluate alternative hypotheses. As a laboratory technique, this procedure is very promising in submersed aquatic macrophytes because lignin content is reduced or negligible, presumably due to reduction of support constraints, whereas similar application in terrestrial plants usually totally inhibits growth.

At present, the details of phenolic synthesis are surprisingly well known (Conn 1986). Of the various enzyme-specific inhibitors that can be applied to macrophytes (APEP, AOPP, glyphosate; see Kerfoot 1988a,b), glyphosate (N-(phosphonomethyl)glycine) seemed the most selective and useful for initial tests. This nonselective, broad-spectrum, postemergence herbicide agent selectively inhibits 5-enolpyruvylshikimate 3-phosphate synthase by blocking PEP (phosphenolpyruvate) activity (Amrheim 1986). It can be administered in aqueous solution, is mobile in phloem tissue, and is readily translocated to active meristematic areas (Sprankle, Meggitt, and Penner 1975; Haderlie, Sliffe, and Butler 1978; Gougler and Geiger 1981). Preliminary screening tests in the laboratory with low-level concentrations ($<1\ \mu M$) of analytical grade glyphosate (obtained from Monsanto) showed that in monocots with low lignin content, treatment can selectively alter production of anthocyanins (*Hydrilla*), other phenolics (*Elodea*), and glucosinolates (*Nasturtium*). In the case of *Elodea*, nearly normally appearing plants are biochemically quite distinctive, both from a quantitative and qualitative standpoint. Treated plants grow normally, but are paler with slightly thinner stems and greater internode distances. The marked differences, however, are in aqueous and ether extracts of leaf tissue. Phenolic end products are suppressed, as evidenced by loss of brownish pigments and ether-extractable spots on TLC plates (Figure 4). Alteration of phenolic products is consistent with previously reported findings for *Lemna* (Jaworski 1972) and terrestrial plants (e.g., Hoagland and Duke 1982). In the case of *Nasturtium*, clear evidence for differences in grazing by snails was associated with glyphosate treatment (Figure 5).

If anthocyanins, p-coumaric acid, and ferulic acid are among the principal antiherbivore, anti-UV, and antipathogen compounds of monocots, as has been asserted by some (e.g., Rice and Pancholy 1974; Valiela et al. 1979; Woodhead and Cooper-Driver 1979; Buchsbaum, Valiela, and Swain 1984; Valiela and Rietsma 1984), then production of low levels of these compounds in plants and plant tissues will allow direct tests of tissue susceptibility. If, as in the case of



a. *Elodea canadensis* after glyphosate treatment



b. Growing tips appear nearly normal, yet are biochemically quite distinct

Figure 4. Example of metabolic blockage technique

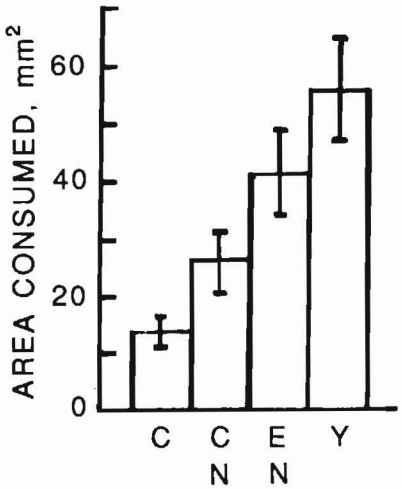


Figure 5. Snail grazing on control, glyphosate-treated (0.6×10^{-6} M), and senescing (yellow) leaves of watercress

Elodea, lignin content is so low that treated plants physically resemble untreated controls, then entire plants offer ideal experimental material.

The design for future experiments (*Nasturtium*, *Elodea*, *Hydrilla*, *Myriophyllum*) is to grow both control and treated plants in the laboratory, to ensure uniformity of environmental effects, and then mix treated and control plants for exposure to various variables (grazers, pathogens, UV radiation, periphyton accumulation). Preliminary results on *Elodea* suggest enhanced susceptibility of fungal infection as one of the important consequences of phenolic blockage.

IMPLICATIONS

Recognition of antipathogen/herbivore compounds in aquatic macrophytes means that these plants could be playing a much more active role in plant-herbivore or plant-pathogen interactions. While the ultimate effects of "allelopathic" substances require much more research, suppression of pathogenic infection, inhibition of periphyton, and resistance to herbivory must be considered prime possibilities, along with root "competition."

Whereas metabolic blocking techniques represent a promising protocol for laboratory experiments, this basic approach has potentially important implications for macrophyte control programs, especially chemical and exotic insect control programs. Rather than killing plants directly, in theory these compounds can operate indirectly by increasing susceptibility to traditional nonlethal agents (pathogens, periphyton, invertebrate grazers, detritivores). Applications would probably complement exotic insect control operations, since plant enzyme-blocking agents often have low animal toxicity (e.g., glyphosate). Yet the use of exotic consumers is not necessary, for if macrophytes routinely utilize diverse compounds for protection against pathogens and herbivores, use of metabolic blocks in the field increases susceptibility to naturally occurring agents. If the protective compounds are specific, then surgical metabolic blockage could selectively target a set of macrophyte species, helping to manage community composition.

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The Habitat Value of Aquatic Macrophytes for Macroinvertebrates: Benthic Studies in Eau Galle Reservoir, Wisconsin

by
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INTRODUCTION

Background

For decades, submersed macrophytes were viewed as nuisances with little functional role in the aquatic ecosystem, and management strategies often included their complete eradication. Although the complex mechanisms by which macrophytes interact with sediments, water, and biological organisms are still not well known, scientists and resource managers are now beginning to understand some of the interactions involving aquatic macrophytes and to recognize their important role in aquatic ecosystems.

Pandit (1984) has stated that freshwater macrophytes have a more dominant influence on their physicochemical environment than do terrestrial plants on their environment. For example, the area of submersed macrophytes in Lawrence Lake, Michigan, provided almost 10 times more surface area for colonization than did the total benthic area of the lake (Losee and Wetzel 1988). The importance and potential impacts of submersed macrophytes on aquatic ecosystems have been summarized in excellent reviews by Seddon (1972), Gregg and Rose (1982, 1985), McDermid and Naiman (1983), Pandit (1984), and Carpenter and Lodge (1986).

Submersed macrophytes are commonly found in both the lotic and lentic habitats of freshwater and estuarine ecosystems. They tend to predominate in small, shallow basins and in the littoral zone of large lakes. Their complex structures create a diversity of microhabitats for colonizing organisms and provide refuge from predators. As integral components of the aquatic community, submersed macrophytes play a major role in the development and modification of physical aspects of their environment, which promotes adaptations and ecological succession in the communities of organisms.

The relationships between submersed macrophyte communities and macroinvertebrates are varied and include complex interactions relating to macrophyte morphology, invertebrate behavior, life cycles, and predator-prey relationships. Invertebrate abundances on submersed macrophytes tend to be related to plant

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morphology and to physicochemical factors. The most common invertebrates colonizing submersed macrophytes include crustaceans, midges, oligochaetes, and gastropods. Of the approximately 3.0 percent of the insects that have adapted to live part or all of their lives in aquatic habitats, many species have adapted to the habitats provided by submersed macrophytes.

In this study at Eau Galle Reservoir, Wisconsin, small open areas in the littoral zone were sampled for macroinvertebrates. Consequently, it was possible to compare infaunal densities in macrophyte beds to open areas of the lake, both within the littoral zone, and to determine if the presence of macrophytes resulted in enhanced invertebrate densities. In addition, the vertical distribution of benthic invertebrates in these habitats was studied. Although the vertical distribution of benthic invertebrates has been a subject of interest for some time, no other studies have been found that reported vertical distribution below macrophyte beds. One of the objectives of this work was to determine whether the presence of macrophytes resulted in a vertical distribution of invertebrates different from that observed in nonvegetated sediments.

Study area

Eau Galle Reservoir is a small, eutrophic, dimictic reservoir constructed by the US Army Corps of Engineers for flood control. Located in west-central Wisconsin, the lake has a surface area of 0.6 km^2, a maximum depth of 9.5 m, and a mean depth of 3.6 m. The lake receives flow from an agricultural drainage area of 166 km^2, with the Eau Galle River contributing 80 to 90 percent of the inflow. Three smaller streams contribute the remaining flow. The littoral zone, as defined by the presence of aquatic macrophytes, makes up about 17 percent of the total surface of the lake (Gunkel, Gaugush, and Kennedy 1984). The principal submersed macrophyte in the lake is *Ceratophyllum demersum*, with the macrophytes *Potamogeton pectinatus*, *P. nodosus*, *P. foliosus*, and *Najas flexilis* also present (Filbin and Barko 1985).

METHODS

On 5 and 6 August 1986, core samples were obtained from three different habitats within the reservoir: (a) from the lake bottom within *C. demersum* beds, (b) from the lake bottom within *P. nodosus* beds, and (c) from small open areas of no vegetation located among the *C. demersum* beds. The small nonvegetated areas are referred to as "no-plant zones." The no-plant zones were small, approximately circular, with a diameter of 2 m or less, and were located amidst dense beds of *Ceratophyllum*.

Five cores were taken from each of the three habitats (*Ceratophyllum* bed, *Potamogeton* bed, and no-plant zone) at each of three locations along the eastern side of the lake. Therefore, 15 cores were taken at each site, with a total of 45 cores taken during the study. To study the vertical distribution of the benthic invertebrates, each core was separated into three depth fractions (0 to 5 cm, 5 to

10 cm, and 10 to 15 cm) as soon as the bottom sample was extruded from the corer. Each of the depth fractions from each core was preserved and processed separately in the laboratory.

Samples were collected from a boat using a hand-held benthic corer constructed from polyvinyl chloride pipe. This corer, described by Miller and Bingham (1987), has an internal diameter of 2 in. and uses a plumber's pipe test plug to exclude water, aquatic plants, and any other materials from entering the corer before the sample is taken. This corer has several utilitarian features. Because it is stiff-bodied and hand-held, it can be placed exactly on the bottom at a desired position. The exclusion of any water, aquatic plants, or invertebrates from entering the sampler prior to sample collection is important. This prevents infaunal samples from being "contaminated" by epiphytic invertebrates.

All cores were taken in shallow water at depths of approximately 1 m. The lake bottom sampled in the no-plant zones and the *Ceratophyllum* beds was clearly visible from the boat (vision of the lake bottom in the *Potamogeton* beds was blocked by floating leaves of *Potamogeton*). While sampling the infauna within the macrophyte beds, the corer was lowered slowly and carefully to keep from "knocking" invertebrates off the plants onto the lake bottom. Likewise, the sampler was lowered slowly to minimize sampler "blowout."

The core samples were processed using a US Standard No. 35 sieve (openings = 500 μ). The samples were stained with a rose bengal solution (Mason and Yevich 1967) to facilitate the separation of invertebrates from vegetation, detritus, and any remaining sediment. Invertebrates were picked under 6× to 12× magnification. Larval chironomids and oligochaetes were prepared for identification using the procedure of Beckett and Lewis (1982).

RESULTS

The bottom fauna of Eau Galle Lake is typical of the benthic invertebrates found in most lakes. Fine-grained sediments (silts and clays) that typically comprise the substrate in slack-water habitats are usually numerically dominated by chironomid larvae, oligochaetes, and molluscs (McCall and Tevesz 1982). In Eau Galle Reservoir, tubificid oligochaetes made up 20 percent of the invertebrates below the *Ceratophyllum* beds, whereas chironomid larvae made up 9 percent and molluscs 39 percent of the benthic organisms. Similarly, in the *Potamogeton* beds, tubificid oligochaetes made up 39 percent of the infauna, with chironomid larvae contributing 11 percent and molluscs 17 percent of the fauna. In the no-plant zones, the dominant taxa were tubificids (33 percent), chironomid larvae (29 percent), and molluscs (4 percent).

Cores from the *Ceratophyllum* beds consistently had higher numbers of organisms than those from the *Potamogeton* beds or from no-plant zones. Among the nine estimations of mean number of invertebrates per square metre of bottom (three habitats with three replicated sites each), all three of the highest means

(40,556 invertebrates/m^2; 39,174 invertebrates/m^2; and 26,050 invertebrates/m^2) came from the *Ceratophyllum* bed (Table 1). Invertebrate densities estimated for the infauna below the three *Potamogeton* beds were quite similar (16,183 invertebrates/m^2; 19,833 invertebrates/m^2; and 19,143 invertebrates/m^2) and were, in turn, all lower than the three means for the infauna in the *Ceratophyllum* beds, and much higher than the means for the infauna in the no-plant zones. Invertebrate densities in the no-plant zones were very low (grand mean in the no-plant zones equaled 2,730 invertebrates/m^2) in comparison to the infaunal densities

Table 1
Mean Number of Invertebrates (Individuals/m^2) in Three Sediment-Depth Fractions from *Ceratophyllum* and *Potamogeton* Beds and from "No-Plant" Zones in Eau Galle Reservoir, Wisconsin

	Site			
Depth, cm	*A*	*B*	*C*	*Grand Mean*
		***Ceratophyllum* Bed**		
0-5	38,681 (95.4)*	38,582 (98.5)	22,695 (87.1)	33,320 (194.5)
5-10	1,480 (3.6)	493 (1.3)	3,256 (12.5)	1,743 (4.9)
10-15	395 (1.0)	99 (0.3)	99 (0.4)	197 (0.6)
Total	40,556	39,174	26,050	35,260
		***Potamogeton* Bed**		
0-5	15,788 (97.6)	18,748 (94.5)	16,972 (88.7)	17,170 (93.4)
5-10	395 (2.4)	592 (3.0)	1,974 (10.3)	987 (5.4)
10-15	0 (0.0)	493 (2.5)	197 (1.0)	230 (1.2)
Total	16,183	19,833	19,143	18,387
		No-Plant Areas		
0-5	2,862 (87.9)	2,862 (87.9)	1,677 (100.0)	2,467 (90.4)
5-10	296 (9.1)	197 (6.0)	0 (0.0)	164 (6.0)
10-15	99 (3.0)	197 (6.0)	0 (0.0)	98 (3.6)
Total	3,257	3,256	1,677	2,730

Note: Five cores were taken from each of three sites (A, B, and C) from each plant bed and the no-plant zone.

*Number inside parentheses indicates percentage of invertebrates in that depth fraction as compared to the total number of invertebrates collected from all three depth fractions (0-15 cm).

present below the macrophytes. A comparison of the grand means of total invertebrate densities among the three habitats shows that mean infaunal invertebrate densities in the *Ceratophyllum* beds were approximately 13 times those of the no-plant zones. Similarly, infaunal densities in *Potamogeton* beds were approximately seven times the densities of the no-plant zones.

The striking difference in infaunal densities between vegetated and nonvegetated habitats can be seen clearly among the common benthic invertebrates. The most commonly collected oligochaete taxon in all three habitats, "immature tubificids without capilliform chaetae," was seven times more common in the sediments below *Ceratophyllum* than in the no-plant zones. These were found below *Potamogeton* beds at over nine times their densities in the no-plant zones. The gastropod *Amnicola limnosa* was very abundant in the lake bottom below *Ceratophyllum*, fairly abundant in the bottom in the *Potamogeton* beds, and much less common in the no-plant zones. Density of *A. limnosa* in the bottom of the lake below *Ceratophyllum* was approximately 162 times that found in no-plant zones. Density of *A. limnosa* in *Potamogeton* beds was about 39 times that of the no-plant zones. Another gastropod, *Gyraulus parvus*, was also much more abundant in cores from vegetated areas than in the no-plant zones.

Sediments immediately below the macrophytes also supported appreciably higher numbers of species than did the nonvegetated areas. The cores from the *Ceratophyllum* beds produced a total of 44 taxa, with 45 taxa present in the samples from the *Potamogeton* bed. In contrast, cores in the no-plant zones produced less than half the number of species found in the vegetated areas. Only 18 distinct species were present in the cores from the no-plant zones.

The overwhelming majority of invertebrates present in the no-plant zones were found near the sediment surface. In the no-plant zones, a mean of 90.4 percent of all the collected invertebrates were present within the top (0- to 5-cm) depth fraction (Table 1). Six percent of the invertebrates in the no-plant zones were found in the 5- to 10-cm depth fraction, whereas only 3.6 percent of the invertebrates collected were in the 10- to 15-cm depth fraction.

The presence of *C. demersum* or *P. nodosus* did not appear to alter the vertical distribution pattern of littoral-zone invertebrates beyond that exhibited in the no-plant zones. As in the no-plant zones, over 90 percent of the invertebrates collected below the *Ceratophyllum* and *Potamogeton* were in the 0- to 5-cm depth fraction (94.5 percent in the *Ceratophyllum* beds; 93.4 percent in the *Potamogeton* beds). Only 0.6 percent of the invertebrates present in the cores in the *Ceratophyllum* beds were in the 10- to 15-cm depth fraction; similarly, only 1.3 percent of the invertebrates in the cores taken below the *Potamogeton* were found in the 10- to 15-cm depth fraction.

Although relatively few organisms were found deeper than the 0- to 5-cm depth fraction, the individuals that were present in depths of 5 to 15 cm below the water-sediment surface were numerically dominated by nematodes, tubificids, and

the gastropod *A. limnosa*. Among all the core samples taken in this study, nematodes made up 27 percent of the invertebrates found between 5 and 15 cm, while tubificids made up 23 percent and *A. limnosa* contributed 21 percent. In the *Ceratophyllum* beds, 29 percent of all the nematodes collected were found below the 0- to 5-cm depth fraction, with 20 percent of the nematodes in the *Potamogeton* beds below 5 cm and 20 percent of the nematodes collected in the no-plant zones in the 5- to 15-cm depth fraction. Although tubificids did make up a sizable percentage of the invertebrates that were present below a depth of 5 cm, the majority of tubificids were found near the surface. Only 5 percent of the tubificids were found below 5 cm in the sediments under the *Ceratophyllum*. Likewise, under the *P. nodosus*, only 5 percent of the tubificids were found at sediment depths of 5 to 15 cm, while in the no-plant zones, 11 percent of the collected tubificids were present at 5 to 15 cm.

DISCUSSION

A number of investigators have shown that the macrophytes in littoral zones increase invertebrate production and diversity in lakes by serving as additional structure for invertebrate colonization, besides that offered by the lake bottom (Muttkowski 1918; Krecker 1939; Andrews and Hasler 1943; Rosine 1955; Gerking 1957; Dvorak and Best 1982; Engel 1985; Schramm, Jirka, and Hoyer 1987). This investigation demonstrated that the presence of macrophytes further enhances invertebrate production and diversity by increasing infaunal invertebrate densities in vegetated areas. This phenomenon of increased infaunal densities and species richness had been reported elsewhere (Watkins, Shireman, and Haller 1983; Wisniewski and Dusoge 1983; Engel 1985), but in each of these cases, the comparison was between vegetated areas in the littoral zone versus nonvegetated areas in profundal or sublittoral zones.

In this study, the presence of infaunal invertebrate densities in the *Ceratophyllum* beds at 13 times that of nonvegetated areas, and in *Potamogeton* beds at 7 times that of nonvegetated areas, occurred at the same depth within the littoral zone, in areas located approximately a few metres apart. The tremendous difference between infaunal densities in the bottom of the *Ceratophyllum* bed (grand mean = 35,260 invertebrates/m^2) and the no-plant zones (grand mean = 2,730 invertebrates/m^2) was especially striking since the no-plant zone and *Ceratophyllum* bed core samples were usually separated by a distance of less than 1 m. It is interesting that the presence of both *Ceratophyllum* and *Potamogeton* resulted in enhanced infaunal invertebrate densities since these macrophyte species are morphologically very dissimilar. *Ceratophyllum demersum* has small leaves positioned in whorls with all of its leaves submersed. In addition, it is rootless, although portions of plant stems are often embedded in the bottom. *Potamogeton nodosus*, in contrast, is a rooted plant with a few narrow submersed leaves and a few broad, floating leaves.

A key feature in this study was the presence of the no-plant zones. However,

the occurrence of small, nonvegetated areas within dense macrophyte beds is not unusual. During an intensive 6-year study of a lake in southwestern Wisconsin, Engel (1985) described "windows" or openings of nonvegetation within nearly continuous plant beds. Macan (1977) noticed the same phenomenon in his study of lakes in northwestern England, stating that "a soil which, though illuminated, watered and stable, is devoid of vegetation is a peculiar but often characteristic feature of freshwater situations." Macan (1977) hypothesized that these bare areas may be caused by an overaccumulation of detrital macrophyte remains in the lake's sediments.

Although the cause of the no-plant zones in Eau Galle Reservoir is not known, these bare areas are ephemeral. In late May of 1988 a stake was placed in the eastern end of each of these small bare patches. By August, all of the staked no-plant zones had been colonized by *Ceratophyllum*. At the same time, other no-plant zones were visible in the lake.

It could be hypothesized that these no-plant zones might be deficient in organic detritus in comparison to sediments below the *Ceratophyllum* and *Potamogeton* beds. Such a deficiency might result in the loss of an important energy source and therefore act as the cause of reduced infaunal densities in the areas devoid of vegetation. This hypothesis was tested by taking three additional core samples from each of the three habitat types during the invertebrate sampling. Each core was separated into the three depth fractions, just as during the invertebrate sampling, and percent organic matter and percent moisture in these sediment samples were then determined. There was no significant difference in percent organic matter among the *Ceratophyllum* bed, *Potamogeton* bed, and no-plant zone cores.

Plant detritus could also be seen in the no-plant zone cores during the microscopic inspection for invertebrates. These findings concur with the determination that the no-plant zones are of an ephemeral nature and that, over time, detritus accumulates in the no-plant zones at about the same rate as within the dense plant beds. It is reasonable to reject the hypothesis that low invertebrate densities in no-plant zones are caused by a lack of a detrital energy base.

A partial explanation for the increased infaunal densities in the *Ceratophyllum* bed is the presence of *Ceratophyllum* stems embedded in the bottom sediments. Care was taken during sampling not to "pin" any *Ceratophyllum* stems to the bottom. Since it was possible to see the sampler move through the *Ceratophyllum* and see the lake bottom, it was easy to avoid trapping any of the *Ceratophyllum* growing in the water column beneath the corer. However, almost all 0- to 5-cm depth fractions from cores in the *Ceratophyllum* beds had some living *Ceratophyllum* stems and leaves in them.

The caddisfly *Leptocerus americanus* is often found in high numbers living epiphytically on *Ceratophyllum* (Wiggins 1977). In Eau Galle Reservoir, these caddisflies were also found at fairly high densities in the cores taken beneath the

Ceratophyllum (grand mean = 3,487 *L. americanus* larvae/m^2). *Ceratophyllum* anchors itself through the development of stems in the lake bottom (Prescott 1969), and it is probable that *Leptocerus* sp. colonizes these stems as well as the upright stems in the water column.

Gastropods in the littoral zone are found epiphytically on the macrophytes as well as on the bottom, and it is possible that the presence of the *Ceratophyllum* stems in the bottom may also result in increased numbers of snails in the cores. However, an attempted explanation that total infaunal densities in *Ceratophyllum* beds are high because many epifaunal animals live on embedded stems is not satisfactory. Tubificid densities in sediments below both the *Ceratophyllum* beds and the *Potamogeton* beds were eight times the tubificid densities in the no-plant zones. Tubificids are infaunal, subsurface deposit feeders (Fisher 1982); extensive studies of the invertebrates living on macrophytes in Eau Galle Reservoir have shown that tubificids do not exist as epiphytes. It is clear, then, that one or more factors besides that of epiphytic colonization of embedded plant stems is responsible for enhanced invertebrate numbers in the bottom beneath *Ceratophyllum* plant beds, at least for tubificids. In addition, enhanced invertebrate numbers under the *Potamogeton* beds (seven times that of the no-plant zones) were not due to embedded *Ceratophyllum* stems since a sprig of *Ceratophyllum* was found in only 3 of the 15 cores taken from the *Potamogeton* beds.

The factor or factors causing increased tubificid densities (and increased densities of other invertebrate taxa) in plant beds remain unclear. Olsson (1981) found in freezing experiments that all chironomid larvae in a box containing many plants survived, while all those in boxes with few plants died. If the no-plant zones sampled in August had persisted as nonvegetated areas since the winter, then initial invertebrate numbers in these zones in the spring may have been much lower than invertebrate densities in the sediments below *Ceratophyllum* (*C. demersum* persists throughout the winter in Eau Galle Reservoir). Another possible explanation is that the macrophytes may stabilize the bottom sediments near them. The no-plant zones were in the middle of dense *Ceratophyllum* beds, however, and were not really "exposed" to extensive water movement.

The presence of plants did not appear to modify the vertical distribution of benthic invertebrates since over 90 percent of the invertebrates were in the top 5 cm of the sediments in the macrophyte beds as well as in the no-plant zones. Although nematodes and tubificids were found predominantly in the top 5 cm of the sediments, the dominance of these two groups among the few organisms found at depths of 5 to 15 cm is consistent with the results of other studies. Nalepa and Robertson (1981) found immature Tubificidae without hair chaetae (the most commonly collected tubificid in Eau Galle Reservoir) and nematodes to be among the taxa with some representatives at deeper strata within the bottom of Lake Michigan. In Eau Galle Reservoir, nematodes seemed particularly able to colonize the deeper strata within the sediments. Nematodes can tolerate very low oxygen concentrations (Weiser and Kanwisher 1961) and have been found deep within the

sediments in a variety of aquatic habitats (Weiser and Kanwisher 1961, Fenchel and Jansson 1966, Sarkka and Paasivirta 1972, Arlt 1973, Tietjen 1979, Nalepa and Robertson 1981).

SUMMARY

Although submersed macrophytes are intimately related to the physical, chemical, and biological environments, they may become overabundant to the extent that significant negative impacts on the ecosystem occur. An excess of submersed macrophytes may contribute to harmful effects by causing wide fluctuations in dissolved oxygen and pH. In addition, luxuriant growths of macrophytes can interfere or prohibit recreational activities and sport fishing. High densities of submersed macrophytes may cause an imbalance in fish populations because the larval and smaller fishes seek refuge in the plant beds to avoid predation.

However, as the results of this study have demonstrated, aquatic vascular plants provide habitat for freshwater macroinvertebrates which in turn are fed upon by larger invertebrates, fishes, waterfowl, amphibians, and reptiles. Management strategies should be designed to control excessive amounts of vegetation and not to eliminate it entirely. Wiley et al. (1984) recommended that the optimal standing crop of submersed macrophytes in ponds in central Illinois should not exceed 52 g dry weight m^{-2}. Because of the potential detrimental effects of submersed macrophytes on water quality, macroinvertebrates, and fishes, it is highly desirable to achieve a well-balanced aquatic ecosystem with no more than 20 to 30 percent of the areal coverage in submersed macrophyte located in suitable and desirable areas of the lake or stream.

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Food Habits of Fishes Associated with Hydrilla Beds and Open Water in Lake Seminole, Florida-Georgia

by
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INTRODUCTION

Aquatic plants are conspicuous features of many water bodies in the southern United States, and the fishes associated with them have been documented in numerous studies. Some species of fish (e.g., *Enneacanthus gloriosus*, *Lucania goodei*, *Lepomis macrochirus*, and *Heterandria formosa*) are substantially more abundant in vegetation, while other species (e.g., *Labidesthes sicculus* and *Dorosoma cepedianum*) are more common in open water (Goin 1943; Swift, Yerger, and Parrish 1977; Killgore, Morgan, and Rybicki 1989). These disparities in abundance have allowed distinctive fish assemblages to be quantitatively described for vegetated and open-water habitats (Barnett 1972; Werner, Hall, and Werner 1978; Guillory, Jones, and Rebel 1979). Such assemblages are not discrete, however, since the majority of species occur in both habitats.

Fish are confronted by substantially different trophic scenarios inside and outside plant beds. Large numbers of fishes frequently occur in vegetation (Ager 1971; Barnett and Schneider 1974; Guillory, Jones, and Rebel 1979; Morgan, Killgore, and Douglas 1989; Killgore, Morgan, and Rybicki, in press), and individual feeding behavior can be influenced by increased intraspecific encounters (Pitcher, Magurran, and Winfield 1982), interspecific competition (Werner and Hall 1979), or predator avoidance (Cerri and Fraser 1983). In plant beds, benthic invertebrates are generally more numerous and more diverse (Gerking 1957; Whiteside, Williams, and White 1978; Mittelbach 1981a,b; Orth, Heck, and Van Montfrans 1984), and zooplankton occurs primarily along the edge or in open-water zones. These habitat-specific differences in invertebrate composition will directly influence habitat selection by fishes and foraging behavior of individual fish (Mittelbach 1981b; Werner, Mittelbach, and Hall 1981). In addition to predator-prey interactions, the physical differences in feeding substrates, the presence of cover, and diel fluctuations in water quality influence fish mobility, prey detection, and feeding strategies (Savino and Stein 1982, Butler 1988).

Several investigators have observed differences in the diet of freshwater fishes among sites where plant growth was qualitatively or quantitatively different

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(e.g., Zimmerman 1970, Crowder and Cooper 1982), or have documented diet shifts coincident with habitat shifts (Cahn 1927, Hall et al. 1979, Werner and Hall 1979). Grazing on plants has been observed in some species (e.g., *Notropis petersoni*) that also frequent nonvegetated habitats (McLane 1955; Swift, Yerger, and Parrish 1977), and vegetation has been associated with vertical segregation and planktivory by some species (Werner, Hall, and Werner 1978). However, quantitative comparisons of diet of individuals inside and outside plant beds within a single location are lacking.

Diet comparisons between pelagic fish and individuals from aquatic plant beds may be conceptually simplistic, but such an approach can also address several important questions. Do most fishes feed opportunistically on available foods, or do they exhibit habitat-independent trophic fidelity? Is intraspecific variability in food habits comparable between habitats? Are community patterns of food utilization substantially different in two settings representing very different invertebrate availabilities and competitor densities? To address these questions, we collected fishes from two habitats (a hydrilla bed and open water) in a large southeastern reservoir and compared the gut contents from each.

STUDY SITE

Lake Seminole is a reservoir formed by the impoundment of the Chattahoochee and Flint Rivers. It has a total watershed of 4.5 million hectares and a surface area at maximum pool level of 15,182 ha (US Army Engineer District, Mobile 1974). Mean depth is 3 m, and maximum depth is 10.7 m. A variety of aquatic plants occur in the reservoir, but hydrilla and Eurasian watermilfoil dominate most of the littoral zones.

Fishes were collected 14-15 July 1987 from two stations in Fish Pond Drain, a cove of Lake Seminole. One station was densely vegetated; the other was not. The water was clear (3 NTU) at both sites with a temperature of 32° C, a pH of 8.0, and a conductivity of 70 μmhos/cm. Dissolved oxygen at the surface was similar at both sites (6 to 8 mg/ℓ), but decreased to 3 mg/ℓ near the bottom at the vegetated site. The average depth at both sites was approximately 1.5 m. The station with aquatic plants was dominated by hydrilla (*Hydrilla verticillata*) with some growths of pondweed (*Potamogeton illinoensis*) and waterlily (*Nuphar luteum*); mean biomass of aquatic plants was 910 g/m^2 dry weight (SD = 607, N = 10). The nonvegetated station was located adjacent to Seminole State Park approximately 1 mile from the vegetated station. This site had no conspicuous growth of aquatic plants, except for musk-grass (*Chara* sp.) that occurred in patches along the bottom with a biomass of 292 g/m^2 dry weight (SD = 27, N = 5).

METHODS

Fishes were collected at both sites with seines and boat-mounted electroshocker. Specimens were preserved in the field in 10-percent formalin and later washed and transferred to 55-percent isopropyl alcohol. Fish were identified according to Carr and Goin (1955) and Wiley (1977) and counted; total length was measured to the nearest millimeter. Food habits were determined for 10 abundant species: *Notropis petersoni*, *Fundulus chrysotus*, *Fundulus escambia*, *Lucania goodei*, *Heterandria formosa*, *Labidesthes sicculus*, *Enneacanthus gloriosus*, *Lepomis macrochirus*, *Lepomis microlophus*, and *Micropterus salmoides* (see Table 1 for a list of common names).

Table 1
Common and Scientific Names of Most Abundant Fishes Collected in Lake Seminole

Species	*Common Name*	*Ranked Abundance*	*Species Abbreviation*
Notropis petersoni	Coastal shiner	5	PETE
Fundulus chrysotus	Golden topminnow	8	CHRY
Fundulus escambia	Eastern starhead topminnow	10	ESCA
Lucania goodei	Bluefin killifish	3	GOOD
Heterandria formosa	Least killifish	9	FORM
Labidesthes sicculus	Brook silverside	1	SICC
Enneacanthus gloriosus	Bluespotted sunfish	4	GLOR
Lepomis macrochirus	Bluegill sunfish	2	MACR
Lepomis microlophus	Redear sunfish	7	MICR
Micropterus salmoides	Largemouth bass	6	SALM

The guts of each fish were removed and examined under a dissecting microscope. The stomach or anterior loop of the gut was separated from the lower tract and bisected; fullness was estimated and food items removed. Prey were identified to the lowest practical taxon and counted; plant foods were recorded as a single prey item. In most cases, the sample size was 15 individuals/species/site. This sample size was considered adequate since at least 80 percent of all prey taxa eaten by a species at a site were recorded after examining 10 individuals (N = 15, SD = 2). For several species (*F. chrysotus*, *F. escambia*, *H. formosa*, and *E. gloriosus*), fewer than 11 individuals were collected at one of the sites. However, these data are also presented because prey numbers were relatively high (i.e., 39 to 130), and the food habits of these four species have received little attention from fish ecologists (but see Breder and Redmond 1929, Hunt 1952, Flemer and Woolcott 1966, Reimer 1970).

Diets were described based on mean number of prey and were compared within species between habitats, and among species within habitats. Taxonomic richness of diets was quantified according to Margalef (1958). Diet breadth, which incorporates components of taxonomic richness and evenness (distribution of individuals within a taxa), was quantified according to Pianka (1973). These values range from 1 (complete specialization on a single prey type) to N (equitable

feeding on all prey taxa). Both richness and breadth were calculated in two ways: as mean values and as overall values for pooled individuals of a single species from a particular habitat. Significant differences between mean values were determined using Student's t (Sokal and Rohlf 1981). Substantial differences between pooled sample values were determined by the magnitude of difference. Differences between richness values >60 percent and breadth values >140 percent were deemed substantial, while differences in richness <35 percent and breadth <90 percent were judged minor. Similarity of diets was quantified using the Percent Similarity Index (PSI), sometimes referred to as the Schoener index; values for this index range from 0.000 (diets completely distinct) to 1.000 (diets completely identical) and are believed to provide the best estimates of "real" overlap (Linton, Davies, and Wrona 1981).

Food habits for each species were ordinated by principal component analysis (PCA) using ORDIFLEX (Gauch 1977). Ordinations were performed within fish species, using individual fish as "samples" and prey taxa as "species," and plotting each fish in multivariate prey space. Only the first two principal components (PCI and PCII) were considered in this study. For 9 of the 10 fishes, PCI and PCII accounted for a substantial percentage (50% and greater) of eigenvariance; for the 10th species (*L. macrochirus*), additional PC axes (PCIII, PCIV, PCV) did not account for a comparable cumulative eigenvariance (42%). Coordinates of all individuals of a species from each site were used to generate 95-percent confidence ellipses (Sokal and Rohlf 1981); this allowed simultaneous evaluations of diet shifts between sites (i.e., degree of spatial separation between two ellipses) and variability in food habits within a site (i.e., relative size of each ellipse). Stepwise regressions (Wolfe and Koelling 1984) of prey numbers and principal component scores were used to identify factors (prey taxa) significantly correlated with PCI and PCII.

RESULTS

The 10 fishes studied represented several trophic guilds, although no species was exclusively herbivorous (Table 2). *Labidesthes sicculus* and *L. macrochirus* were midwater and surface-film feeders. Over two thirds of their diets were zooplankton and emergent and terrestrial insects, with zooplankton predominating. *Enneacanthus gloriosus* and *L. goodei* were midwater and bottom feeders, with over half of their diet composed of zooplankton and chironomid larvae. *Lucania goodei* was more generalized in its food habits, however, feeding to a greater extent on benthos than did *E. gloriosus* and on several foods that comprised only marginal portions of the diet of *E. gloriosus* (i.e., Trichoptera, terrestrial invertebrates, and plants). *Notropis petersoni* and *H. formosa* were omnivores, feeding to a greater extent on plants (over 20% of the diet) than did the other species (less than 7% of the diet). *Fundulus chrysotus* was a benthivore, with more than two thirds of its diet made up by larval chironomids and ostracods. *Lepomis microlophus* was a molluscivore-benthivore; molluscs and larval chironomids (in near equal

Table 2
Food Habits of 10 Species of Fish from Lake Seminole

	Species*									
Sample Size	*PETE* 15-15	*CHRY* 4-7	*ESCA* 10-11	*GOOD* 15-15	*FORM* 4-15	*SICC* 15-15	*GLOR* 4-15	*MACR* 14-15	*MICR* 15-15	*SALM* 15-15
Plant foods										
Algal filaments	14		T**	T	8			1	1	
Diatoms					5					
Desmids				T	4					
Macrophytes		1	1		1			2	1	7
Detritus	21		T	2	5	T		2	T	
Aquatic invertebrates										
Gastropoda		2		1				2	21	
Bivalvia									17	
Hydracarina	32		1	1	14	1	2	4		
Oribatidae	1	1	5	6	T	T		1		2
Amphipoda		1	1	T				1	7	
Ostracoda		43		12	2		1	2	1	
Copepoda			T	4	12	9	16	2		
Cladocera	1		3	32	34	60	65	35		
Ephemeroptera	6	4	T	1	T		1	2	1	15
Odonata		1		T			1		1	9
Hemiptera			1	T						10
Coleoptera		11	1	T			1	1		
Trichoptera		8	T	8			1	1	3	3
Diptera (miscellaneous)			6	1	T	T	1	T	4	
Chironomidae										
Pupae		1	30	1		T		1	T	2
Larvae	14	24	1	21	7	1	8	12	38	5
Other			2	1	6	1		T	1	2
Fish, fish eggs				T			T	1	1	40
Terrestrial invertebrates										
Homoptera			9						T	2
Hymenoptera	1		5			1		7		
Diptera (miscellaneous)			5					1		
Chironomidae	7		23			25	T	4		
Other	2	3	5	8	T	T	1	17	1	2
Total number of prey	112	140	343	488	211	1,389	279	670	285	58

Note: Numbers represent numerical percentage of prey. Sample sizes are for collections made at a nonvegetated and a vegetated station, respectively.
*See Table 1 for species abbreviations.
**Trace.

proportions) made up more than 75 percent of its diet. *Fundulus escambia* was a specialized surface-film feeder, with emergent and terrestrial insects making up 80 percent of its diet. *Micropterus salmoides* was mainly piscivorous; 40 percent of its prey were fish, but no single taxon or prey group dominated the remainder of its diet.

Mean diet richness values were comparable among the majority of the species studied, although pooled values showed substantial variation (Figure 1). Mean diet richness for eight species ranged from 2.901 to 5.323, indicating that those species were trophic generalists. Values for *L. sicculus* and *M. salmoides* were less than 2.5000 because they specialized on few prey items. Pooled richness ranged

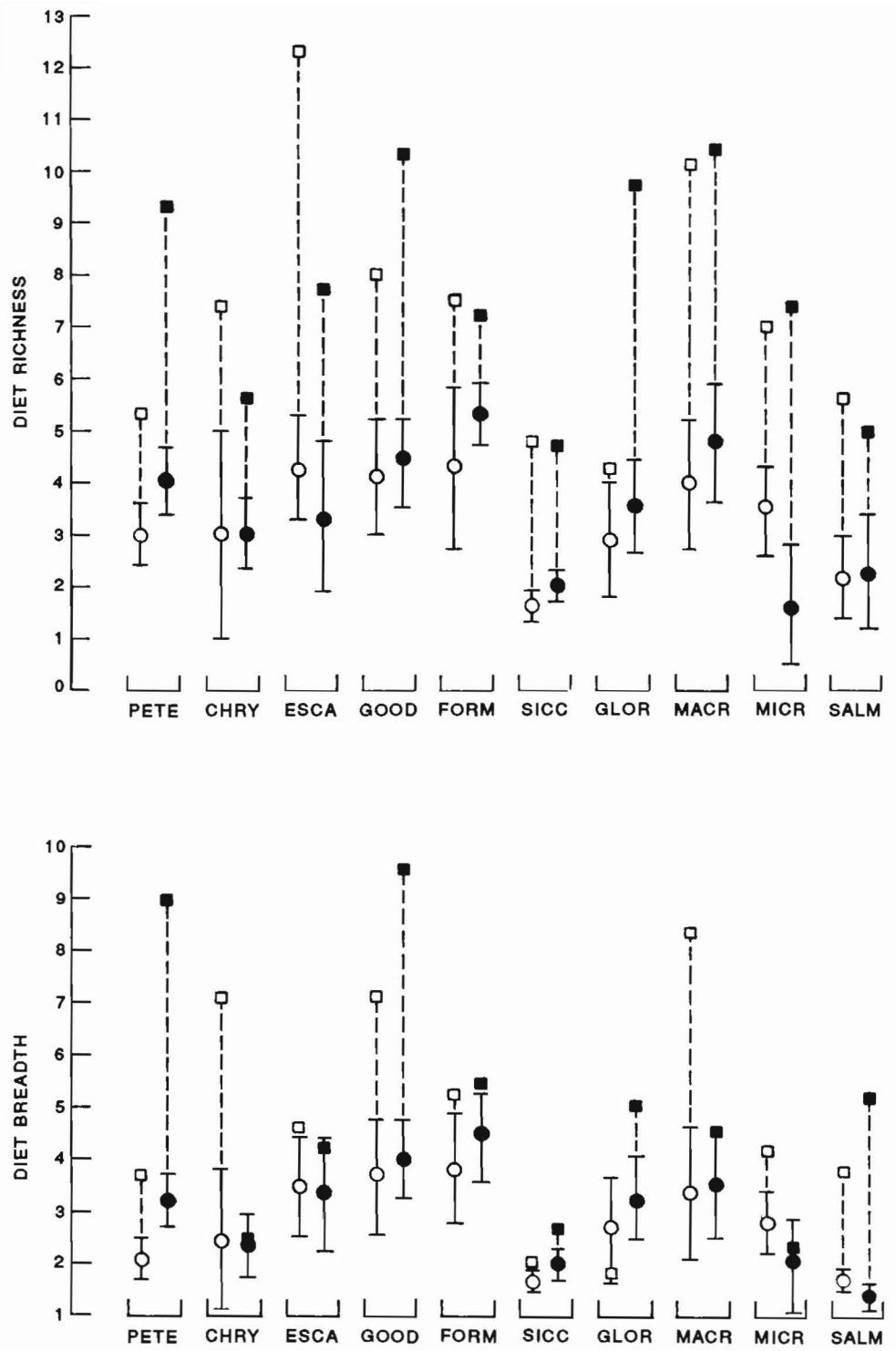

Figure 1. Diet richness and breadth for 10 species of fish collected in Lake Seminole outside (open symbols) and inside (shaded symbols) a hydrilla bed. Circles are mean (individual) values ±2 standard errors; blocks are pooled (sample) values

from 4.257 to 12.339; low values (<6.000) were obtained for *L. sicculus* and *M. salmoides*, and high values (>10.000) for *L. macrochirus*.

Diet richness for 6 of the 10 species did not differ appreciably between the two habitats (Figure 1). For *N. petersoni*, mean diet richness was significantly higher ($t = 2.198$, d.f. = 28, $p < 0.05$), and pooled diet richness was substantially higher at the vegetated site. In contrast, *Fundulus escambia* exhibited substantially lower pooled diet richness at the hydrilla bed and no significant difference in mean diet richness. This indicated that individual *F. escambia* fed on comparable numbers of food types in both habitats, but that individuals from the vegetated site were more likely to feed on different types of foods. *Lepomis microlophus* also exhibited lower diet richness in hydrilla, but consumed higher numbers of individual prey types at the nonvegetated site. Pooled richness was comparable at both sites, but overall mean richness was significantly lower for the vegetated site ($t = 2.515$, d.f. = 28, $p < 0.05$). Diet richness for *E. gloriosus* was very similar to that of *N. petersoni* among the two habitats, but the low number of individuals collected from the nonvegetated location precluded quantitative interpretations.

Mean diet breadth ranged from 2.10 to 4.36 for eight species but was less than 2.00 for *L. sicculus* and *M. salmoides*. Pooled breadth ranged from 1.99 to 9.48; low values (<3.00) were obtained for *L. sicculus*, and high values (>8.00) were obtained for *N. petersoni*, *L. goodei*, and *L. macrochirus* in at least one of the habitats.

Seven species did not exhibit important differences in diet breadth between habitats (Figure 1). Diet breadth of *N. petersoni* was higher in hydrilla than at the nonvegetated site; the mean value was significantly higher ($t = 3.53$, d.f. = 28, $p < 0.01$), and the pooled value was nearly three times higher for individuals from the vegetated site. For *F. chrysotus*, mean diet breadth in the two habitats was nearly identical, but pooled breadth was three times higher at the nonvegetated site. This indicated that individual *F. chrysotus* tended to show the same degree of specialization in both habitats but individual fish were specializing on different prey at the nonvegetated site, while fish feeding in the hydrilla bed were specializing on the same prey types. Pooled diet breadth of *E. gloriosus* was higher at the vegetated site, but this may represent a sample size phenomenon, since larger numbers of fish were collected in hydrilla.

Intraspecific similarity in diets between the two habitats was not high (PSI <0.750) for any species, but there was substantial variation among the fishes. Moderate values (PSI = 0.564 to 0.604) were obtained for *L. goodei*, *L. sicculus*, and *M. salmoides*, and low values (PSI <0.350) for the remaining species. The diet of *L. microlophus* showed the lowest degree of similarity between habitats (PSI = 0.177).

Principal component analysis indicated that intraspecific variability in diet composition was comparable at the two sites for most species (Figure 2). Paired ellipses could not be reliably created for *F. chrysotus*, *H. formosa*, and *E. gloriosus*

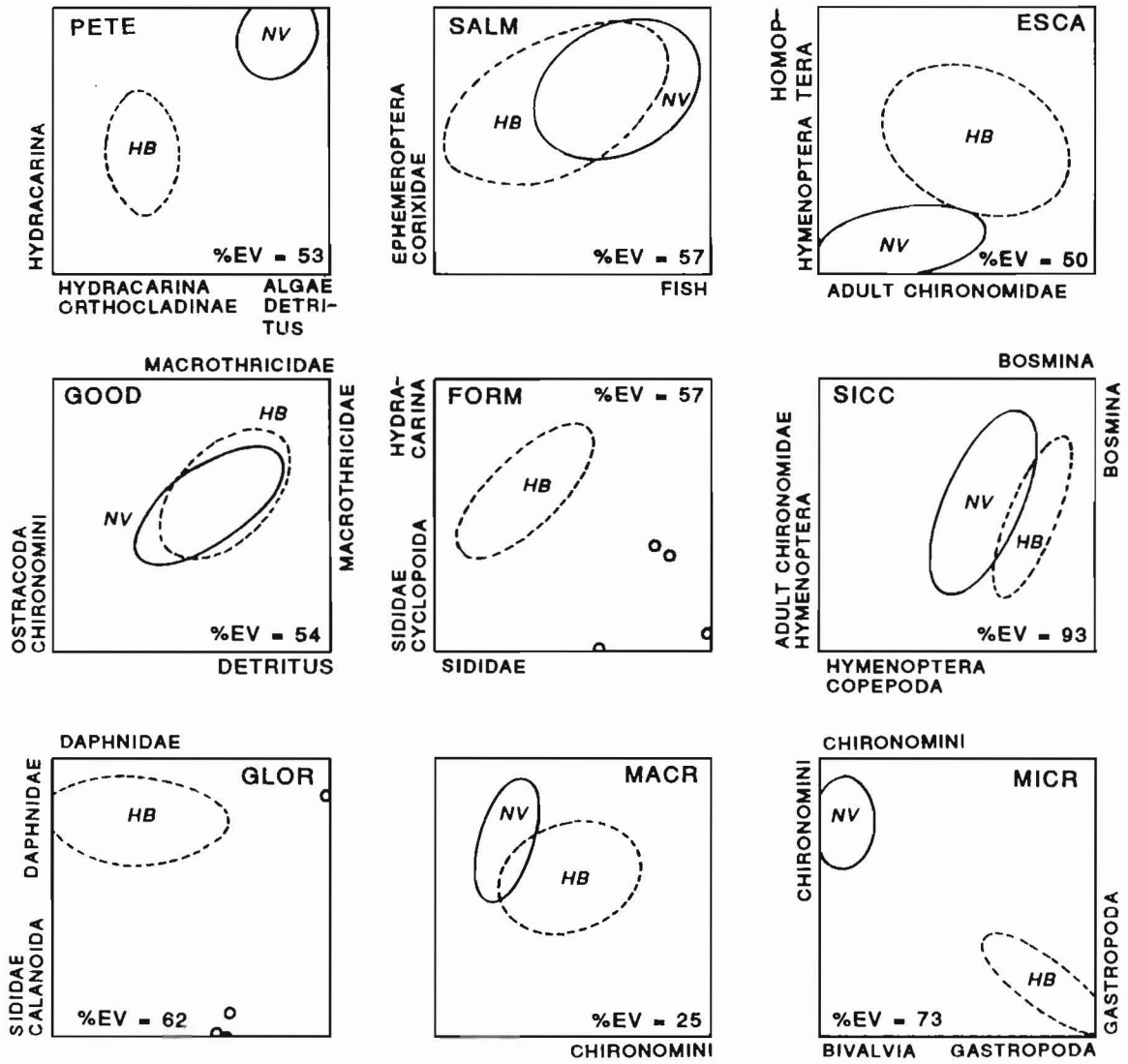

Figure 2. Principal component analysis of diets of fish collected in Lake Seminole (NV, nonvegetated station; HB, hydrilla bed). Ellipses based on point spread of individual fishes within each habitat (outside and inside a hydrilla bed) (%EV, percent eigenvariance). Prey taxa indicated were significantly correlated with PC coordinates along that axis; positively correlated taxa listed at right, and negatively correlated taxa listed at left

due to low sample sizes (N < 8), but those generated for the other seven species did not vary greatly in size (i.e., ellipse size differences were substantially less than an order of magnitude). However, there was a trend for ellipse area to be greater for fishes collected in hydrilla, suggesting a tendency for increased intraspecific variability in diet composition for fishes in vegetation. Only *L. sicculus* exhibited a larger ellipse for the nonvegetated site. The remaining six species had larger ellipses (point spread) in the hydrilla collections. Ellipse size disparity was minimal (<10%) for *L. goodei* and *L. microlophus*, moderate (approximately 45%)

for *N. petersoni* and *M. salmoides*, and greatest (>100%) for *F. escambia* and *L. macrochirus*.

The PCA ellipse separation was usually consistent with the PSI values. The PSI values were low (<0.300) for several species, and this was reflected by ellipses that were well separated (e.g., *N. petersoni* and *L. microlophus*) or overlapped only marginally (e.g., *F. escambia* and *L. macrochirus*). However, when PSI values were higher, but within a small range (i.e., 0.564 to 0.604), ellipses overlapped marginally (*L. sicculus*), moderately (*M. salmoides*), or almost completely (*L. goodei*). Complete agreement between the two measures (PCA and PSI) would occur only if prey numbers and frequencies were equivalent between habitats and if low-frequency prey did not make up an important percentage of the diet.

Some prey (e.g., Bosminidae, bivalves) were consumed preferentially by a single species of fish, but no taxon was eaten in large numbers by all species (Table 2). Of 58 prey types eaten, 17 showed significant correlations with PCI or PCII (Figure 2). Larval chironomids were important to four species (*F. chrysotus*, *L. goodei*, *L. macrochirus*, *L. microlophus*) and copepods to three species (*H. formosa*, *L. sicculus*, and *E. gloriosus*). Other invertebrate taxa (e.g., larval Orthocladinae, Hydracarina, Homoptera, and various Cladocera) showed significant correlations with principal component loadings for only one to two species.

Habitat-associated differences in diet varied with each species of fish, although several species in hydrilla fed on larger numbers of Hydracarina and lower numbers of copepods and terrestrial insects than did conspecifics at the non-vegetated site (Figure 2). *Notropis petersoni* collected at the vegetated site fed less frequently on algae than did individuals from the nonvegetated site and more frequently on Hydracarina and larval Orthocladinae. *Fundulus escambia* at the vegetated site ate fewer adult chironomids and hymenopterans, and consumed more homopterans. *Heterandria formosa*, *L. sicculus*, and *E. gloriosus* all consumed larger numbers of copepods and cladocerans at the nonvegetated site than at the vegetated station. The *H. formosa* and *E. gloriosus* ate over four times as many cladocerans at the vegetated site, respectively, than from the nonvegetated site. Mean numbers of cladocerans eaten by *L. sicculus* were higher at the non-vegetated station (35.47/individual) compared to the vegetated site (17.93/individual), although this appears contradictory to PCA results that indicated greater bosminid utilization at the vegetated site. Cladocerans occurred more frequently and in less variable (albeit lower) numbers in the diets of hydrilla-collected *L. sicculus* (C.V. = 83%) than in those individuals from the nonvegetated site (C.V. = 129%). The *L. sicculus* at the nonvegetated station also consumed significantly lower numbers of adult chironomids (5.27/individual) than those fish in the hydrilla bed (17.87/individual) ($t = 2.74$, d.f. = 29, $p < 0.05$). The *L. macrochirus* ate large numbers of hymenopterans and low numbers of larval chironomids at the nonvegetated site; this pattern was reversed, however, at the vegetated site where low numbers of hymenopterans and high numbers of chironomids were eaten. Consumption of several other prey taxa by

L. macrochirus was also different in vegetation, but was not significantly correlated with principal components: plant foods (1.46/individual versus 0.66/individual), sidid Cladocera (12.60/individual versus 0.00/individual), and Hydracarina (1.67/individual versus 0.27/individual).

Three of the remaining four species ate fewer larval chironomids in the hydrilla bed than at the nonvegetated station (Figure 2). Although only a few *F. chrysotus* were collected from each habitat, there was an indication that individuals from the hydrilla bed were eating fewer chironomids (1.0/individual versus 2.3/individual) and larger numbers of other benthic prey (i.e., ostracods, beetles, and caenid mayflies). Major prey of *L. goodei* at the nonvegetated site were also important dietary components for individuals collected in the hydrilla bed; differences in prey numbers for taxa correlated with principal components (i.e., macrothricid cladocerans, larval chironomids, and detritus) were not greatly disparate; hence, there was little separation of PC ellipses. There were some other differences in food habits that were not associated with PC axes that accounted for moderate similarity in diet between habitats. The *L. goodei* at the nonvegetated station ate large numbers of larval hydroptilid caddisflies (2.27/individual), while individuals collected in the hydrilla bed ate none. The *L. microlophus* that occurred at the nonvegetated site ate large numbers of bivalve molluscs (3.27/individual) and chironomids (5.60/individual), but smaller numbers of snails (0.60/individual); in vegetation, though, this species ate large numbers of snails (3.47/individual), few chironomids (0.20/individual), and no bivalves.

Diet partitioning among species was pronounced in both habitats (Figure 3). Of 90 pairwise comparisons among the 10 species, 73 resulted in interspecific overlaps (PSI) of less than 0.200, and none resulted in overlaps greater than 0.500. Species pairs exhibiting even slight similarity in diet (PSI > 0.250) were relatively uncommon (8 of 90). *Notropis petersoni*, *L. sicculus*, and *M. salmoides* did not exhibit moderate overlap with any species in either habitat, probably due to their distinctive specializations on water mites (Hydracarina), cladocerans, and fish, respectively (Table 2). Only two instances of overlaps greater than 0.400 occurred: *F. chrysotus* and *L. microlophus* both consumed large portions of larval chironomids in areas without vegetation; *H. formosa* and *L. macrochirus* both ate substantial numbers of sidid cladocerans in vegetation.

DISCUSSION

The diets of Lake Seminole fishes were comparable with previously published food habits for those species, with two exceptions. Diet information is not yet available for the recently described *F. escambia*, and only limited information is available on the food habits of lentic *N. petersoni*. Davis and Louder (1971) reported that *N. petersoni* fed primarily on copepods. Individuals in Lake Seminole fed chiefly on Hydracarina, detritus, larval chironomids, and filamentous algae. Davis and Louder (1971) also reported algae and detritus from the gut

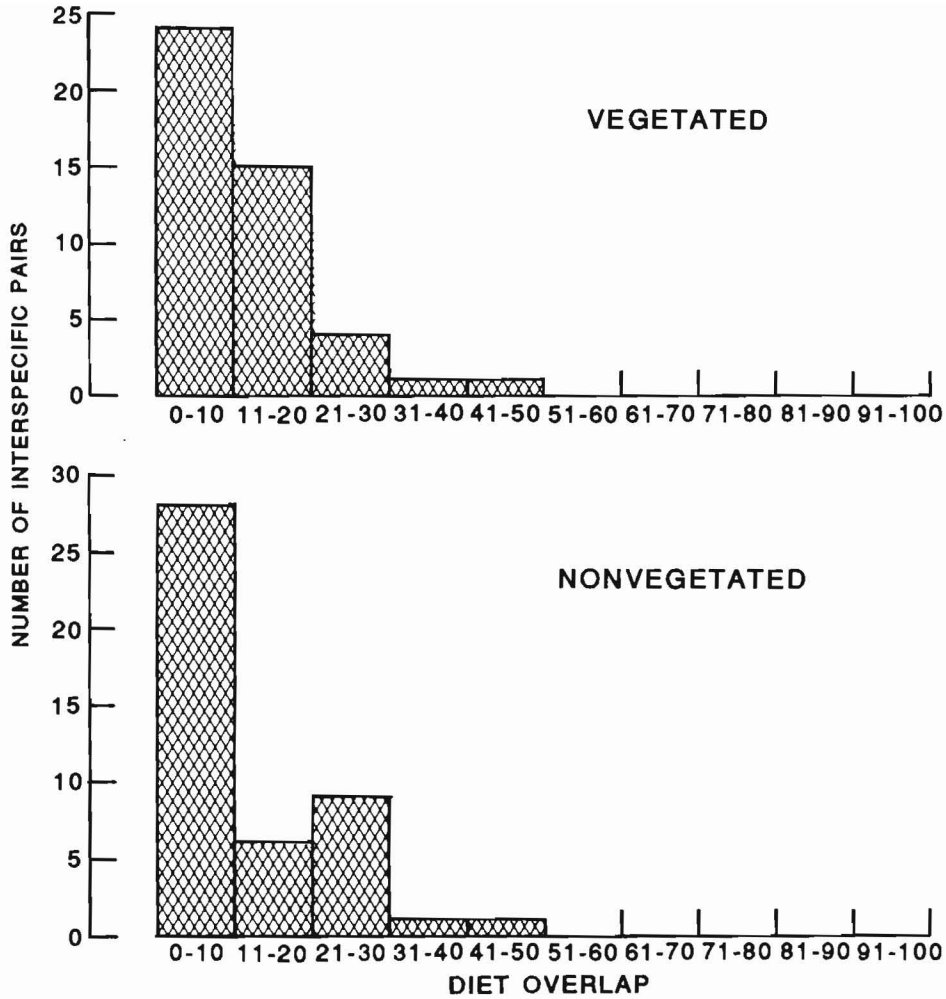

Figure 3. Frequency distributions of interspecific overlaps among Lake Seminole fishes

contents of *N. petersoni*, but suggested that plant materials were consumed incidentally and were of negligible nutritional value.

Several studies have surveyed food habits for large assemblages of fishes (e.g., Hunt 1952, Flemer and Woolcott 1966, Keast and Webb 1966, Zaret and Rand 1971, Keast 1985), but these have focused primarily on interspecific resource partitioning and have not emphasized small-scale variations in diet, such as those existing among different habitats. Investigators have noted substantial shifts in diet composition for some species coinciding with changes in habitat, but these observations have been restricted to one to three species (Cahn 1927, Hall et al. 1979, Werner and Hall 1979, Crowder and Cooper 1982). An assemblage-level evaluation and a community-level generalization regarding degree of diet change between habitat types are lacking. Our results suggest that diet shifts can be substantial between habitats (i.e., >40%) within an entire assemblage, irrespective of trophic guilds and degree of dietary specialization.

Substantial habitat-associated differences in diet existed for all species (Figure 2), and yet there were only a few significant changes in mean diet richness and diet breadth (Figure 1). This suggests that such differences in diet composition were largely attributable to differences in invertebrate availabilities (Quade 1969, Mittelbach 1981a) than to structure-induced changes in feeding behavior or feeding efficiencies (Savino and Stein 1982, Butler 1988).

Sidid cladocera tend to be less abundant in open water than in vegetation (Quade 1969, Fairchild 1981). At the nonvegetated site, *H. formosa* and *L. macrochirus* specialized on benthic invertebrates and daphnid cladocerans, but in the hydrilla bed, both species fed on sidids more so than any other prey (Figure 2). In contrast to sidids, bosminid cladocerans are more abundant in open water than in vegetation (Quade 1969, Fairchild 1981). This was reflected in the diet of the specialized predator, *L. sicculus*; bosminids were consumed more frequently in open water. In the case of *L. sicculus*, however, factors other than prey availability may influence their diet composition. This species is morphologically specialized for open-water swimming and feeding (Keast and Webb 1966) and is almost always more abundant in open waters than in vegetation (Goin 1943; Reid 1950; Ager 1971; Barnett 1972; Guillory, Jones, and Rebel 1979). Also, silversides are known to select bosminids by visual discrimination of the cladoceran's eyespot (Zaret and Kerfoot 1975). It seems reasonable to assume, then, that the physical impediments (to swimming) and the visual impediments (to prey detection) presented by the hydrilla resulted in lower feeding efficiency of some individuals. This is supported by our estimates of gut fullness of *L. sicculus*, which were significantly higher in open-water specimens (74% full) than in specimens from the hydrilla bed (57% full) ($t = 2.634$, d.f. $= 28$, $p < 0.05$).

Habitat-associated differences in the diet of Lake Seminole fishes may also be influenced by intraspecific interactions. Larger group sizes have been shown to impact feeding activity probably through passive information exchange (Pitcher, Magurran, and Winfield 1982) and reduced vigilance (Pitcher and Magurran 1983). If larger populations of fish are less sensitive to predator effects (sensu Pitcher, Magurran, and Winfield 1982) and to interspecific competitive restraints (sensu Werner and Hall 1979), and if they experience greater degrees of intraspecific "information exchange" (sensu Pitcher, Magurran, and Winfield 1982), they should also exhibit higher similarity in diets between habitats. This speculation is supported by our observations. Among the nine fishes that consume primarily invertebrates, there is a significant positive correlation between abundance (total numbers of fish collected) and similarity of diets (PSI) between stations ($r = 0.666$, $N = 9$, $p < 0.05$). Such a relationship underscores the importance, and the difficulty, of evaluating multiple factors which may be responsible for small-scale changes in the diet of fishes.

Aquatic plants provide an important forage base to fishes and can influence their distribution and condition (Hall and Werner 1977, Colle and Shireman 1980, Holland and Huston 1984). Although this study indicates that many fish species

alter feeding habits when they encounter aquatic plants, their trophic dynamics are not yet fully understood. However, as new information on this subject becomes available to aquatic plant managers, the functional relationship between plants and the diet of fish can be considered when choosing the appropriate level of control.

SUMMARY

Diets were compared for 10 species of fishes collected from a nonvegetated station and a hydrilla bed in a large southeastern reservoir. Although most of the species did not exhibit significant differences in diet richness or diet breadth between the two habitats, all species exhibited moderate to substantial changes in diet composition. *Notropis petersoni* and *Lepomis microlophus* exhibited the greatest degree of habitat-associated diet dissimilarity; *Lucania goodei* and *Micropterus salmoides* showed the least. Habitat-associated differences in diet composition were not uniform among the species in that fishes did not converge on a few types of prey in either habitat. Resource partitioning was pronounced at both stations, despite differences in physical structure and ichthyofaunal composition.

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Effects of Submersed Macrophytes on Patterns of Sedimentation in the Potomac River

by
Harry L. Eakin* and John W. Barko*

INTRODUCTION

The recolonization of the freshwater tidal Potomac River by native submersed macrophytes after a four-decade absence with the subsequent introduction and proliferation of the exotic, nuisance species *Hydrilla verticillata* (L.f.) Royle (Steward et al. 1984, Rybicki et al. 1986) has possibly altered patterns of sedimentation occurring there. *Hydrilla* has become the dominant species within several large areas of the tidal Potomac River; its distribution now extends from Hunting Creek downstream to Dogue Creek (Figure 1). Impediment of water movement by large beds of *Hydrilla*, resulting in decreased velocities and loss of carrying capacity of riverine and tidal flows, may enhance sedimentation. Increased sedimentation in the area of these macrophyte beds may also affect nutrient availability, colonizable substrate, and other environmental factors such as light and water temperature which influence macrophyte distribution and growth.

Nutrient availability, inorganic carbon supply, sediment properties, light, water temperature, and interactions of these factors influence the growth of submersed aquatic macrophytes (Barko and Smart 1981, 1983, 1986; Barko, Adams, and Clesceri 1986; McFarland and Barko 1987). Sediments that are finely textured and relatively low in refractory organic matter promote increased growth of selected macrophyte species. Conversely, decreased growth is evident on sediments with high bulk densities (e.g., sand) and high refractory organic content. Changes occurring within the sediment may have pronounced effects on the growth and distribution of submersed macrophytes, which in turn may alter patterns of sedimentation within the tidal Potomac River.

An investigation of nutrient uptake by *Hydrilla* from sediment over two successive growth periods demonstrated dramatic reductions of exchangeable nitrogen (95%) and extractable phosphorus (36%) concentrations in sediment (Barko et al. 1988). Notably, a 30-percent reduction in growth of *Hydrilla* was observed on sediment following sediment nutrient reductions. A subsequent investigation designed to evaluate regeneration of nutrients in sediments by decompositional and diagenetic processes demonstrated insignificant replenishment of nutrients following two periods of removal by previous macrophyte growth (Barko et al. 1988, unpublished). These results suggest that major nutrient

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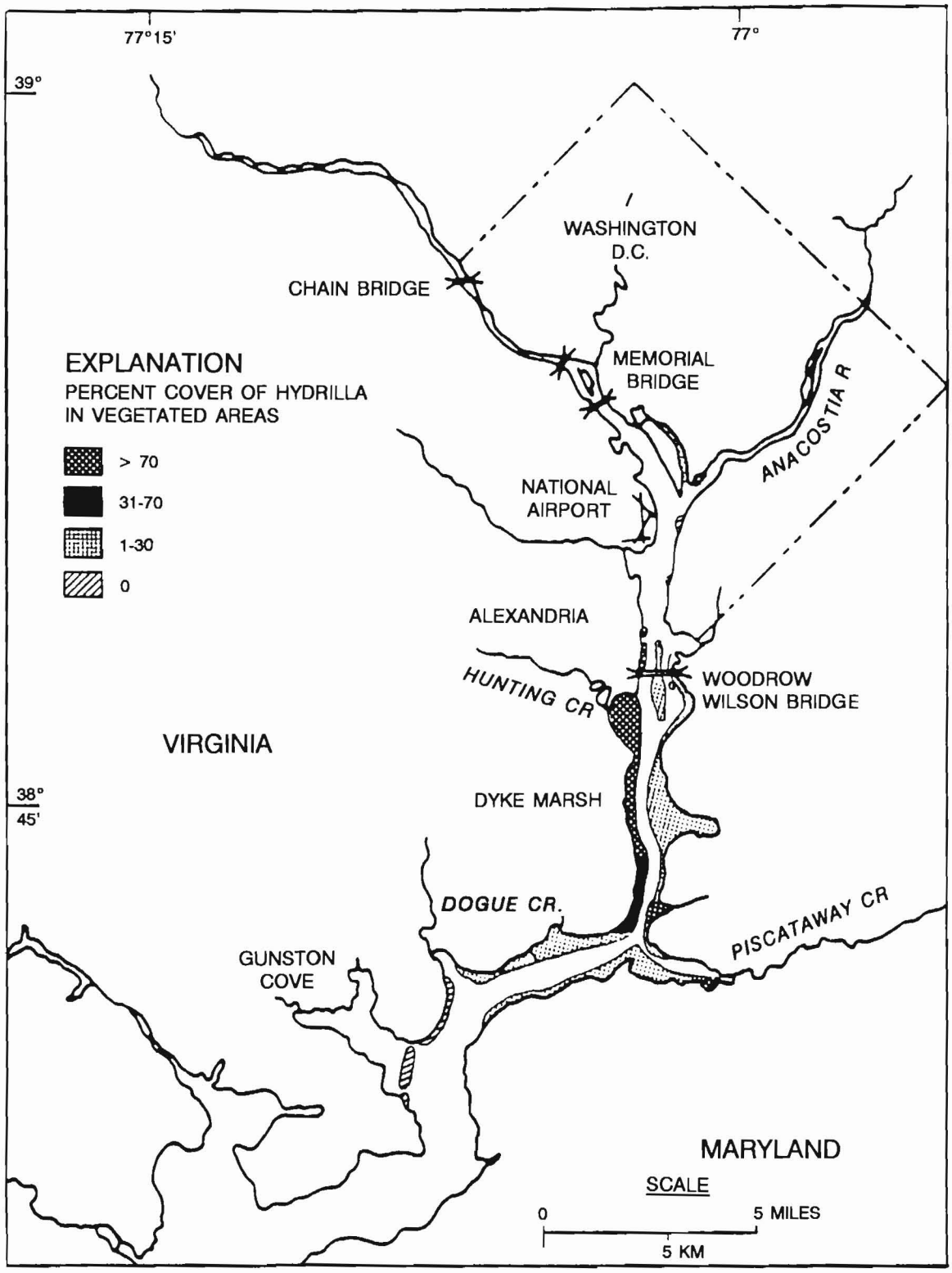

Figure 1. Distribution of *Hydrilla* in the tidal Potomac River

replenishment to the sediments may be due to sedimentation. Thus, the continued growth and expansion of macrophyte beds may be influenced by the quantity and quality of the sedimenting material.

Objectives of this study were to quantify spatial and temporal patterns of sedimentation associated with *Hydrilla* macrophyte beds and to evaluate qualitative differences in the chemical nature of the sedimenting material that might affect macrophyte growth and distribution.

MATERIALS AND METHODS

The investigation was conducted within the tidal Potomac River near Washington, D.C., in an extensive submersed macrophyte bed dominated by *Hydrilla*. Sampling was conducted along a transect perpendicular to the shoreline extending through the macrophyte bed into the open water in an area known as Dyke Marsh. The macrophyte bed extended from the shoreline to 360 m along the transect. Water depth at high tide ranged from about 2.0 m at the nearshore site (180 m) to about 2.7 m at the open-water site (380 m), with tidal fluctuations of approximately 1 m. Sampling sites were located on the transect at 180, 260, 340, and 380 m.

Apparatus for collecting sedimenting material consisted of plastic (polyvinyl chloride) cylinders (Hargrave and Burns 1979), constructed with an aspect ratio of 6.0 (Lau 1979) to minimize resuspension of settled material. Cylinders were deployed in triplicate and were allowed to rest vertically (cylinder length = 39 cm) on the sediment surface for periods of 2 weeks during June and September 1988.

Retrieval of collected sediment was accomplished by quantitatively transferring the solid contents of each cylinder into 1-ℓ plastic bottles for transport and storage at 4° C. Sample analyses were undertaken within 36 hr of collection.

Sample aliquots of collected sediment were analyzed for dry weight, ash content, total nitrogen, and total phosphorus. Physical analyses were performed according to Standard Methods (American Public Health Association 1980). Chemical analyses, using Technicon Autoanalyzer II procedures, followed sulfuric acid/potassium persulfate digestion (Raveh and Avnimelech 1979). Sedimentation rates were calculated by the equation

$$R = \frac{C \times V}{A \times D}$$

where

R = sedimentation rate, mg dry weight/m^2/day

C = sediment concentration, mg/ℓ

V = volume of collection cylinder, ℓ

A = area of cylinder mouth, m^2

D = time, days

RESULTS AND DISCUSSION

Sedimentation rates

Rates of sedimentation differed spatially and temporally along the transect during the two deployment periods (Figure 2). Material deposition during June was substantially greater at the 380-m open-water site than within the macrophyte bed. Sedimentation within the macrophyte bed was greatest at the 340-m site (located 20 m from the open water) and least at the 180-m site.

Sedimentation rates at the 380- and 340-m sites during September showed patterns similar to those occurring in June (Figure 2). However, there were great differences in magnitudes of sedimentation. The open-water site (380-m) again exhibited the greatest rate of sediment deposition among the sampling sites. Sedimentation there in September was about 36 percent of that occurring during June. The sedimentation rate at the 340-m site, near the edge of the macrophyte bed, was about 26 percent of the rate in June. Rates of sedimentation at the 260- and 180-m sites during September were almost identical, about 7 and 13 percent of the measured rates at the respective sites during June.

Temporal differences in rates of sedimentation probably reflect both the condition of the macrophyte bed and climatological conditions during each of the deployment periods. During June, the submersed macrophytes had not attained full canopy, and riverine and tidal flows were able to traverse the entire macrophyte bed with little impedance, especially near the water surface. Also, the effects of early summer storm-related activity on runoff, i.e., increased turbidity and elevated levels of suspended solids, were still evident. In contrast, the plants had attained full canopy by September, resulting in a potentially great reduction of riverine and tidal flows over the expanse of the macrophyte bed. Visual observations of riverine and tidal flows during September revealed the diversion of the majority of flows around and along the outer edge of the plant bed as a result of physical obstruction of water movement by the plants. Paradoxically, greater deposition of suspended material near the outer edge of the plant bed and adjacent open water occurred, even though increased water velocities and carrying capacities tend to decrease deposition. The relative increases in deposition at these sites may have been induced by the increase in volume associated with the diverted flows. Lessened rainfall, under drought conditions during late summer, resulted in reduced turbidity and suspended solids in riverine and tidal flows (based on visual observations).

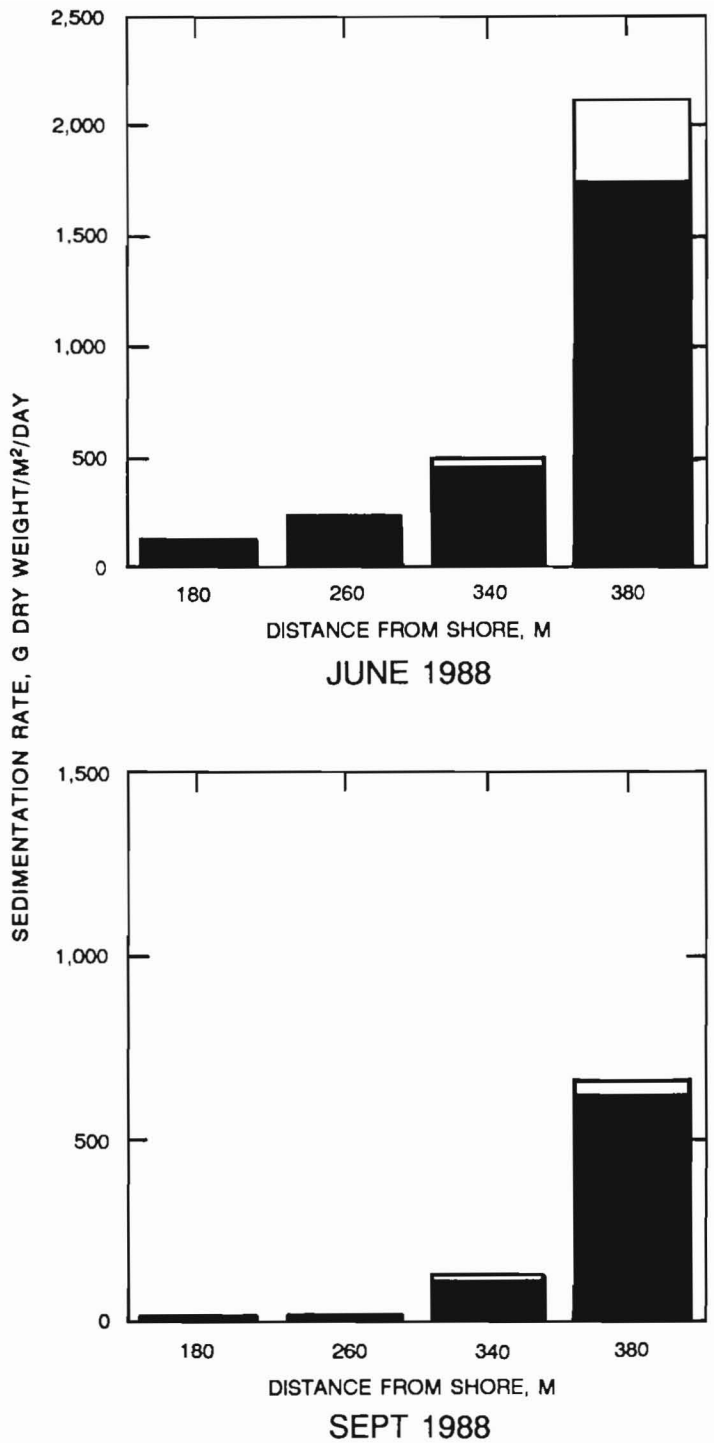

Figure 2. Mass sedimentation rates. Vertical bars represent means (n = 3) with associated standard deviations (open portions of bars)

Ash content

There were seasonal changes in ash content of deposited material between June and September (Figure 3). During June, the uniform ash content of sediment collected at all sites indicated essentially no differences in organic content. However, during September, a 17-percent decrease in the ash content at sites deep within the macrophyte bed (180 and 260 m) indicated a relatively higher organic content of sedimenting materials. Contributions of dislodged epiphytic algal populations to the sedimenting material, evident upon microscopic examination, appeared to have caused the increase in organic content. The 340- and 380-m sites showed essentially no change in ash content between June and September, suggesting the deposition over time of similar materials with a relatively low organic content.

Nitrogen and phosphorus

Spatial variations in rates of total nitrogen and total phosphorus sedimentation (Figure 4) paralleled spatial variations in rates of total mass sedimentation (Figure 2). The 380-m site exhibited the greatest deposition of nitrogen and phosphorus during both deployment periods; the 180-m site, nearest to shore, experienced the least deposition of these nutrients.

Among studied sites, rates of nitrogen and phosphorus deposition were much greater during June than September. Ratios of nitrogen to phosphorus concentrations were essentially 5:1 at all sites during June (Figure 5). In September, the ratio of nitrogen to phosphorus concentrations again approximated 5:1 at the 340- and 380-m sites, but increased to about 10:1 at the 180- and 260-m sites.

The difference in ratios observed during September appears to reflect differences in the composition of sedimenting material. The open water of the Potomac River experiences greater water velocities and thus greater sediment carrying capacity than within the macrophyte beds (Carter et al. 1988). Thus, sedimenting particles collected at this site were probably much larger than particles collected within the macrophyte bed. Relatively fine-grained materials deposited in the macrophyte bed had a significantly higher nutrient content than sediments deposited in the open water (Figure 6). Thus, reduced flows within the macrophyte bed appear to induce deposition of material containing higher nutrient concentrations.

SUMMARY AND CONCLUSIONS

Spatial and temporal patterns of sedimentation within and immediately adjacent to an extensive macrophyte bed were examined in the tidal Potomac River. Physical impedance of riverine and tidal flows by *Hydrilla* resulted in reduced velocities and loss of sediment carrying capacity, which effected increased deposition of suspended material. Greatest deposition was focused along the

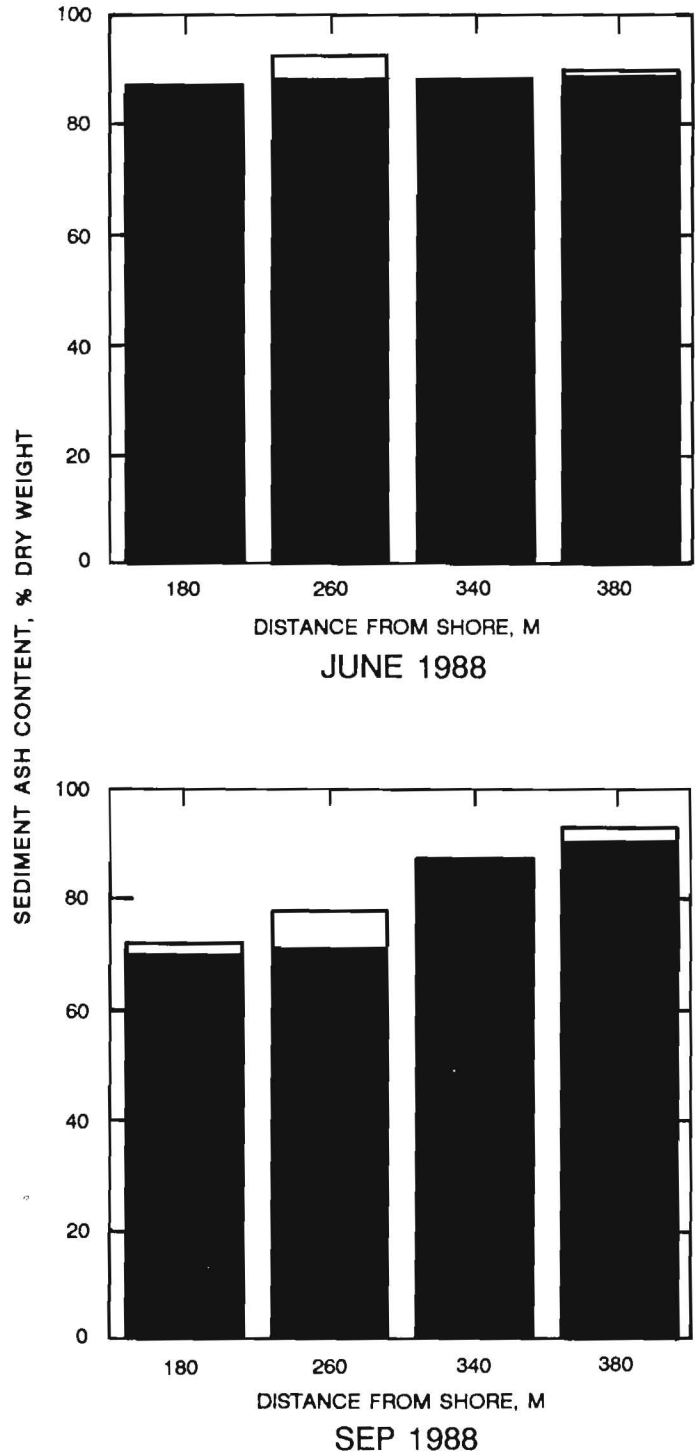

Figure 3. Percent ash content of sediment. Vertical bars represent means (n = 3) with associated standard deviations (open portions of bars)

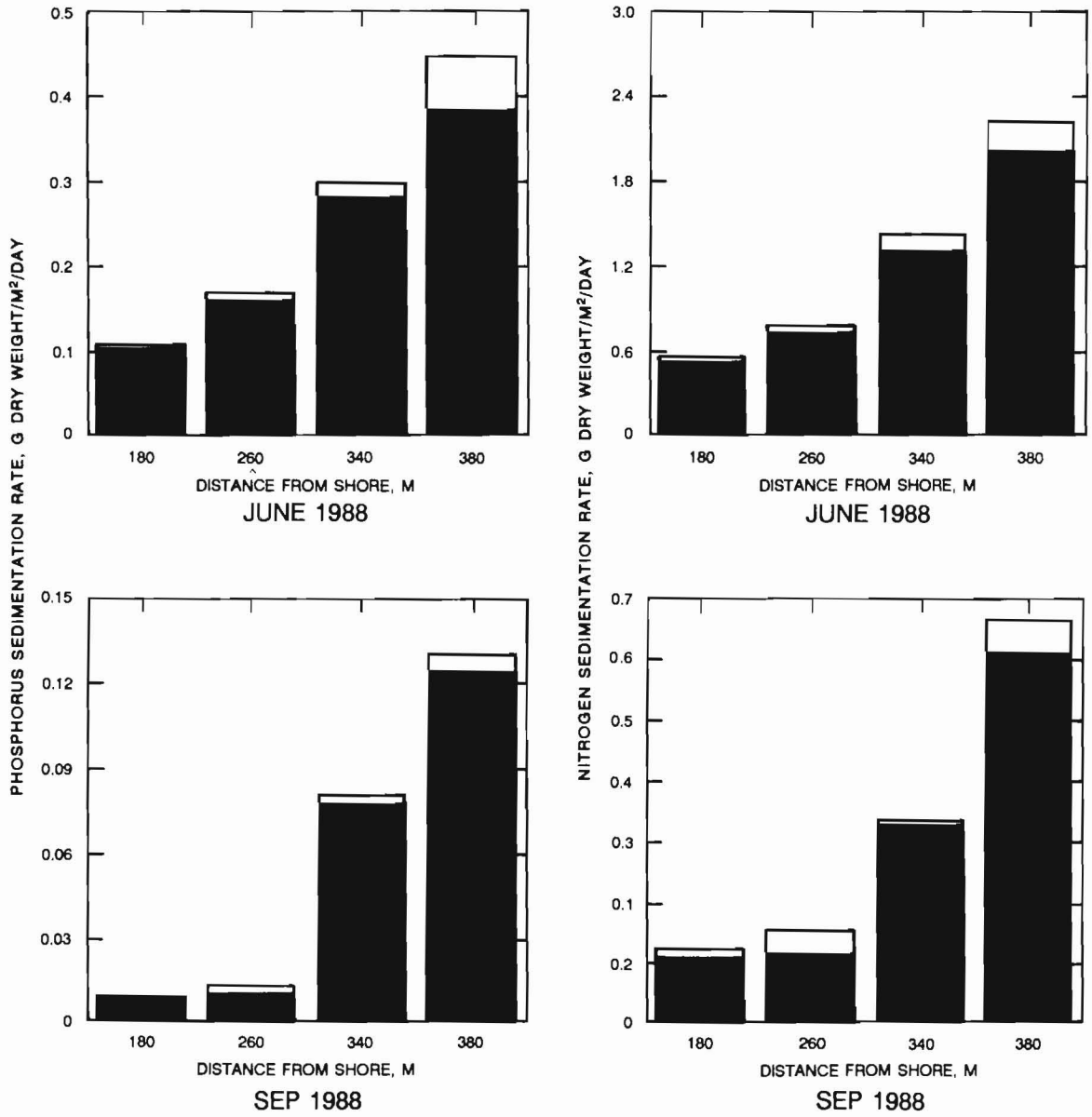

Figure 4. Total phosphorus and total nitrogen sedimentation rates. Vertical bars represent means (n = 3) with associated standard deviations (open portions of bars)

outer edge of the macrophyte bed, due to possible redirection of riverine and tidal flows toward open water, i.e., path of least resistance.

Continued growth and expansion of submersed macrophytes in the tidal Potomac River may be enhanced by sedimentation induced as a direct physical result of the plants' presence. Increased deposition of sediment along the outer edge of the macrophyte bed may result in increased colonizable substrate and continued expansion of the bed. Sustained growth of macrophytes may result from the deposition of nutrient-rich sediment within the plant beds.

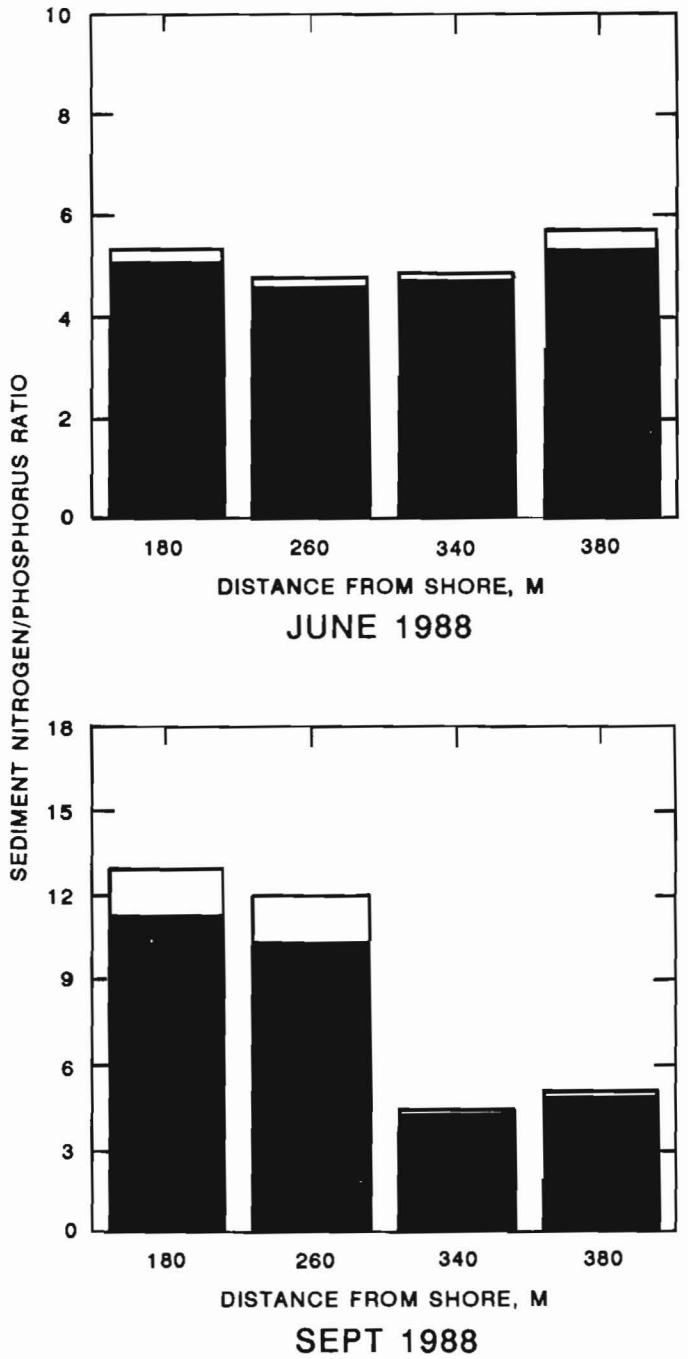

Figure 5. Ratios of nitrogen to phosphorus concentrations (mg/g dry weight). Vertical bars represent means (n = 3) with associated standard deviations (open portions of bars)

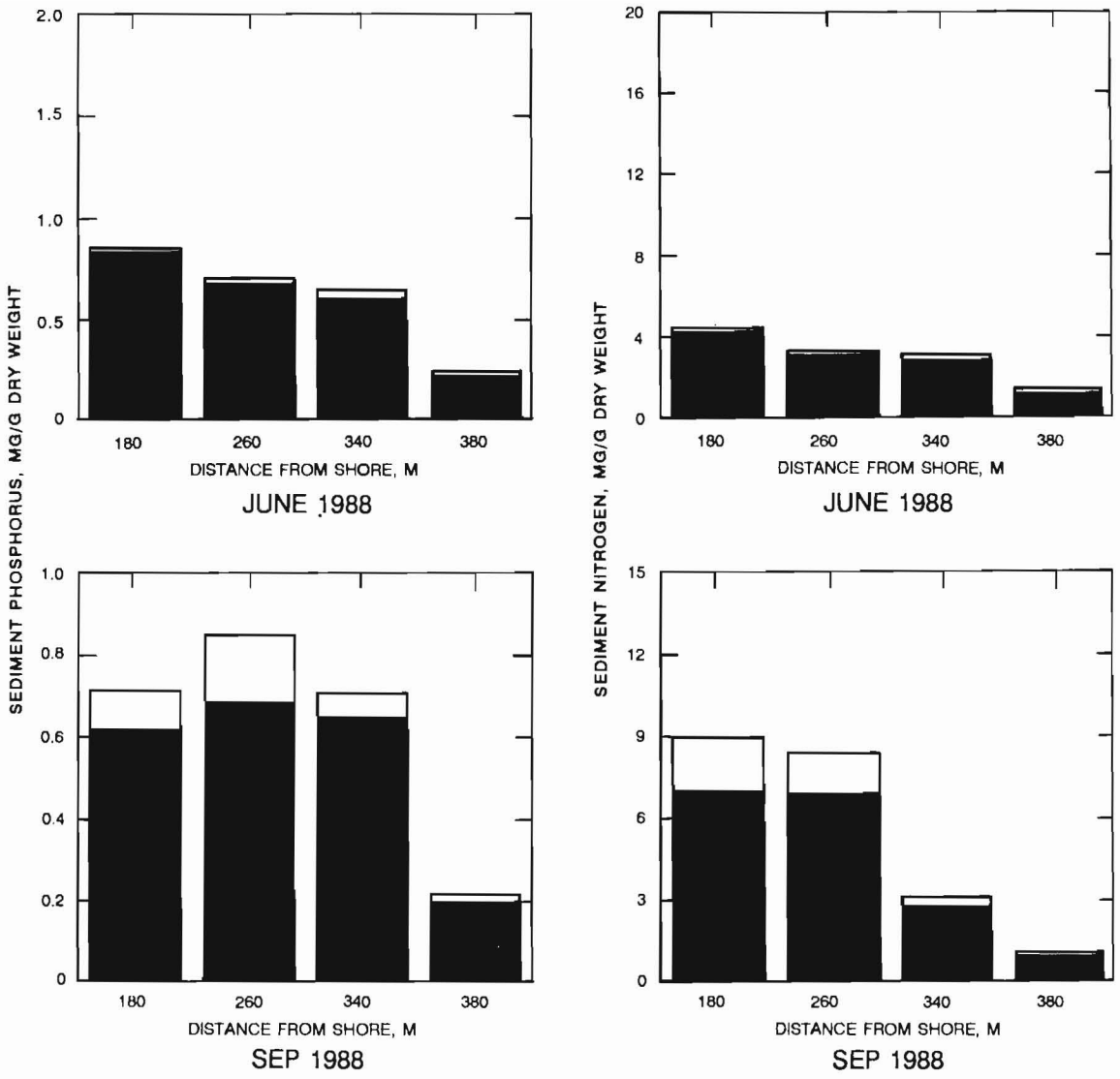

Figure 6. Concentration of total phosphorus and total nitrogen. Vertical bars represent means (n = 3) with associated standard deviations (open portions of bars)

ACKNOWLEDGMENTS

Grateful appreciation is extended by the authors to V. Carter and N. Rybicki of the US Geological Survey, H. Snyder of the US Park Service, and W. Taylor of the US Army Engineer Waterways Experiment Station (WES) for their contributions to study design and sampling and logistical assistance. Appreciation is also extended to G. Bird and K. Piggot of the WES for their analytical assistance and to G. Bird for her assistance with data management. W. Taylor and D. McFarland of the WES provided technical review of this manuscript.

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Influence of Submersed Macrophytes on Sedimentation Rates in a North-Temperate Reservoir: An Update

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

INTRODUCTION

Background

The filling of a lake basin with sediment is important to the expansion of aquatic macrophyte communities. As lakes age, productivity shifts in importance from phytoplankton to macrophytes due to decreased water storage capacity and associated expansion of colonizable area for macrophyte growth. Littoral vegetation may play an important role in stabilizing the sedimentary environment, promoting sedimentation, and mobilizing nutrients. Macrophytes stabilize their sedimentary environment by reducing turbulence that can resuspend and remove fine particles from the littoral sediments (Madson and Warncke 1983). Carpenter (1981) demonstrated that macrophytes are an important link in a positive feedback system that accelerates the accretion of colonizable sediment. Nutrients mobilized from the littoral sediments by submersed macrophytes (Barko and Smart 1980, Moeller and Wetzel 1988) can stimulate productivity and further sedimentation. Macrophyte decomposition products are also an important source of material input to littoral sediments (Godshalk and Wetzel 1984).

Since the littoral zone is subject to turbulence, sediment accretion can be reduced by erosional forces that act to remove fine particulate sediments to deeper areas of the lake. Many workers have observed greater sediment accretion in deeper areas than in shallow areas of lakes due to several mechanisms that influence the distribution of sediment (Likens and Davis 1975; Hakanson 1977; Hilton, Lishman, and Allen 1986; Moeller and Wetzel 1988). This pattern and differences in the physical and chemical composition of surficial sediment (Kamp-Nielson and Hargrave 1978) have been attributed to sediment focusing (Likens and Davis 1975). Since sediment accretion in a potentially erosional environment has received little attention, it was the purpose of this study to examine the role macrophytes play in stabilizing their sedimentary environment, promoting sediment accretion, and increasing their growth potential as a lake ages. This role was evaluated by comparing deep- and shallow-water areas of Eau Galle Reservoir.

Study site

Eau Galle Reservoir is a small US Army Corps of Engineers impoundment

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

located in west-central Wisconsin. Initially flooded in 1968, the reservoir is small (0.62 km^2), circular, and has a mean and maximum depth of 3.2 and 9 m, respectively. The sediment surface area is sinusoidal in shape, and over 60 percent of the sediment surface area is exposed to depths less than 4 m (Figure 1).

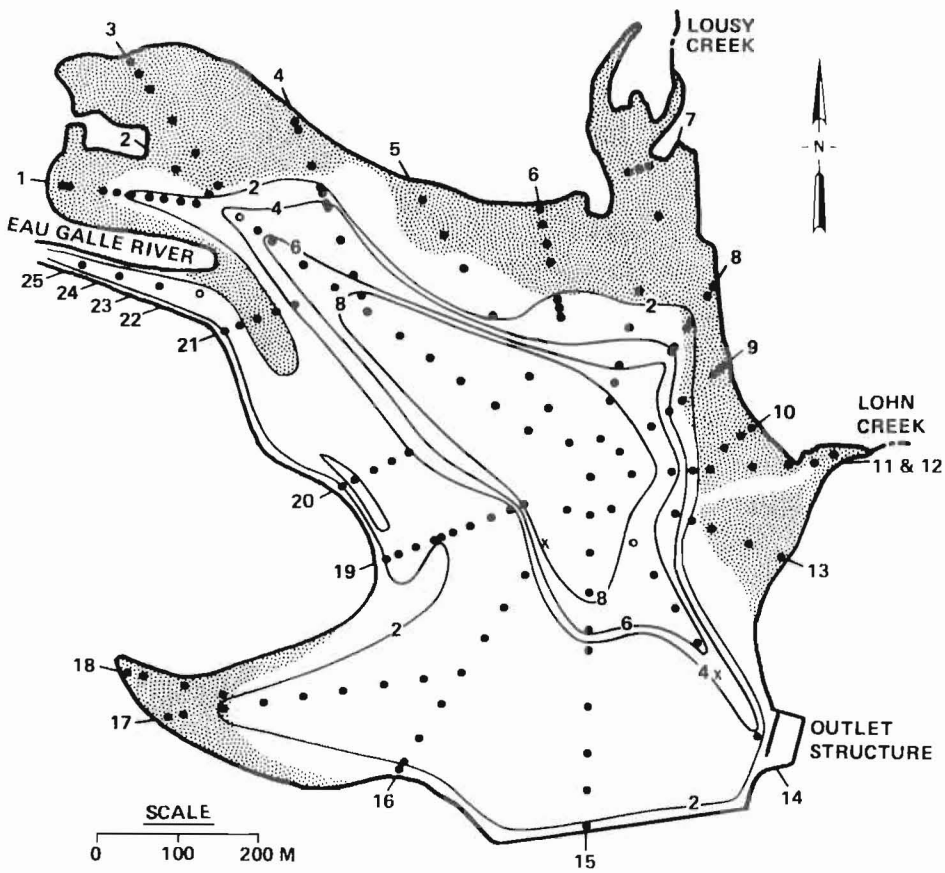

Figure 1. Morphometric map of Eau Galle Lake with transect and sampling locations. Shaded area represents extent of macrophyte growth

Steep depth gradients surround the deepest area of the reservoir between the 3.5- and 6-m depths. The Eau Galle River provides nearly 80 percent of the reservoir's gaged water income and drains a predominantly agricultural watershed (166 km^2). Littoral vegetation, mostly submersed, is extensive around the perimeter of the reservoir, with densities reaching 500 g dry weight/m^2 in some areas (Filbin and Barko 1985). *Ceratophyllum demersum* and *Potamogeton* sp. are the dominant species. The distribution of submersed macrophytes is irregular around the shoreline of the reservoir. Densities are greatest near the mouths of creeks and in sheltered bays. Submersed macrophytes are restricted by light to depths less than 2.5 m.

METHODS

Long-term sedimentation study

Sediment cores were collected under the ice in 1987 and 1988 at 50-m intervals along transects that radiated from the center of the reservoir to the shoreline (Figure 1). A total of 150 stations were sampled. Additional transects were placed near the mouth of the Eau Galle River (Nos. 20 and 21). These ran perpendicular to the shoreline and intersected transect 2 to determine riverine influences on sedimentation. Additional cores were collected within the zone of steep depth gradients and in the littoral zone. The littoral zone was delineated from aerial photographs, routine biomass determination (Godshalk, unpublished data), and grab sampling (Figure 1). A Wildco KB Sediment Core Sampler (Wildco Wildlife Supply Company), provided with a polyvinyl chloride core liner (5.08-cm O.D., 4.45-cm I.D., and either 50.8 or 91.4 cm in length), was used to collect samples. The sampler was dropped from the reservoir'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 reservoir. The depth of sample collection was recorded for each station.

Each core sample was sectioned at 5-cm intervals until parent material (i.e., preimpoundment soil) was encountered. Parent material consisted of hard clays, sands, pebbles, and roots and was characterized as having a moisture content of less than 30 percent. The sediment above the parent material was that which had settled during the reservoir's existence. Long-term sedimentation rates were calculated as the dry mass of sediment accreted above parent material divided by the area of the core tube and the reservoir age. Each core section above parent material was weighed immediately, then dried to a constant dry weight at 105° C. Subsamples were combusted at 550° C for 24 hr to determine the ash-free dry weight concentration (organic matter). Total iron, manganese, and calcium concentrations were determined by atomic absorption spectrophometry after digestion and reflux with nitric acid (American Public Health Association (APHA) 1985). Total phosphorus concentration was measured on a Technicon Autoanalyzer using the ascorbic acid method (APHA 1985) after digestion with sulfuric acid, potassium sulfate, and red mercuric oxide (Plumb 1981). The elemental concentration was multiplied by the respective dry weight mass for each core section above the parent material to obtain an elemental mass. The elemental masses were summed for each core and divided by the area of the core tube and reservoir age to determine elemental sedimentation rates.

Short-term sedimentation study

From the sediment core study (see results section), it was determined that long-term sedimentation rates were greater in the littoral zone and much lower in immediately adjacent nonvegetated areas (erosional zone). The erosional zone generally occurred just beyond the plant beds at depths less than 3.5 m. A secondary study was conducted to measure short-term seasonal variations in the

sedimentation rate in these two zones using sediment traps deployed in the reservoir between May and September 1988. Traps were positioned in the littoral zone at the 1-m depth and in an erosional zone at the 3-m depth in the northwest bay (near transect 4). Sedimentation traps consisted of three cylinders (33-cm height, 7-cm diameter, aspect ratio of 4.5) symmetrically attached to a metal frame. They were kept near the lake bottom with an anchor and held vertically with a buoy at the 3-m depth sites. At the 1-m depth sites, a pole driven into the sediment was used to anchor the traps. Two traps (three cylinders each) were sampled and redeployed at each depth at 2- to 3-week intervals. The contents were removed, homogenized, and subsampled for dry weight. Subsamples were filtered onto a precombusted glass fiber filter (Gelman A/E) and dried at 105° C to a constant weight. The dry weight mass (in grams) was divided by the days of deployment and area of the trap cylinder to calculate a sedimentation rate.

Short-term sediment dispersion study

The potential erosion of fine-textured sediment from the littoral and erosional zone (see results for delineation of depositional zones) was determined during summer 1988 by measuring the loss of sediment from pans. Bulk quantities of fine-textured sediment (characterized as having a moisture content of 74 percent and a bulk density of 0.29 g/ml) were collected from the littoral zone with a ponar sediment sampler. The sediment was screened (49-mm^2 mesh size) of large debris and completely homogenized by manual stirring. A known mass (2,700 g fresh weight) was placed into 15 pans measuring 6 × 23 × 32 cm. Sediment was filled to a depth of 1.5 cm from the top of each pan. Six of the pans were dried immediately at 105° C to a constant weight to determine a dry weight conversion factor. Three pans filled with fresh, wet sediment were deployed both in the littoral zone (1 m) and in the erosional zone (3 m and immediately adjacent to the littoral zone) near the sediment trap stations. The pans were secured to a 100-cm^2 mesh screen attached to a metal tubing frame. Each frame held the pans approximately 5 cm above the sediment to prevent contamination from disturbance of the bottom sediments during pan placement. Each sediment-filled pan was sealed with a lid and carefully placed onto a rack using scuba. The lids were removed after 10 to 15 min. This period allowed the settling of any sediment disturbed during the placement process. Three additional sediment-filled pans were subjected to the same deployment procedure, then dried to a constant weight (105° C) to determine any change that may have occurred during deployment. Less than 3 percent was lost from any pan and a mean of 0.6 percent was lost for all sampling periods. Pans were replaced on a 2- to 3-week rotational period. Sediment dispersion rates were calculated as the change in dry weight mass divided by the deployment period and the area of the pan.

RESULTS

Pronounced variations in the long-term sedimentation rate were apparent within the reservoir (Figure 2). Sedimentation rates were greatest in the deep, central

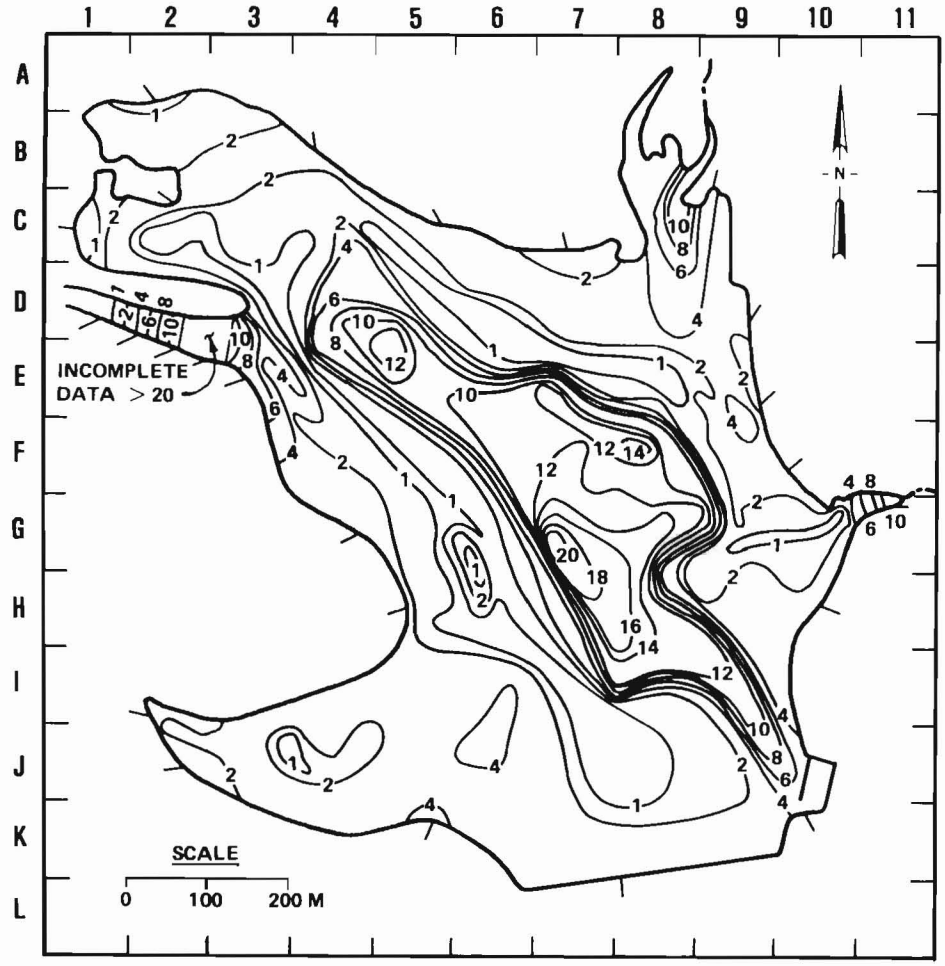

Figure 2. Contour map of spatial variations in the net dry weight sedimentation rate. (Each contour represents a sedimentation rate in units of kilograms per square metre per year)

area of the reservoir (>6 m, near area 7G) and declined with both decreasing depth and decreasing distance from the shoreline. Sedimentation was also high at the mouth of the Eau Galle River (2D and 3D) and the various creeks (8C and llG). Steep sedimentation gradients were observed between the 3.5- and 6-m depth contours. Within shallow, nonvegetated areas (less than 3.5 m), sedimentation rates were relatively low as illustrated for areas 2C, 6D, 8E, and 3J. This area was typically located just beyond the littoral zone. Within the littoral zone between the shoreline and the 3.5-m depth, however, elevated sedimentation rates occurred. Four distinct depositional zones could be defined within the reservoir from these observations: an accumulation zone, a transition zone, an erosional zone, and a littoral zone (Figure 3). Mean organic matter and elemental sedimentation rates were greater in deep water than in shallow water (Figure 4). However, sedimentation rates in shallow water were elevated in the littoral zone relative to the erosional zone. In particular, dry weight, ash-free dry weight

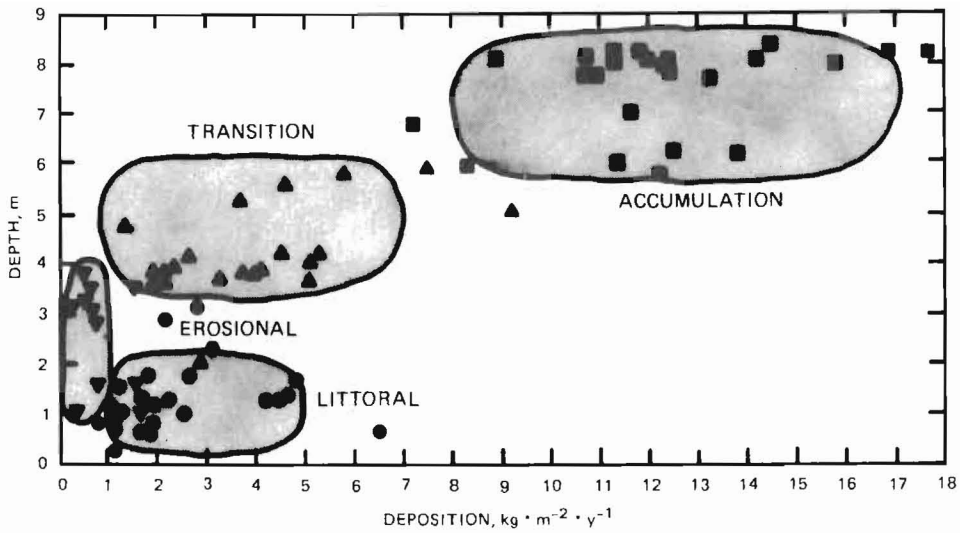

Figure 3. Variations in the dry weight sedimentation rate (kilograms per square metre per year) with depth (metres) for the lacustrine area of the lake. Riverine stations are not included. Shaded areas represent the accumulation, transition, erosional, and littoral depositional zones

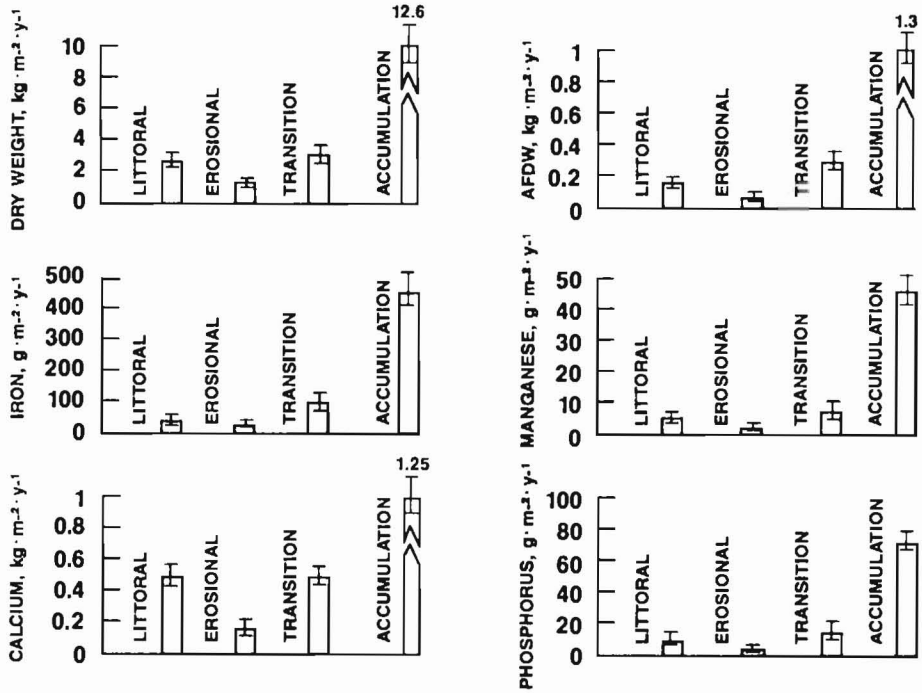

Figure 4. Annual means and standard errors for dry weight, ash-free dry weight, total iron, total manganese, total calcium, and total phosphorus sedimentation rates within the accumulation, transition, erosional, and littoral depositional zones

(AFDW), and calcium sedimentation rates were substantially higher in the littoral zone than in the erosional zone.

The nutritive and physical quality of surficial sediments (0 to 5 cm) in the littoral and erosional zones exhibited very different characteristics (Figure 5). Mean moisture content was high in the littoral zone (64 percent) and less than 50 percent in the erosional zone. Conversely, mean bulk density was only 0.41 g dry weight/ml in the littoral zone and very high in the erosional zone (0.70 g dry weight/ml). These contrasting characteristics indicated that sediments in the littoral zone were of predominantly fine-textured clays and silts while those of the erosional zone were coarser and more dense. Mean concentrations of organic matter, manganese, calcium, and phosphorus of the surficial sediment were also higher in the littoral zone. Surficial sediments in the erosional zone exhibited the lowest elemental and organic matter concentrations.

Sedimentation rates were divided by depth to correct for differences in water volume (i.e., depth above sediment surfaces). This calculation essentially normalized sedimentation rates with depth and allowed for direct comparisons between depositional zones. Mean elemental depth-weighted sedimentation rates were very high in the littoral zone and comparable to those measured in the accumulation zone (Figure 6). In contrast, the erosional zone displayed the lowest elemental depth-weighted sedimentation rates.

Marked differences in the loss of fine-textured sediment were apparent between the littoral and the erosional zone. Seasonal variations in the short-term sediment dispersion rates, measured from pans placed on the reservoir bottom, are shown in Figure 7. At the station in the erosional zone, the rate of sediment dispersion was high in May (272 $g/m^2/day$), during vernal mixing, and remained greater than 100 $g/m^2/day$ throughout the summer. Rates of sediment dispersion increased to 242 $g/m^2/day$ during convective cooling and fall overturn. At the littoral zone station, the rate of sediment dispersion was high in May when macrophyte biomass was low. However, the sediment dispersion rate decreased to a minimal value as macrophyte biomass increased during the summer. Sediment gains in the pans were observed at the littoral zone station in July, August, and September 1988.

The trends in short-term sedimentation rates, measured directly with sedimentation traps, were opposite to the patterns suggested by the dispersion rate studies (Figure 8). Trap rates were higher at the littoral zone station than at the erosional zone station from June until late August 1988. During periods of water column mixing (May and September), trap rates were higher at the erosional zone station.

DISCUSSION

The most striking pattern observed in this investigation was the delineation of distinct depositional zones within the reservoir. These were separated physically (i.e., with respect to water column depth and distance from shoreline) and by

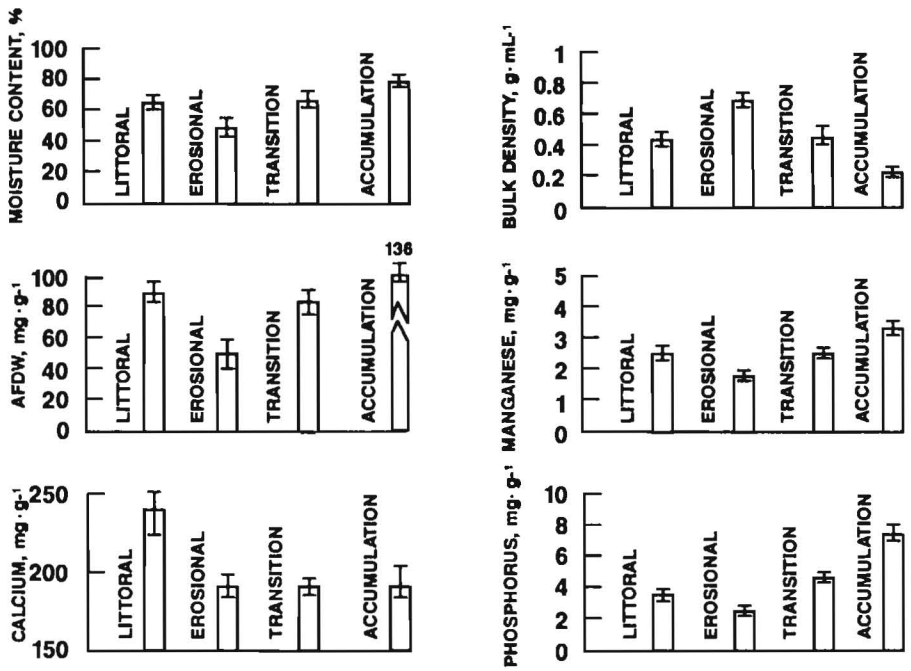

Figure 5. Means and standard errors for percent moisture content, bulk density, and ash-free dry weight, total manganese, total calcium, and total phosphorus concentrations (milligrams per gram dry weight) for the surficial sediments (0 to 5 cm) within the accumulation, transition, erosional, and littoral depositional zones

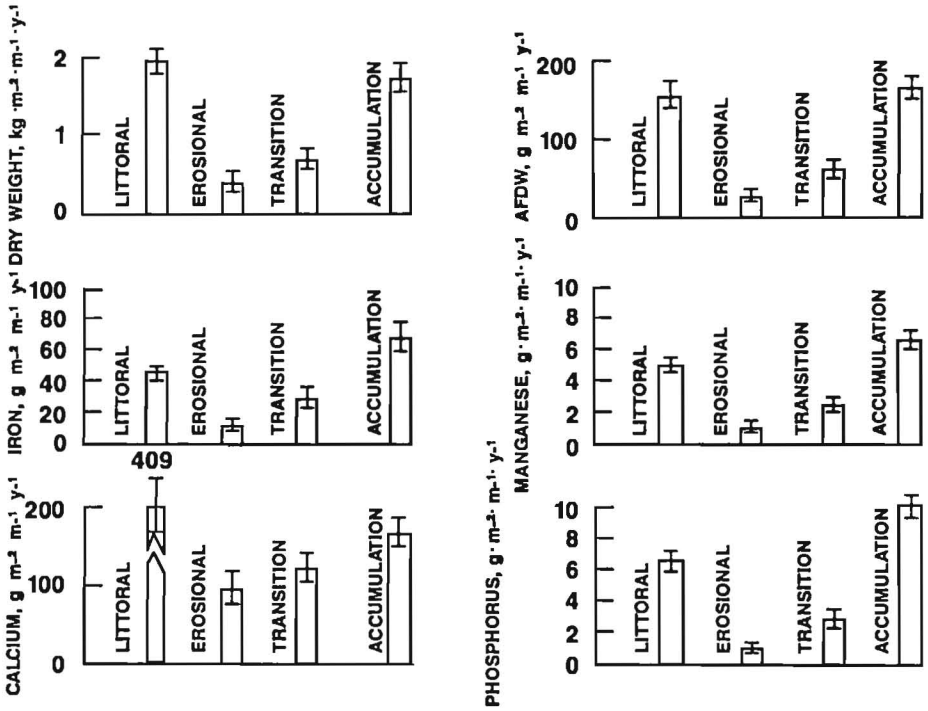

Figure 6. Annual means and standard errors for dry weight, ash-free dry weight, total iron, total manganese, total calcium, and total phosphorus depth-weighted sedimentation rates within the accumulation, transition, erosional, and littoral depositional zones

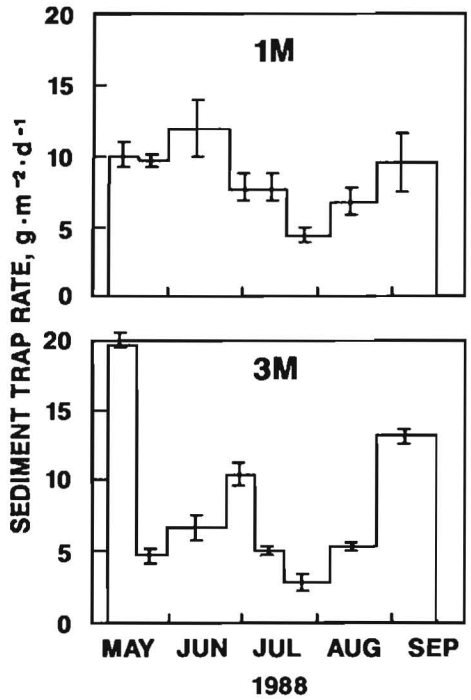

Figure 7. Mean sediment dispersion rates and standard errors measured from sediment pans placed at the 1-m depth in the littoral zone and the 3-m depth in the erosional zone

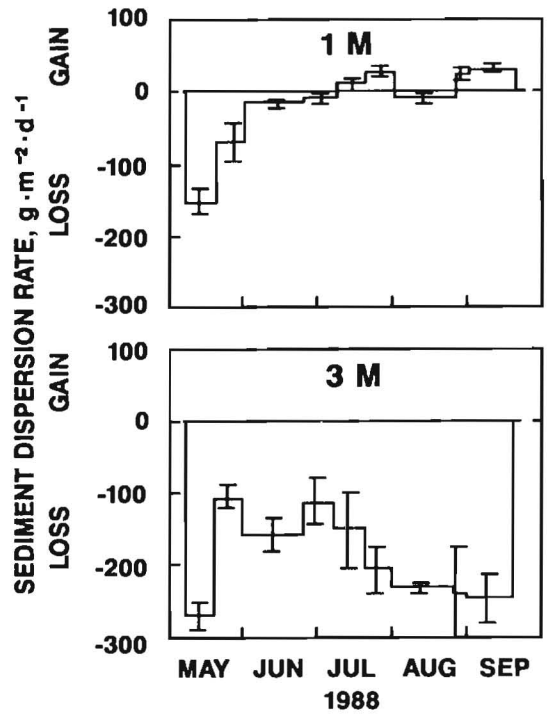

Figure 8. Mean sediment trap rates and standard errors measured from cylinders placed at the 1-m depth in the littoral zone and the 3-m depth in the erosional zone

sediment composition. Hakanson (1977) found similar depositional patterns in Lake Vanern, Sweden. He indicated that sedimentation was influenced by basin morphometry and water turbulence, which create erosional and depositional environments. Sediments in the erosional zone of Lake Vanern were found to be poorly sorted, exhibiting a moisture content ranging from 40 to 50 percent. Sediment in the depositional zone consisted of fine-grained particles and had a moisture content of 60 to 75 percent.

Although sediment in Eau Galle Reservoir is potentially subjected to erosional forces at depths of less than 3.5 m (Gunkel, Gaugush, and Kennedy 1984), littoral areas exhibited markedly higher sedimentation rates than shallow, nonvegetated areas. These differences strongly suggested that macrophytes were decreasing erosion and/or enhancing sedimentation in the littoral zone. The percent moisture content and bulk density of the surficial sediments within the littoral zone reflected the occurrence of fine particles which would not be expected to remain in an erosional environment (Hakanson 1977). Percent moisture content, chemical composition, 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 erosional zone. Barko and Smart (1986) found that sediment composition strongly affects macrophyte growth. Sediments exhibiting a high percent moisture content, a bulk density less than 0.9 g/ℓ, and a low organic matter content appear to provide conditions that are favorable for nutrient uptake

by rooted submersed plants. They found that macrophyte growth can be restricted on sandy sediments exhibiting a bulk density greater than 0.9 g/ℓ.

While mechanisms of erosion and sedimentation are important in explaining spatial variations in the sedimentation rate, the depth of the water column, and thus mass of particles above the sediment surface, can also be important. For instance, a higher rate of sedimentation in the deep basin of a lake or reservoir can be reflective of a greater mass of particles settling from the water column as well as sediment focusing (Likens and Davis 1975). Sedimentation rates could be lower in shallow regions, even though the seston concentrations and settling velocities do not differ from those of the deeper region, because there is less water volume above the sediment surface, and hence less mass that can settle. Variations in the depth-weighted sedimentation rate at Eau Galle Reservoir suggested that sediment accretion was very high in the littoral zone and comparable to depth-weighted rates observed in the accumulation zone, indicating that both areas of the reservoir were zones where sediment accretion was enhanced. The erosional zone, however, exhibited the lowest depth-weighted rates, indicating that erosional forces were reducing sediment accretion in shallow, nonvegetated areas of the reservoir.

Macrophyte biomass was important in the retention of fine particulate material in the littoral zone. This is of ecological significance to plant growth in a potentially erosional environment. Loss of fine-textured sediment from sediment dispersion pans was substantially reduced in the littoral zone as macrophyte biomass increased. In other studies, dense macrophyte beds have been shown to effectively reduce current velocities and erosional forces that would otherwise act to resuspend fine particulate material (Madson and Warncke 1983). As a consequence, macrophytes can accelerate the deposition of fine particulate material (Gregg and Rose 1982).

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 (Carpenter 1981).

ACKNOWLEDGMENTS

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COMPUTER-AIDED SIMULATION PROCEDURES FOR AQUATIC PLANT MANAGEMENT

Simulation Technology Development: An Overview

by
R. Michael Stewart*

INTRODUCTION

Designing and conducting effective aquatic plant management operations is extremely complex. Not only must managers ensure that treatment techniques are effective under prevailing environmental conditions, they must also prevent detrimental impacts to nontarget components of the ecosystem. To complicate matters further, time and monetary restrictions hinder managers' efforts to evaluate the effects of modifying proven treatment techniques, even when circumstances/results indicate that minor modifications may improve efficacy, reduce costs, or limit nontarget impacts.

To help aquatic plant managers overcome these restrictions, the Waterways Experiment Station (WES) is developing personal computer (PC)-based simulation procedures that will quickly provide information necessary for reaching a sound decision. Information generated by the software systems will furnish answers to "what if" questions managers most probably would ask in the process of selecting a treatment technique for application. Some example questions are (a) When will this particular aquatic plant population reach a problem level? (b) Since I have a particular mechanical control system, how expensive and time consuming will it be to harvest submersed plants from this particular water body? (c) How effective will a particular biological control agent be during this growing season and during the following years? and (d) If I treat with herbicides on this day, how long before I get control, and when will I need to treat again during the growing season?

Development of computer-based simulation procedures was initiated by WES in the 1970s. Two software packages developed by WES and made available to interested users are HARVEST and AMUR/STOCK. HARVEST (Version 1.0) is a mechanical control simulation model that provides information on time and cost requirements of mechanical harvesting operations for submersed aquatic plants (Hutto 1982, 1984; Sabol 1983; Sabol and Hutto 1984). AMUR/STOCK (Version 1.0) is a biological control simulation model that provides information useful for determining proper stocking regimes of white amur for control of hydrilla (Miller and Decell 1984). Widespread interest in these two models resulted in transfer of over 150 IBM PC-compatible copies to users between Fiscal Years (FY) 1983 and 1988 (Figure 1). As illustrated in Figure 2, various organizations are interested in using these simulation models.

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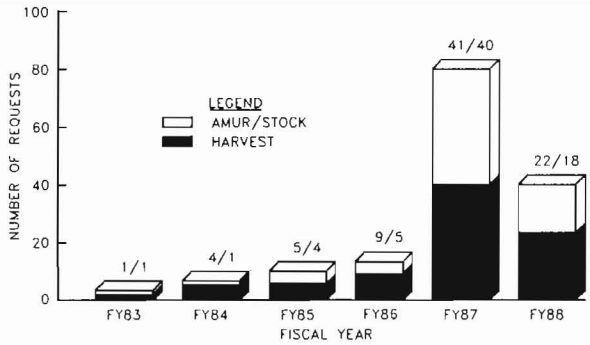
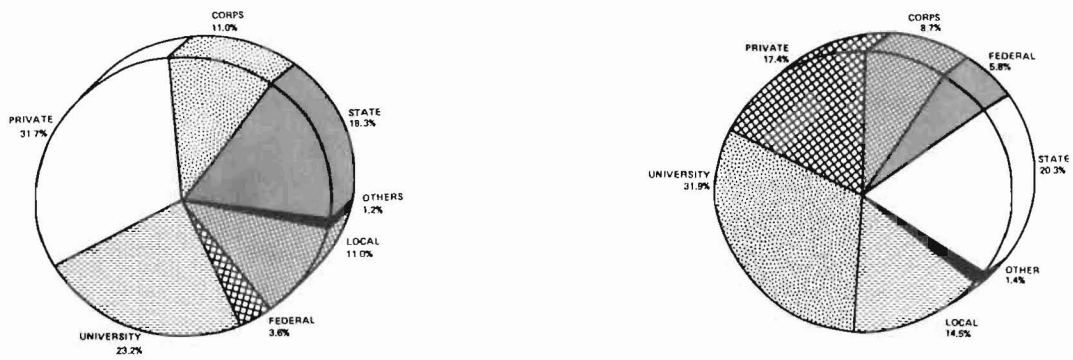


Figure 1. Numbers of diskette copies of HARVEST and AMUR/STOCK models transferred to users, FY 83-88



a. HARVEST model **b. AMUR/STOCK model**

Figure 2. Percentage, by organization type, of total requests for aquatic plant control simulation models, FY 83-88

Work is currently in progress to develop simulation procedures for additional problem aquatic plant species and treatment techniques. Stewart (1988) gives a listing of the specific aquatic plants and control techniques for which simulation procedures are planned. To accomplish these tasks, WES has established technical areas in (a) plant growth simulation research, (b) biological control simulation research, (c) chemical control simulation research, and (d) aquatic plant data base development. Work completed under each of these technical areas during FY 88, and work planned for FY 89, is briefly presented in the following sections.

PLANT GROWTH SIMULATION RESEARCH

FY 88 accomplishments

Waterhyacinth plant growth model. Validation and improvement of the waterhyacinth plant growth model was continued using a 2-year data set collected from six sites in north and south Florida. Additional tests of the software were conducted using data from a field site in southeastern Texas (Grodowitz and Stewart 1989). Model simulations compare well with these field data during the growing season; however, comparisons indicate that relationships for respiration,

detritus production, and biomass distribution (within plant parts) need improvement to depict unique conditions during cold-weather (i.e., low-temperature) months.

Hydrilla plant growth model. The hydrilla plant growth model was tested using data collected during 1977 from Pool 4, Lake Conway, Florida. This work is presented in greater detail in Wooten (1989). Improvements made to the hydrilla growth model during this effort include the addition of (a) graphics routines that allow visual interpretation of simulation results and (b) relationships that simulate winter "dieback" of hydrilla plants in the water column. In most respects, the hydrilla growth model produced simulations that compared well with the limited field data available from Lake Conway. Differences between simulation results and field data have not been fully analyzed. A possible factor that needs future evaluation deals with relationships used for establishing initial tuber density. In general, further improvements of the model are required and will be accomplished.

FY 89 scheduled work

Existing plant growth models. The main focus of planned research on existing plant growth models will be collection of field data for continued validation and improvement. Cooperative efforts will he initiated with other WES research groups at the newly acquired Lewisville Aquatic Plant Research Facility. This facility will allow collection of waterhyacinth growth data under controlled field conditions, including no impacts from *Neochetina* and other control agents. The facility will include a fully functional laboratory that will allow collection of growth data needed for refinement of all major algorithms in the model. If the currently proposed cooperative efforts with the Tennessee Valley Authority are initiated, extensive data collection will be conducted at Guntersville Reservoir, Alabama, in support of continued improvement of the hydrilla plant growth model.

Eurasian watermilfoil plant growth model. Development of a first-generation plant growth model for Eurasian watermilfoil will be initiated. This model will be structured similarly to the hydrilla model, allowing it to function either as independent plant growth model or as an interactive component within selected biological and chemical control models.

BIOLOGICAL CONTROL SIMULATION RESEARCH

FY 88 accomplishments

The INSECT model was tested against 2-year data sets collected from six sites in Florida and one site in southeastern Texas. Initial validation efforts with these data indicated that the simulated *Neochetina* development through the four life stages did not fully agree with field observations. Modifications were made to *Neochetina* initialization algorithms during FY 88. Additional work on the *Neochetina* module will be conducted in FY 89, focusing on improvement of algorithms for *Neochetina* development and interactions with waterhyacinth.

FY 89 scheduled work

Improvements to INSECT. Validation efforts to date demonstrate the complexity of the waterhyacinth/*Neochetina* system. Specific areas needing additional basic research are given in Akbay, Wooten, and Howell (1988) and in Grodowitz and Stewart (1989). Cooperative efforts by WES will be initiated during FY 89 to collect this basic information.

Development of AMUR/STOCK. The AMUR/STOCK model generates simulations useful in determining proper stocking regimes of the white amur for control of submersed aquatic plants. Currently, two versions of this model have been developed. As stated earlier, AMUR/STOCK (Version 1.0) was designed for determining proper stocking regimes for control of hydrilla and is available to interested users. AMUR/STOCK (Version 2.0) has been under development by WES since FY 87. This model has previously been identified in the literature as HYDAMUR (Wooten and Akbay 1988). Further improvement of this model will continue during FY 89 using data from planned research at Lake Marion, South Carolina, and Guntersville Reservoir, Alabama. Additionally, development will be initiated on AMUR/STOCK (Version 3.0). This model will contain algorithms useful in determining stocking regimes for control of Eurasian watermilfoil. Field data from Guntersville Reservoir will be used in development of this model.

CHEMICAL CONTROL SIMULATION RESEARCH

FY 88 accomplishments

Studies were conducted to validate 2,4-D fate and effects algorithms included in HERBICIDE (Version 1.0). This model (previously referred to as FATE) is described in Rodgers, Clifford, and Stewart (1988). In addition to the work described by Clifford, Rodgers, and Stewart (1989), extensive pre- and post-application field sampling was conducted in association with a 2,4-D treatment to waterhyacinth near Wallisville, Texas. These data will provide a data set that is representative of operational field conditions.

FY 89 scheduled work

Validation of HERBICIDE (Version 1.0). Samples collected during the Wallisville, Texas, field study will be analyzed. Data from this study will be used to validate the fate/effects algorithms. Based on this effort, the need to develop algorithms for herbicide interception by the waterhyacinth canopy (see Clifford, Rodgers, and Stewart 1989) will be determined. Additionally, the model will be modified to allow simulation of multiple herbicide applications in a single growing season.

Further development of HERBICIDE. Plans for the model include the addition of algorithms for other herbicides and target plants. Fate/effects algorithms will be developed for diquat application to waterhyacinth and for 2,4-D (BEE), diquat, endothall, and fluridone application to hydrilla and Eurasian watermilfoil. Plant

response (i.e., regrowth) modules for hydrilla and Eurasian watermilfoil will be added as the plant growth models for these two aquatic plants are completed.

AQUATIC PLANT DATA BASES

FY 88 accomplishments

A digital data base structure was designed that will provide a method for storing environmental data related to aquatic plant management activities. To meet planned application requirements, the data base structure will allow (a) accessing of stored data by aquatic plant control simulation models, (b) information from specific geographic locations to be cross-referenced to all other information for that location, and (c) easy data update and addition. Kress, Ballard, and Causey (1989) describe the data base structure and provide example applications for water bodies in central Florida.

FY 89 scheduled work

Environmental data from a site in the southeastern United States will be compiled and included in the data base for demonstration. Likely sites include Lake Marion, South Carolina, and Guntersville Reservoir, Alabama. Site-specific environmental data will include meteorological conditions, water depth, and available water quality and substrate information, as well as water body use information. Aquatic plant distribution data will be interpreted from historical survey records, including aerial photography and digital LANDSAT-TM data.

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Applications of INSECT, A Computer Model of Waterhyacinth and *Neochetina* Population Dynamics

by
Fred G. Howell* and R. Michael Stewart**

INTRODUCTION

INSECT is a computer simulation model designed to reflect certain aspects of the population dynamics of waterhyacinth and *Neochetina* over extended time periods. The model has been under development by the Waterways Experiment Station (WES) since 1985. The philosophy of model development, the general model structure, and the intended applications of INSECT are presented in Akbay, Wooten, and Howell (1988). A brief overview of the model can be found in Howell, Wooten, and Akbay (1988). Results of validation efforts with field data from north and south Florida are included in Howell, Akbay, and Stewart (1988) and in Howell and Akbay.† Additional validation of INSECT is being conducted by WES with field data from Texas (Grodowitz and Stewart 1989).

Several changes to the INSECT model have been made during these validation efforts. In many cases, only minor changes have been required to produce close agreement between simulations and field data. As should be expected, however, our current understanding of the waterhyacinth/*Neochetina* system is limited in many areas. Thus, additional basic research will be necessary before a final software package of INSECT can be completed.

As development continues, applications of this model will be emphasized. Intended applications of INSECT are presented in this paper under two broad categories: (a) research and development and (b) technology transfer.

RESEARCH AND DEVELOPMENT

Embodiment of current knowledge

When properly constructed, simulation models become monuments to the progress and contributions made by a research program toward its stated goals. Simulation models function as the embodiment of knowledge generated from a diversity of sources that explain system dynamics. In this regard, the model provides ready access to pertinent information that defines how components of a

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†F. G. Howell and K. S. Akbay. 1988. "Computer Simulation Modeling of Biological Control Agents of Nuisance Aquatic Plants," prepared for US Army Engineer Waterways Experiment Station, Vicksburg, Mississippi.

system interact. In INSECT, an attempt has been made to incorporate relationships which define the interactions between waterhyacinth and *Neochetina* and their physical and biological environments. The synthesis of information collected by researchers of waterhyacinth and *Neochetina* over the years into a functional system should be regarded and appreciated as the initial application of the INSECT model.

Examination of current knowledge

Once a workable model is developed, researchers are able to examine the complexities of the system more deeply. The model provides a systematic method for evaluating relationships derived from results of previous research and observation. In many cases, simulation models support and strengthen confidence in previous conclusions and highlight specific relationships needing further investigation through basic research. Though most programs with active research and development elements would have prior knowledge of system relationships not completely understood, a simulation model, through its ability to represent system interactions, provides a tool for understanding the consequences of the information and relationship deficiencies. In cases where simulations do not compare well with field data, model results are useful in determining areas for additional research.

In this regard, INSECT has successfully highlighted several components of the waterhyacinth/*Neochetina* system that need enhancement. Specific research areas are presented in Akbay, Wooten, and Howell (1988), Howell, Akbay, and Stewart (1988), and Grodowitz and Stewart (1989).

TECHNOLOGY TRANSFER

Illustration of plant/insect interactions

In its current form, INSECT can be used to illustrate some of the expected impacts of a variety of environmental factors on the operation of the waterhyacinth/*Neochetina* system. Simulation results can strengthen the conceptual understanding of the importance of weather conditions, geographic region, and other factors on the development and growth of waterhyacinth and *Neochetina*. A user might ask, "What impact does adverse weather have on waterhyacinth biomass and weevil densities?" To illustrate the effects of weather on waterhyacinth and *Neochetina* populations as estimated by the model, two simulations were conducted in which all input data were held constant except for weather data. One simulation used a 1976 north Florida weather data file; the other used a 1979 north Florida weather data file. In comparison, winter conditions were more severe and continued longer into the growing season in 1979 than in 1976. Figure 1 illustrates the differences in predicted plant biomass for the two simulations. Biomass in 1979 did not begin its seasonal increase until much later in the growing season. Additionally, though maximum biomass in 1979 approached 1976 simulated levels, the delay in spring regrowth initiation resulted

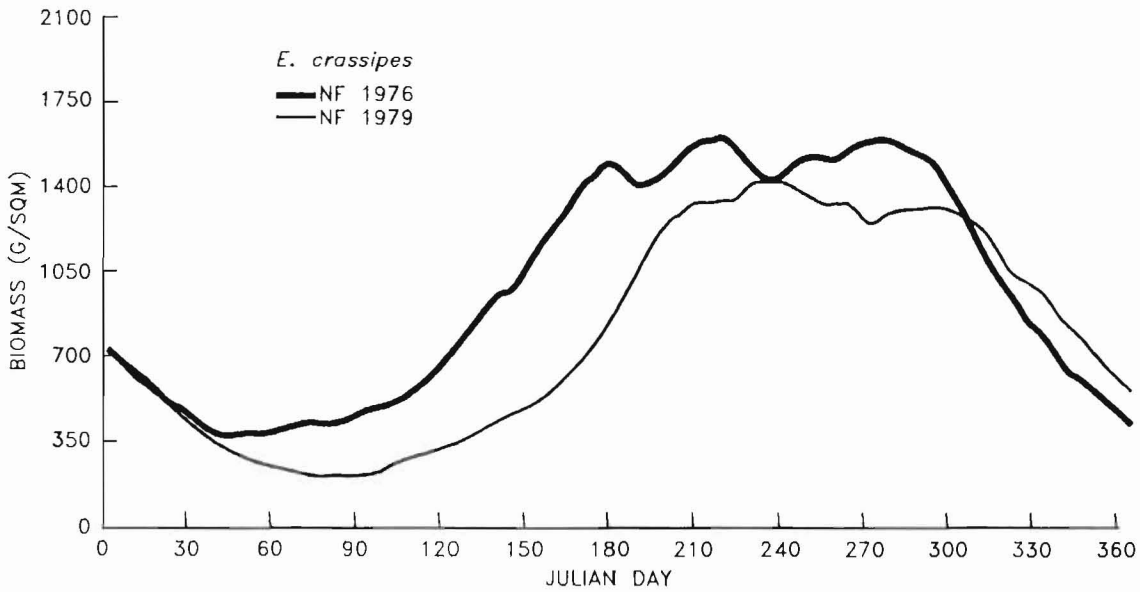

Figure 1. Simulation results for waterhyacinth biomass over a 1-year period (365 Julian days). Simulations were conducted using different weather data files: 1976 north Florida and 1979 north Florida. Other initialization data were identical for both simulations

in a shorter duration at the maximum biomass level before initiation of fall senescence. Figure 2 shows the model predictions of the two weather sets for adult weevil densities. Weather conditions (i.e., average daily temperatures) were more favorable to weevil development in 1976 than in 1979. The 1976 simulation shows a second adult peak near Julian day (JD) 240 that was absent in the 1979 simulation. Further, the peak occurring near JD 280 was considerably higher for the 1976 simulation.

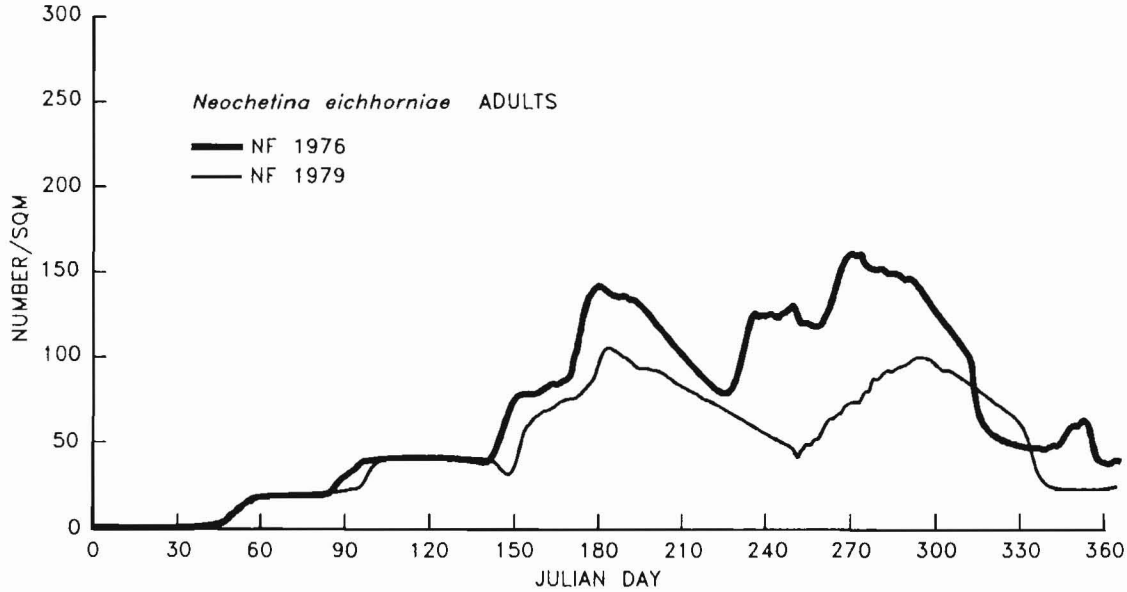

Figure 2. Simulation results for adult *Neochetina* densities over a 1-year period (365 Julian days). Simulations were conducted using different weather data files: 1976 north Florida and 1979 north Florida. Other initialization data were identical for both simulations

From examination of Figure 2, one might assume that differences in weather conditions were primarily responsible for all differences in simulated adult weevil densities. Rather, since the model simulates the interaction between the weevil and plant components of the system, a portion of the differences in weevil densities stems from the direct impacts of weather on the plant population. One point worthy of note is that INSECT users should have an understanding of the assumptions used in the model so that proper interpretation of results can be made.

Predicting impacts to waterhyacinth

The inability to predict the impacts of *Neochetina* on waterhyacinth infestations under field conditions has limited its use in water bodies where plant infestations must be maintained below specific threshold levels. In general, use of *Neochetina* has been restricted to low-use areas where maintenance of waterhyacinth below specific levels is not a requirement. In areas with specific threshold levels, chemical and/or mechanical methods have been used because these control methods produce reliable impacts on treated plant infestations.

The primary objective for initiating development of the INSECT model was to develop a predictive tool for *Neochetina* impacts on waterhyacinth. Once completed, information provided by the model will help users confidently and effectively use *Neochetina* as an operational control agent of waterhyacinth. In practice, data collected from field sites will be used to initialize the model. The simulation can then help to predict the impacts that a resident *Neochetina* population will have on the waterhyacinth infestation through time. With this information, operational control activities can be structured to achieve the maximum benefits from this biological control agent.

Development/application of new control techniques

Research in progress at WES is investigating the effects of chemical applications on waterhyacinth biological control agents (Cofrancesco and Pellessier 1988). One of the more intriguing theories being investigated is that application strategies can be designed which include the concurrent use of chemical and biological control agents of waterhyacinth in a compatible manner. The philosophy here is that chemical applications can be "timed" to critical events in the development cycle of *Neochetina* and, in so doing, result in less direct and indirect detrimental impact to the insect population (see Grodowitz and Pellessier 1989). By limiting the detrimental impact of the chemical control application to the biological control population, continued impact to the target plant is not disrupted.

The INSECT model can be used to predict the timing of the critical events in the development cycle of *Neochetina*. Figure 3 illustrates the dynamics of the pupal and adult stages over a 1-year period. Notice a dramatic shift from the immobile pupal stage to the mobile adult stage near JD 160. If it is true, as previously speculated (Haag 1986), that mobility of the adult life stage will allow it

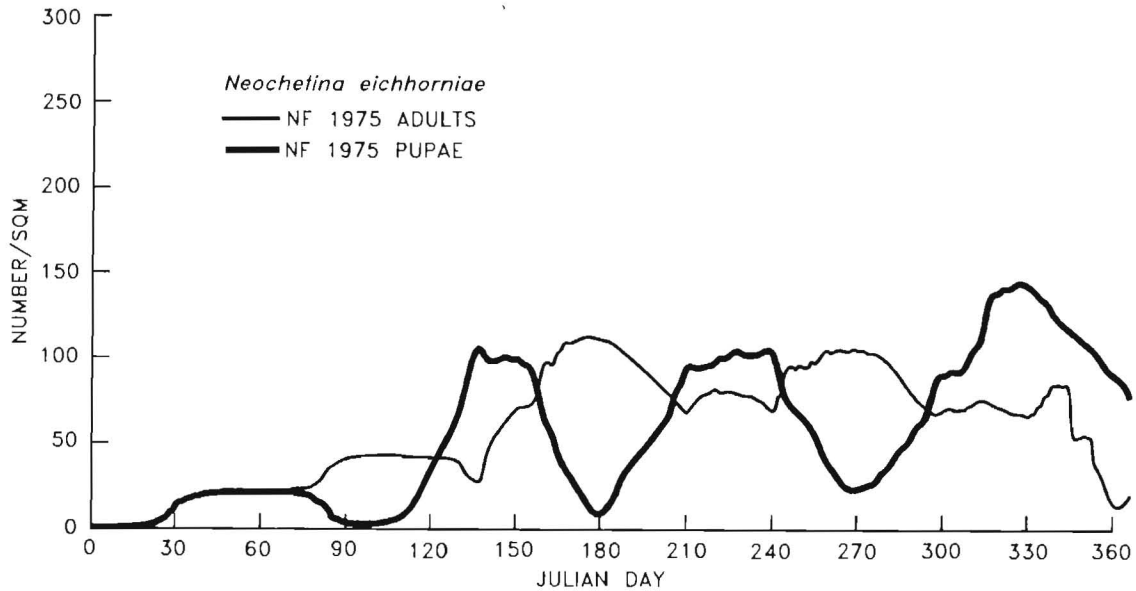

Figure 3. Simulation showing the cycling of pupal and adult development stages over the course of a 1-year period (365 Julian days). Note the dramatic shifts in life stage dominance during the annual cycle

to avoid impact from a chemical application, information available from INSECT simulations can aid in specifying compatible, concurrent applications of biological/chemical techniques for site-specific conditions.

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Validation Studies in Texas for the Model INSECT

by
Michael J. Grodowitz* and R. Michael Stewart*

INTRODUCTION

Background

INSECT is a first-generation mathematical model describing population dynamics and interactions between waterhyacinth and associated insect biocontrol agents, specifically *Neochetina eichhorniae* and *Neochetina bruchi* (Howell, Wooten, and Akbay 1987; Akbay, Wooten, and Howell 1988; Howell, Akbay, and Stewart 1988). With INSECT's development, as with any model, it is important that the predictive ability be checked or validated against actual field data (observed) describing insect and plant population dynamics. Toward this goal, validation data have been or are currently being collected and analyzed from two geographical regions in Florida (Howell, Akbay, and Stewart 1988) and from one location in southeastern Texas (Grodowitz 1988).

An important first step in comparing model output with actual field data is the complete analysis of the observed data. Toward that end, the current paper will concentrate on examining, in detail, the changes in waterhyacinth and associated insect biocontrol agent populations found at a site in southeastern Texas during 1987 and 1988. More detailed comparison of field observations to output from INSECT is presently in progress. In addition, future studies planned at this site and other sites in southeastern Texas will be presented. These studies are basically designed to further the conceptual understanding of the insect/plant interactions for later model incorporation.

Study site

The study site is an approximate 5-acre borrow pit located within the US Army Corps of Engineers Wallisville facility, situated ca. 40 miles east of Houston, Texas, directly off Interstate 10. The borrow pit has been inundated with waterhyacinth for more than 2 years. The site has little access to external flowing water systems, a condition which could confound results.

METHODS AND MATERIALS

Study plan

Samples were taken monthly, beginning in June 1987 and continuing through August 1988. The borrow pit was subdivided into three subsites, or blocks, based

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

on differences in plant canopy height observed in June 1987. At each subsite, three sets of adjacent 0.25-m^2 paired samples were collected. One of each pair was used to determine plant biomass; the other was used to determine insect density. Within the 0.25 m^2, all plants 50 percent or more within the sampling frame were placed into plastic bags; then, plants were taken to a remote facility for processing. This sampling procedure was repeated for each subsite in the borrow pit. The samples from each subsite were averaged and used in a randomized complete block design; i.e., each subsite mean represented a block value.

Weather measurements

Weather data were collected using a Campbell Scientific Model CR-21X data logger. Data were collected once per minute, averaged hourly, and subsequently downloaded on either cassette tape or storage module. The data were then transferred to an IBM-AT computer for final manipulation. Various parameters were measured, including temperature (Figure 1) and solar loading (Figure 2).

Plant measurements

Various plant measurements were determined, including density (plant number/m^2) and total biomass (kg/m^2). Plant biomass was subsequently partitioned into living above-water biomass (LAWB, kg/m^2), below-water biomass (BWB, kg/m^2), and total dead weight (TDW, kg/m^2). Plant material was considered dead if 50 percent or more of the plant part was brown. Weight per plant was calculated from density and total biomass data. All weights were reported dry and were calculated from fresh weight measurements by multiplying by 0.05.

Insect measurements

All plants from the 0.25 m^2 were carefully examined for insects of various life stages. Numbers of adult male and female individuals, pupae, and third instars were quantified, and these counts were subsequently put on a square-metre basis.

Statistical analysis

The data were analyzed as a randomized complete block design where the average of each subsite represented a single block value. Differences between means were determined using a Least Significant Difference test statistic calculated from the pooled standard error of the mean. All analyses were done on the Statistical Analysis System (SAS) using the General Linear Model procedure (SAS Institute 1982).

RESULTS

Significant changes in total dry plant weight were observed ($P < 0.0001$; Figure 3). Total dry plant weight/square metre was relatively constant during 1987, with values ranging from 0.70 to 0.92 kg/m^2 (Figure 3). At the start of the the 1988 growing season, increasing biomass was noted, with peak values of

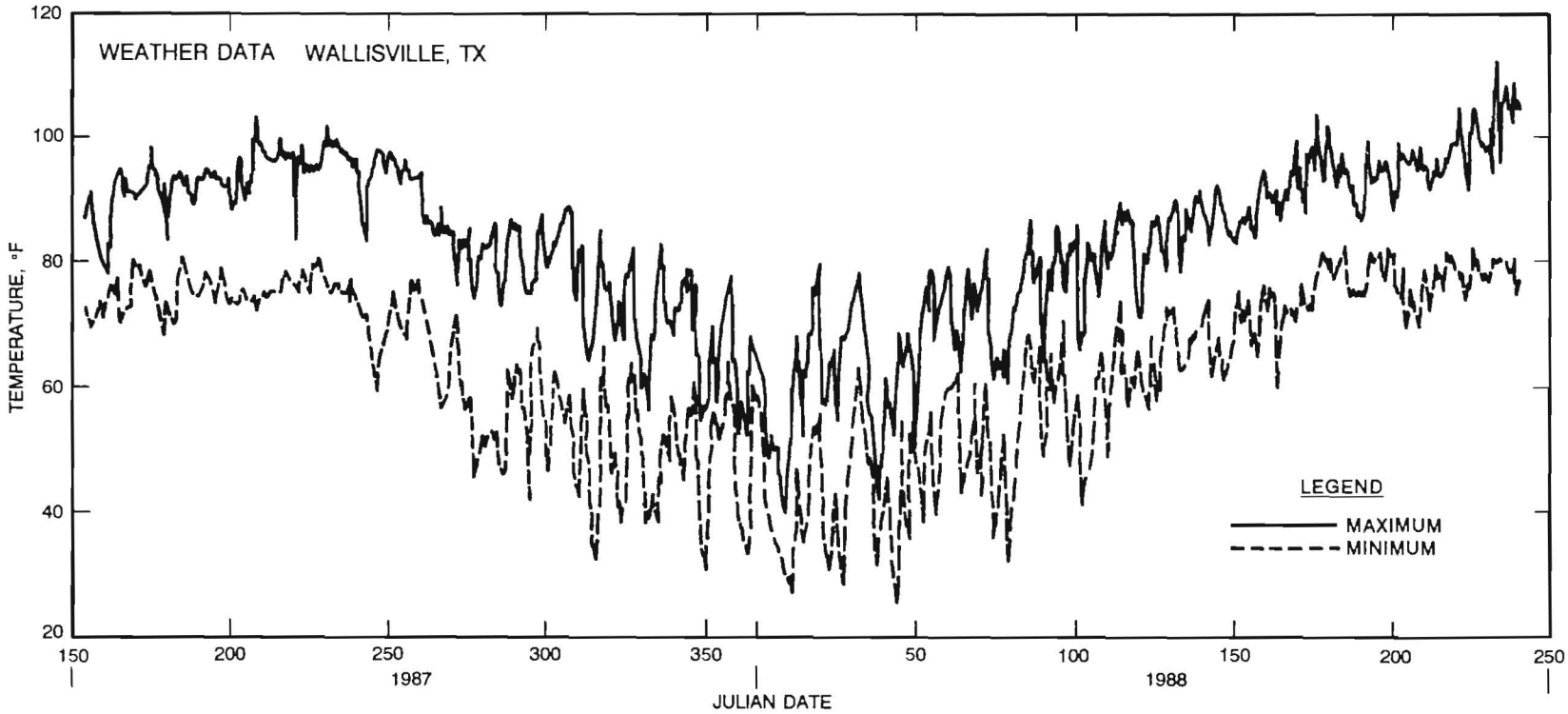

Figure 1. Maximum and minimum temperatures (degrees Fahrenheit) through time at Wallisville

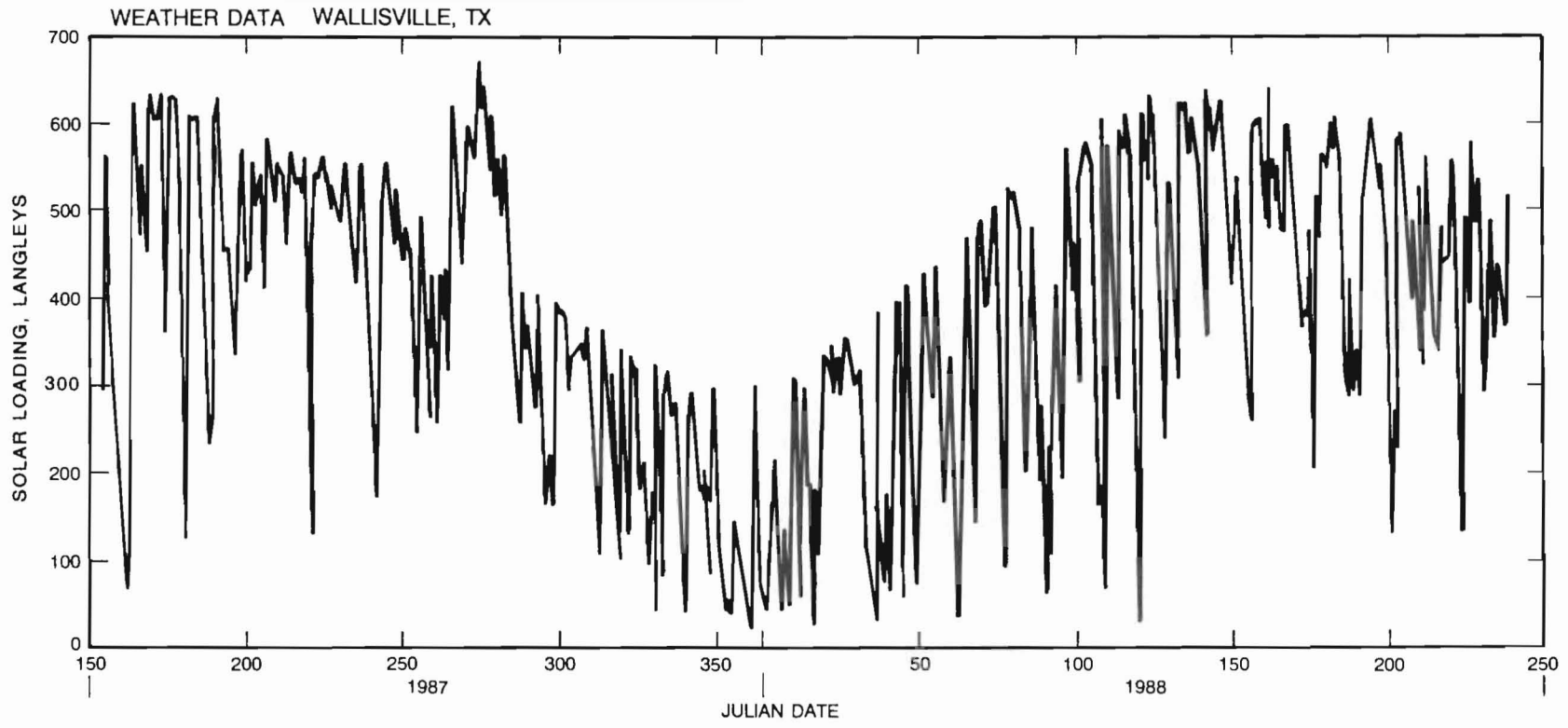

Figure 2. Daily solar loading (Langleys) through time at Wallisville

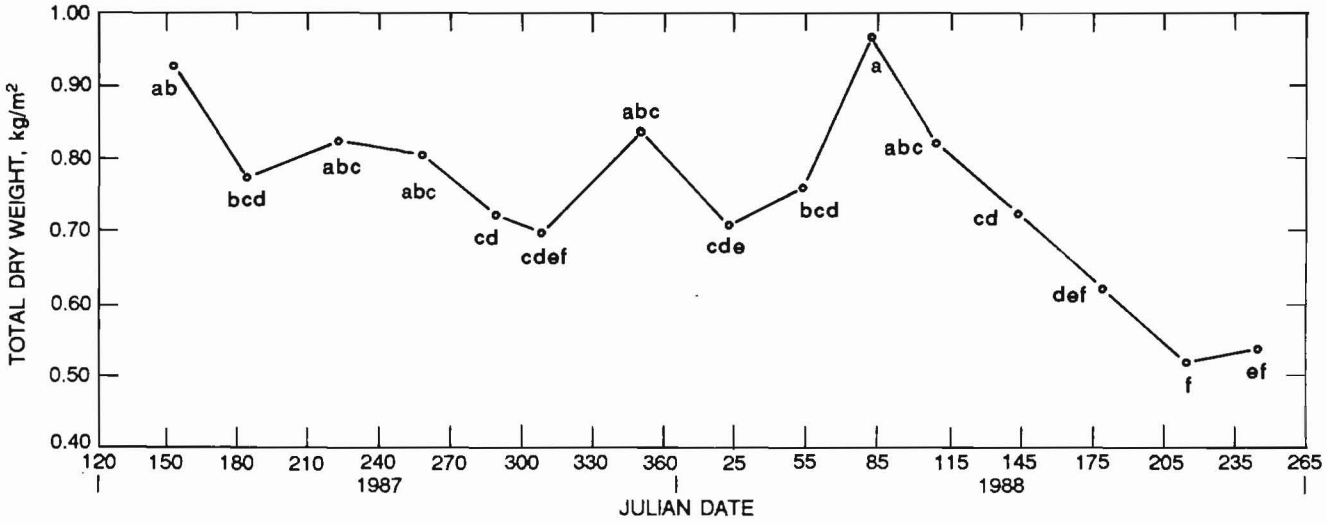

Figure 3. Total dry plant weight (kg/m^2) through time at Wallisville. Means followed by the same letter are not significantly different at P > 0.05 . The F statistic for overall model is 5.00 (df = 16, 28; P < 0.0001) with a pooled standard error of the mean of 0.06

>0.95 kg/m^2 occurring on Julian Day (JD) 88082 (i.e., March 22, 1988). Following this date, significant decreases were noted until JD 88214 (i.e., August 1, 1988), when minimum values of ca. 0.55 kg/m^2 occurred, representing a decrease of approximately 42 percent.

Associated with the decrease in total biomass noted during 1988 was a shift in the proportion of LAWB relative to BWB ($P < 0.001$ for all; Figure 4). For the majority of 1987, LAWB was ca. 27 percent; however, this decreased to ca. 16 percent on JD 87350 and continued at this relatively low value throughout the 1988 sampling. This represents a decrease of approximately 41 percent. Correspondingly, BWB increased from ca. 28 percent on JD 87155 to a relatively constant value of ca. 46 percent throughout 1988, a change of 1.6-fold. Similar trends in TDW were observed for both 1987 and 1988, with lowest values noted during early winter (ca. 25 percent). Total dead weight subsequently increased gradually to highest values (ca. 50 percent) noted during the early part of the 1988 growing season. Decreasing values occurred following JD 88109.

In addition to the shifts in LAWB and BWB, significant changes occurred in plant density and weight per plant ($P < 0.0001$ for both; Figure 5). For example, a relatively constant density of ca. 73 plants/m^2 occurred from JD 87188 to 88054. However, the onset of the 1988 growing season noted increases in density of ca. 1.5-fold, with highest densities occurring on JD 88144. Corresponding to this increase in density were rapid decreases (ca. 50 percent) in plant weight, which began on JD 88082 and continued to the end of the sampling period.

The majority of insect biocontrol agents on waterhyacinth were *N. eichhorniae.* Five percent or less were *N. bruchi*, and no *Sameodes albiguttalis* were collected on any sampling date. The number of adults collected exhibited significant changes over time (male, $P = 0.0002$; female, $P = 0.0014$; Figure 6). For example, highest number of adults in 1987 for both sexes occurred during the later part of the growing season with values >30 individuals/m^2 noted on JD 87258. Gradual decreasing trends were noted following this date, with lowest values for both sexes recorded during the period JD 88054 through JD 88109. Numbers of either sex did not surpass ca. 7 individuals/m^2 on any of these dates. The lowest values were noted following the month with the highest number of days registering below-freezing minimum temperatures, i.e., January (Figure 1). Large increases in adult numbers occurred during the 1988 growing season and were apparently more widely fluctuating than those observed in 1987. Peak values of ca. 30 individuals/m^2 were observed on JD 88214 for both sexes. The sex ratio was relatively constant throughout the 1987-88 collection period, i.e., 1:1.

Significant changes in numbers of third instar larvae and pupae were noted ($P < 0.0001$ for both). Two distinct peaks of numbers of third instar *N. eichhorniae* larvae occurred in 1987: one on JD 87188 (ca. 60 individuals/m^2) and another that began on JD 87350 and continued through JD 88021 (ca. 57 individuals/m^2; Figure 7). Following both peaks was a rapid decline in numbers of third instar larvae. This decline in late 1987 followed the coldest

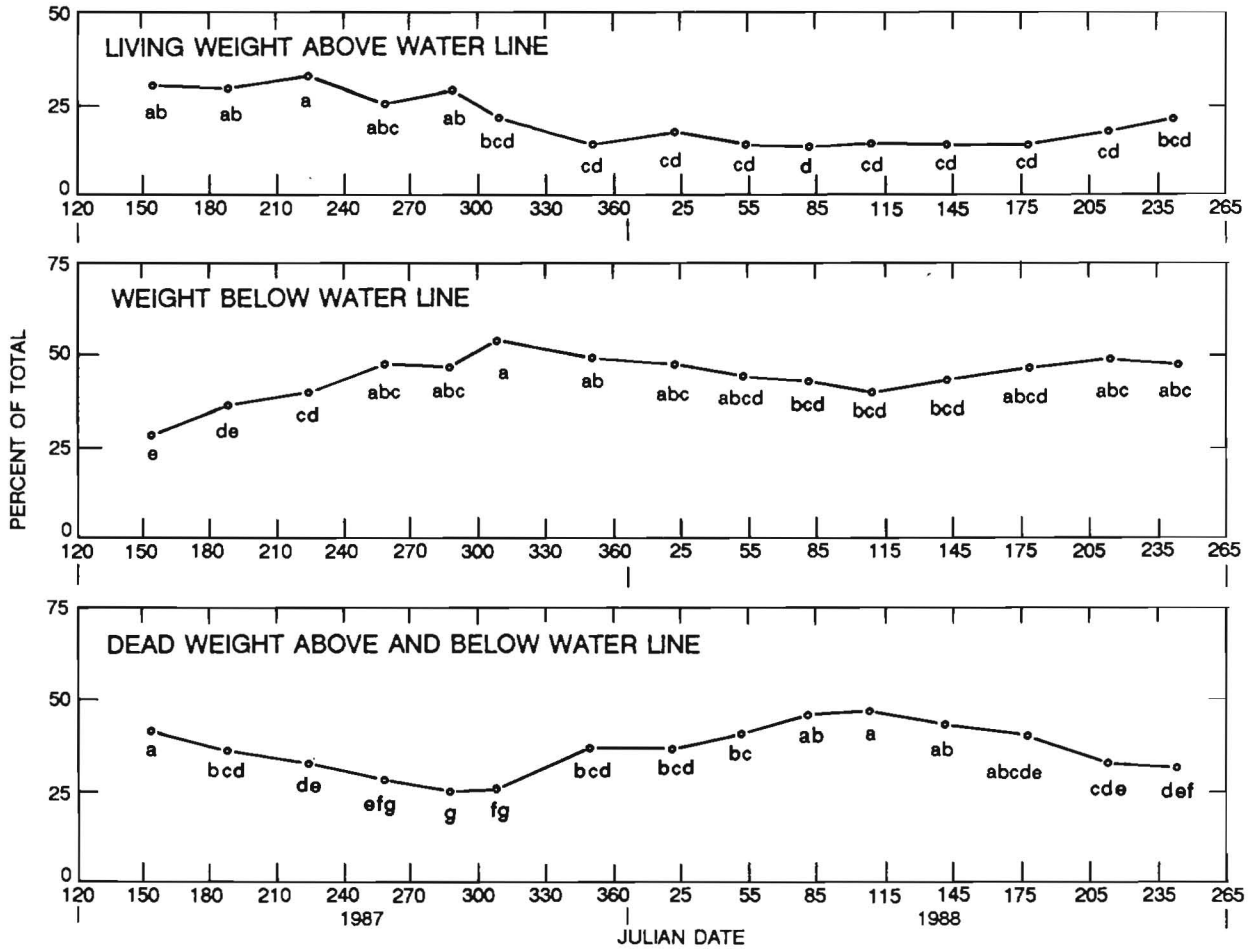

Figure 4. Total biomass partitioned into percent LAWB, BWB, and TDW through time at Wallisville. Means followed by the same letter are not significantly different at P > 0.05. The F statistics for overall models are 3.30 (P = 0.0028), 3.48 (P = 0.0019), and 8.79 (P < 0.0001), respectively, with df = 16, 28 . Pooled standard errors of the mean are 3.69, 3.52, and 2.26, respectively

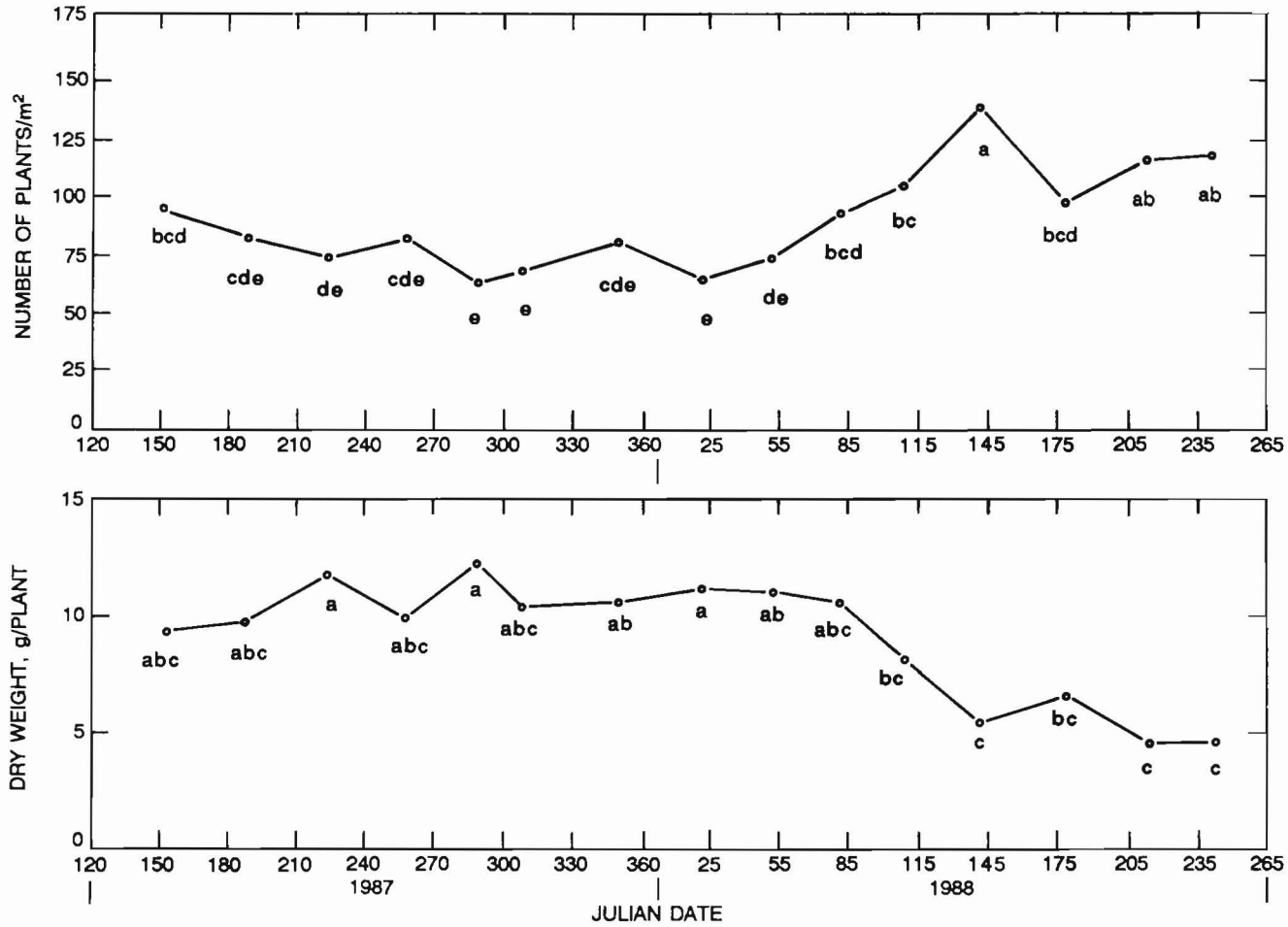

Figure 5. Density (numbers of plants/m^2) and dry weight (g/plant) through time at Wallisville. Means followed by the same letter are not significantly different at $P > 0.05$. The F statistics for overall models are 6.31 ($P = 0.0001$) and 15.98 ($P < 0.0001$), respectively, with df = 16, 28 . Pooled standard errors of the mean are 8.3 and 0.6, respectively

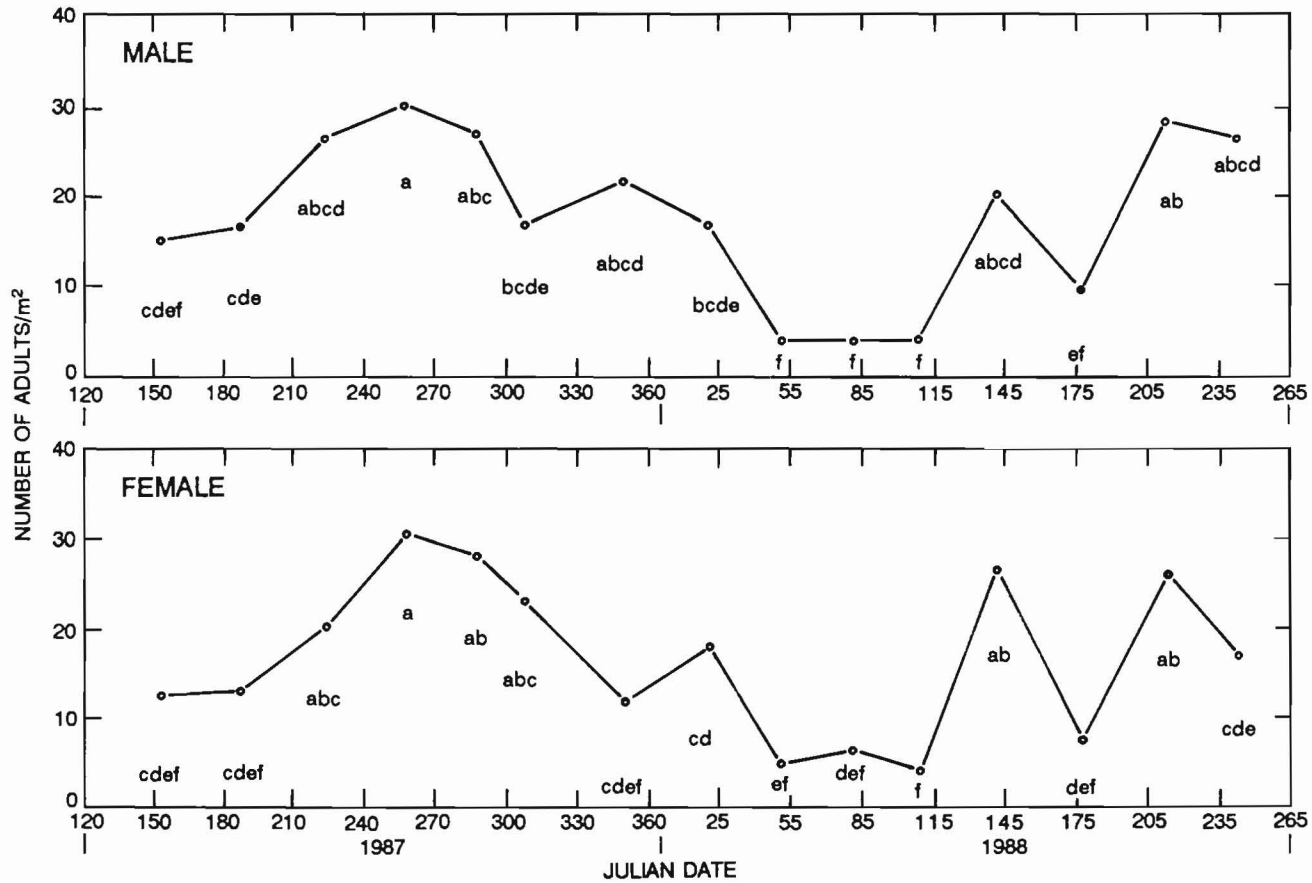

Figure 6. Density of male and female *N. eichhorniae* adults (numbers/m^2) through time at Wallisville. Means followed by the same letter are not significantly different at P > 0.05. The F statistics for overall models are 4.79 (P = 0.0002) and 3.62 (P = 0.0014), respectively, with df = 16, 28 . Pooled standard errors of the mean are 4.08 and 4.35, respectively

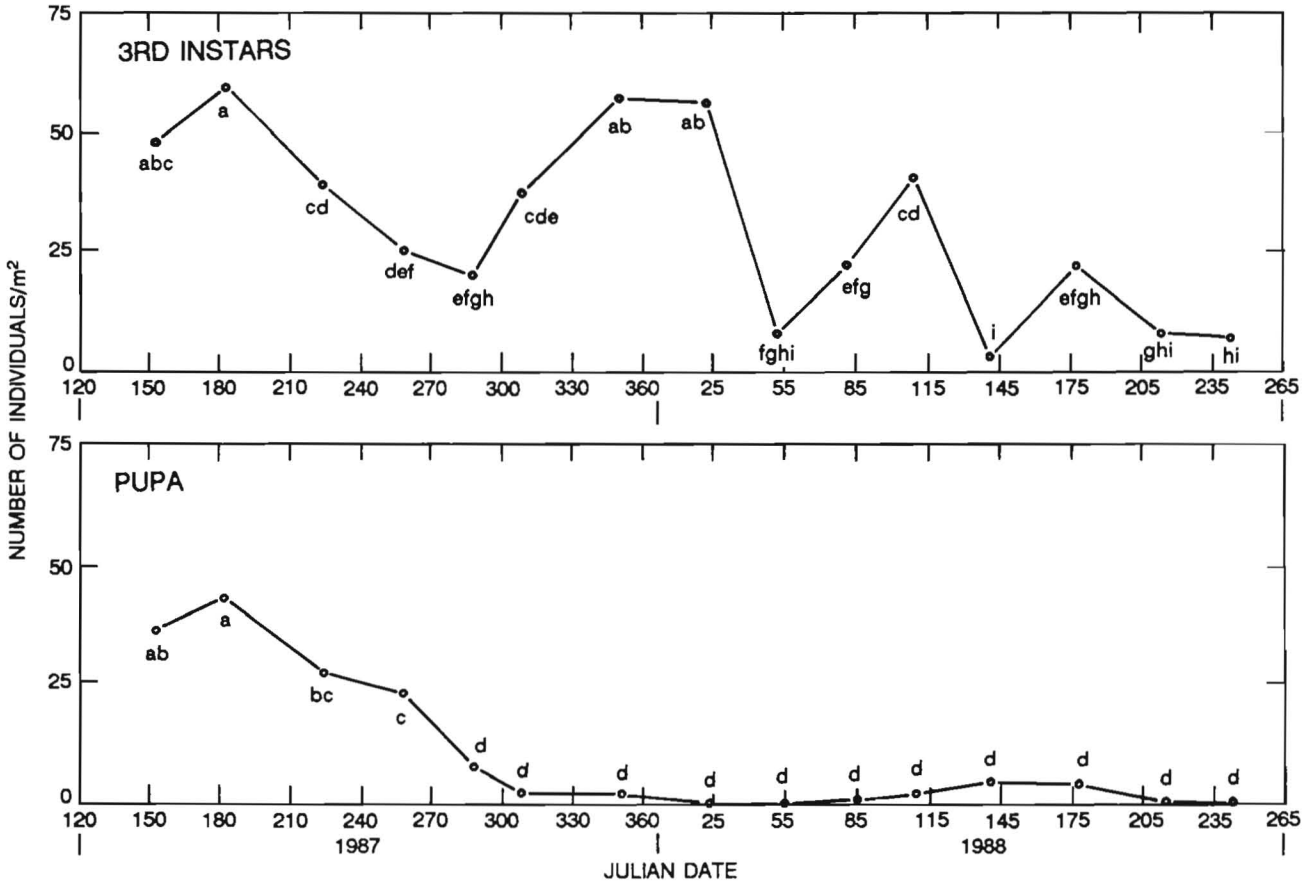

Figure 7. Total number of third instar larvae and pupae (numbers/m^2) through time at Wallisville. Means followed by the same letter are not significantly different at P > 0.05. The F statistics for overall models are 10.17 (P < 0.0001) and 10.01 (P < 0.0001), respectively, with df = 16, 28 . Pooled standard errors of the mean are 5.84 and 4.39, respectively

month, January. Numbers of third instar larvae were lower in 1988; however, two peaks were again observed. One occurred on JD 88109 (ca. 41 individuals/m^2) and the other on JD 88179 (ca. 21.3 individuals/m^2). Note that lower numbers of larvae were found for the second peak. Numbers of pupae were highest on JD 87188 (ca. 42.7 individuals/m^2) and decreased on subsequent sampling dates. Few if any pupae were gathered late in 1987 or throughout 1988 collections. On 11 sampling dates from JD 87288 through JD 88243, the numbers of pupae were not significantly different from zero ($P > 0.05$).

DISCUSSION

There was a general decline in the status of waterhyacinth at the sample site in Wallisville, Texas. This decline occurred gradually throughout the study; however, the biggest change was observed during the 1988 growing season. The decline was manifested by decreased LAWB as well as increases in the proportion of BWB (i.e., root material) relative to LAWB. Associated with the increase in BWB was an apparent change in the condition of the root material, which became relatively more matted and less turgid. There was also an increase in bladderwort (*Utricularia* sp.) interspersed between the root material. Associated with this 1988 decline in LAWB and increases in relative root biomass were smaller plant size and increases in plant density. These changes become more apparent by examining photographs of the site from August 1987 and 1988 (see Figures 8 and 9). Note that in 1987 the plants had a taller canopy relative to the site in 1988.

Along with the decline in waterhyacinth status came an associated decrease in numbers of *N. eichhorniae* larvae and, more significantly, in numbers of pupae. The matted and less turgid nature of the root material may have influenced the ability of *N. eichhorniae* to successfully pupate and eclose to adults. Pupation occurs on lateral root hairs that late third instar larvae use to form a protective pupal covering (Center 1982). In addition, the smaller stature of the plants and their obviously stressed nature may have negatively influenced the capacity of larvae to survive. This may be related to decreased larval habitat area and/or changes in the nutritional status of the plant as would be expected to occur when waterhyacinth is declining.

Such changes in waterhyacinth status are common when pressured by *Neochetina* sp. herbivory. For example, Center and Durden (1986) reported increases in root-to-shoot ratios for plants in sections of a Florida canal with heavy *N. eichhorniae* herbivory. Smaller plants also occurred as indicated by decreased leaf lengths (also an indication of a smaller LAWB) as well as standing crop. Similar observations were made by Goyer and Stark (1984) in Louisiana. The presence of browned and curling plant laminae also indicated heavy *N. eichhorniae* herbivory and subsequent influence to the waterhyacinth. This condition was observed in 1987 and was more apparent in 1988. Adult feeding by *N. eichhorniae* was heavy, and it was not unusual to observe completely girdled

Figure 8. Photograph of Wallisville experimental site, August 11, 1987

Figure 9. Photograph of Wallisville experimental site, August 1, 1988

petioles. Such feeding by the adults ruptures the leaf surface, thereby increasing evaporation and causing browning and curling of the affected plant part (Center and Durden 1986). Similarly, high levels of larval feeding (which were observed) disrupt fluid transport within the petiole and cause the observed lamina condition. Another affected area was the crown, where numerous larvae and larval feeding

galleries were noted. Such feeding in this plant area is believed to cause great damage.*

However, other environmental conditions may have played a role in the waterhyacinth's decline. For example, the winters of 1987 and 1988 were relatively cold, with the month of January 1988 having minimum temperatures of 33° F for 29 percent of the time. Near- or below-freezing temperatures have been shown to impact greatly on waterhyacinth populations (Gopal 1987). In addition, rainfall was greatly decreased in the spring and summer of 1988. Such drought conditions caused a marked decrease in water level at the site. While fluctuations in water level have been shown not to influence waterhyacinth populations greatly (Gopal 1987), low water levels coupled with significant herbivory may have acted in conjunction to cause the observed decline in waterhyacinth. These drought conditions may also have altered the site's nutritional status for adequate plant growth and development. This occurs because a major source of nutrients results from runoff of the surrounding terrestrial ecosystem (Odum 1971). Changes in nutrient conditions may have influenced the plant's ability to keep up with the insect herbivory, hence contributing to the observed decline. Most likely, a complex of environmental factors interact to cause the decline and eventual elimination of a weed species from a particular site.

Actual comparisons between the observed data and predictions of the INSECT model are currently being analyzed. To date, the preliminary comparisons indicate better agreement for insect populations than for plant biomass/growth characteristics. This initial analysis suggests the necessity for additional data related to environmental characteristics and insect-based rate functions. Comparable data needs have been outlined previously (Akbay, Wooten, and Howell 1988; Howell, Akbay, and Stewart 1988), as well as in this section of the current proceedings.

Proposed improvements to INSECT can be included under two major categories: (a) differences due to specific plant/site condition (geographical region) and (b) environmental stresses. For the first, differences due to geographical location may be related to variations in waterhyacinth respiration associated with southeastern Texas environmental conditions.** Currently, the INSECT model uses two different respiration rates, one for Florida plant conditions and the other for Louisiana plant environments (Akbay, Wooten, and Howell 1988). Hence, precedent is already established for using different plant respiration rates. Other geographical differences may include insect strain variations. In addition, this study has confirmed the need for more research in developing an improved understanding of insect reproduction, especially during overwintering time periods and during changes in the plant's nutrient status. Changes in reproductive status

*Personal Communication, 1989, Ted Center, US Department of Agriculture, Aquatic Plant Management Laboratory, Fort Lauderdale, Florida.

**Personal Communication, 1989, Jean Wooten, University of Southern Mississippi, Hattiesburg, Mississippi.

and nutrient levels may also be associated with site-specific (geographical) conditions.

Some of the more important model improvements may be related to understanding how environmental stresses impact plant growth and development. This could include the effect insect herbivory has on plant status, as well as changes that stresses induce in the plant's immediate habitat. For example, the only herbivory effect currently addressed by the model is biomass removal by third instar larvae. Certainly, adult feeding and the removal of critical plant tissues (i.e., apical meristems) may also greatly impact waterhyacinth.* Further, other environmentally induced stresses should be accounted for, including drought conditions (i.e., water-level decreases), fluctuations in site nutrient levels, and associated insect/plant interactions.

FUTURE STUDIES

The first set of studies involves the characterization of the reproductive structures of female *N. eichhorniae* in an effort to understand shifts in reproductive status in field populations. During 1988, research was initiated to (a) describe ovarian morphology, (b) develop a physiological age-grading system based on ovarian structure, and (c) use the age-grading system in southeastern Texas to preliminarily describe physiological age structure of field populations.

Toward this goal, ovarian morphology was described. General ovarian morphology (Figure 10) is similar to other curculionid weevils (Grodowitz and

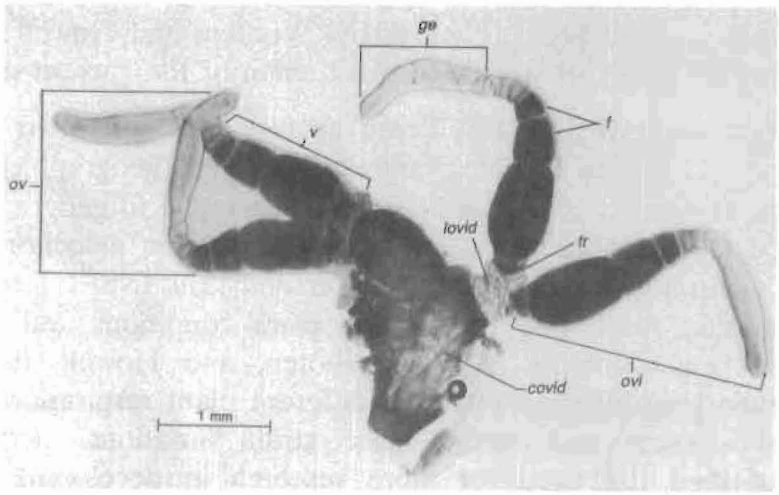

Figure 10. Photomicrograph of *N. eichhorniae* ovary illustrating general morphological features (covid, common oviduct; f, follicle; fr, follicular relic; ge, germarium; lovid, lateral oviduct; ov, ovary; ovl, ovariole; v, vitellarium)

*Personal Communication, 1989, Ted Center.

Brewer 1987). The ovary is really composed of two ovaries, each containing two1 "tubelike" structures, the ovarioles, where the eggs (or follicles) develop and mature. The two ovarioles connect to each other via the lateral oviduct; then, the two lateral oviducts combine, forming the common oviduct.

After the morphology description, the continuum of ovarian development and maturation was divided into a series of classes or categories based on the degree of ovarian development. Such physiological age-grading systems are a way of determining the relative reproductive status of field populations. The continuum can be subdivided into a nulliparous (i.e., no eggs) or parous (i.e., with eggs) condition, based on the presence or absence of follicular relics (Figure 11). These

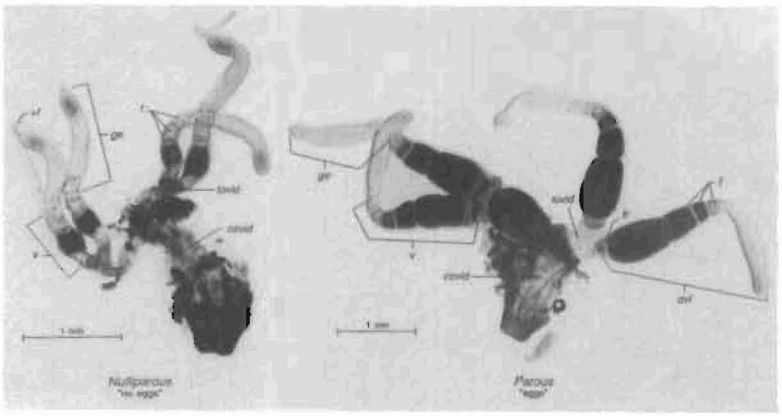

Figure 11. Photomicrograph of *N. eichhorniae* ovaries illustrating differences between nulliparous and parous individuals (covid, common oviduct; f, follicle; fr, follicular relic; ge, germarium; lovid, lateral oviduct; ovl, ovariole; tf, terminal filament; v, vitellarium)

follicular relics are typically formed by the sloughing of follicular epithelium, which surrounds the developing follicles, and its subsequent accumulation in the ovariole base (Grodowitz and Brewer 1987). Their particulate composition is depicted in Figure 12. Follicular relics are typically yellow in color and are relatively easy to detect; their quantity and quality usually indicate the number of previous ovipositions, i.e., number of eggs laid.

The physiological age-grading system underwent preliminary testing at a site near the borrow pit discussed in previous sections. Definitive shifts in reproductive status were observed, and future research will be directed toward understanding environmental conditions that influence these shifts in field populations. This will include biochemical/nutrient profiles of the waterhyacinth populations.

Future studies will also be directed toward quantifying the status of insect biocontrol populations in the Galveston District. These studies will include estimating insect and plant densities and the biocontrol agent impact, and denoting site-to-site variation. Potential factors that may influence site variability will

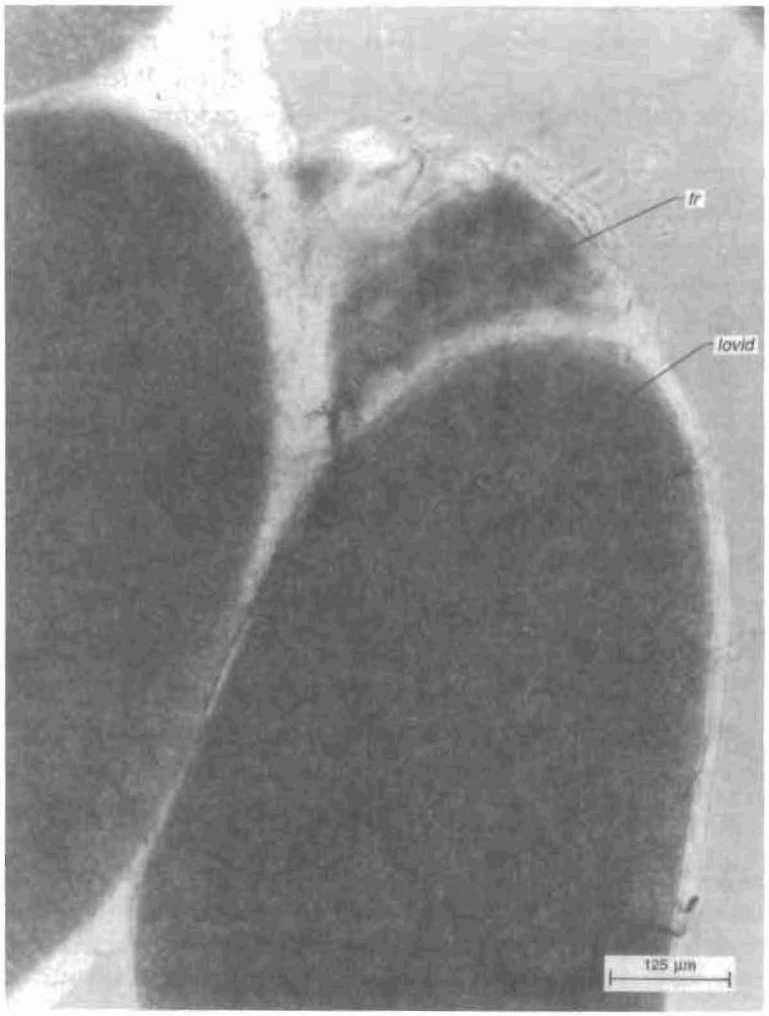

Figure 12. Closeup photomicrograph of *N. eichhorniae* ovary illustrating placement and composition of follicular relics (fr, follicular relic; lovid, lateral oviduct)

include chemical application history, geographical location, infestation type, and water body type.

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Further Development of Coupled Herbicide Fate and Target Plant Species Effects Model

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

INTRODUCTION

During development of the prototype Coupled Herbicide Fate and Target Plant Species Effects Model (Rodgers, Clifford, and Stewart 1988), information gaps regarding control of waterhyacinth (*Eichhornia crassipes* (Mart.) Solms) with 2,4-D (DMA) were identified. Modeling actual field situations requires simulating 2,4-D (DMA) interception by waterhyacinth canopies of different densities, understanding the route of 2,4-D (DMA) uptake by waterhyacinth (uptake both by leaves and roots from water and by leaves from direct contact), parameterizing exposure/mortality relationships, and experimentally verifying data obtained from literature sources.

The goal of the research conducted during Fiscal Year (FY) 1988 was to obtain and verify the information necessary to calibrate and validate the computer simulation model.

EXPERIMENTAL DESIGN

The herbicide formulation used for these experiments was Weed-Rhap A-4D (Vertac). Maximum label application rate of this formulation is 5.25 ℓ/ha. At 46.7 percent active ingredient, this approximates a surface area application rate of 2.45 ℓ/ha of 2,4-D (DMA). The test containers used are 1,750-ℓ cement tanks. Since the average depth of these tanks is 1 m, direct application of this 2,4-D formulation at maximum label rate would produce an aqueous 2,4-D concentration in the tanks of 0.25 ppm, assuming that all of the 2,4-D applied went into solution. Figure 1 illustrates the overall experimental design and layout of the test tanks.

Experiment I

The purpose of the first experiment was to determine whether waterhyacinth leaf contact to 2,4-D concentrations approximating those found in a water body following herbicide application is sufficient to produce a mortality response. Waterhyacinths were exposed to a 2-ppm solution of 2,4-D (DMA) by dipping above-water plant portions into the solution for contact times of either 1, 10, or

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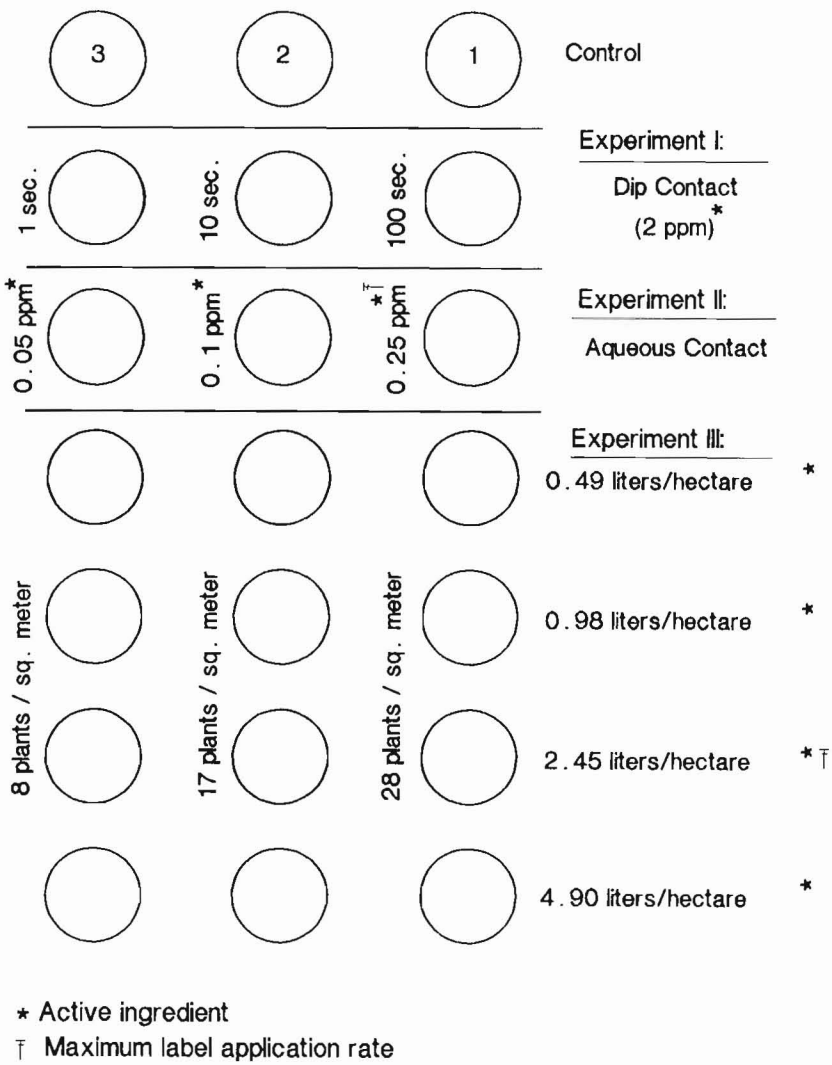

Figure 1. Design for 2,4-D (DMA) and waterhyacinth experiments. Circles represent 1,750-ℓ cement tanks

100 sec. After dipping, plants were placed in test tanks containing no 2,4-D. Plant tissues and water were periodically sampled and analyzed for 2,4-D concentrations over a 3-week period. The contact concentration of 2 ppm is approximately eight times greater than the maximum aqueous concentration (0.25 ppm) achievable for label rate applications of this herbicide formulation to 1-m-depth test containers. The elevated contact concentration was used to prevent significant depletion of 2,4-D from the dipping solution via plant uptake. The contact times chosen were expected to elicit mortality responses needed to describe the inflection points (i.e., zero, partial, and 100 percent) of the exposure/mortality relationship.

Experiment II

The purpose of this experiment was to determine whether waterhyacinth roots are capable of sufficient 2,4-D uptake from water contacted by a spray application

to produce tissue concentrations required for a mortality response. Herbicide application was made via aqueous introduction to minimize leaf contact with the herbicide. The experiment was conducted in tanks with aqueous 2,4-D concentrations of 0.05, 0.1, and 0.25 ppm. These concentrations were used to approximate aqueous concentrations expected to occur in the containers if 20, 40, and 100 percent, respectively, of the tested herbicide formulation, applied at maximum label rates, entered the water.

Experiment III

The third set of experiments was designed to parameterize the exposure-response relationship of waterhyacinth to 2,4-D and to determine the effects of density on interception of 2,4-D by plants when herbicide is applied via aerial spray. Since plant density should affect the amount of herbicide contacting individual leaves and also the amount entering the water and becoming available to roots, this experiment was conducted with several concentrations of 2,4-D (DMA) and several plant densities. The test included 2,4-D application rates (active ingredient) of 0.49, 0.98, 2.45, and 4.90 ℓ/ha. These approximate 0.04, 0.2, 1.0, and 2.0 times the label application rates. Each application rate was applied to test tanks containing waterhyacinth densities of 8, 17, and 28 plants per square meter. The 2,4-D was applied via spray applicator. Estimates of plant mortality were made by comparing the amount of dead plant material in test tanks to that present in the controls (no 2,4-D). Concentrations of 2,4-D in plant tissues, water, and sediments were determined through time. Analytical procedures followed GLC protocols of Nesbitt and Watson (1980a,b) and Hammarstrand (1979).

RESULTS AND DISCUSSION

No waterhyacinth mortality was observed in Experiment I. Analysis of the contact solution verified that the aqueous concentration was approximately 2 ppm. The maximum observed 2,4-D concentration in plant tissues, 0.19 mg/kg plant wet weight, occurred in Day 7 samples. During Experiment I, 2,4-D was detected in water only in Day 7 samples; maximum concentrations were 0.05 ppm. Also, during this experiment, 2,4-D was detected only in Day 0 sediment samples. The maximum concentration measured was 0.08 mg/kg sediment wet weight. The results from this study suggest that leaf contact to 2,4-D at an aqueous concentration approximating eight times that achievable with maximum label application rates is insufficient to produce tissue concentrations required to elicit a mortality response. In actual field situations, at tank-mix dilutions of 1:130 (formulation: water), the concentrations of 2,4-D contacting leaf surfaces approximate 3,600 ppm. These results indicate that leaf tissue not directly contacted by the herbicide spray will probably not uptake sufficient 2,4-D from water to elicit a mortality response.

The second experiment was designed to determine if waterhyacinth roots are capable of sufficient 2,4-D uptake from water contacted by herbicide spray to elicit

a mortality response. No plant mortality was observed. The maximum concentration of 2,4-D in waterhyacinth tissues was 1.07 mg/kg wet weight and was measured in Day 7 samples from the 0.25-ppm test container. Maximum 2,4-D concentration measured in sediment samples was 0.238 mg/kg wet weight and was observed on Day 0 in the 0.25-ppm test container. No 2,4-D was detected in subsequent sediment samples. The results of Experiment II indicate that waterhyacinth roots did not uptake sufficient quantities of 2,4-D from the treated water to elicit a mortality response.

Experiments I and II verify that the primary route of 2,4-D uptake by waterhyacinths is via direct contact of emergent plant portions with the concentrated spray. It appears that 2,4-D uptake by waterhyacinth from water plays an insignificant role in the effectiveness of 2,4-D as a control agent for waterhyacinths.

Concentrations of 2,4-D in waterhyacinth tissues and in water measured during Experiment III are presented in Figures 2 and 3. Significant quantities of 2,4-D

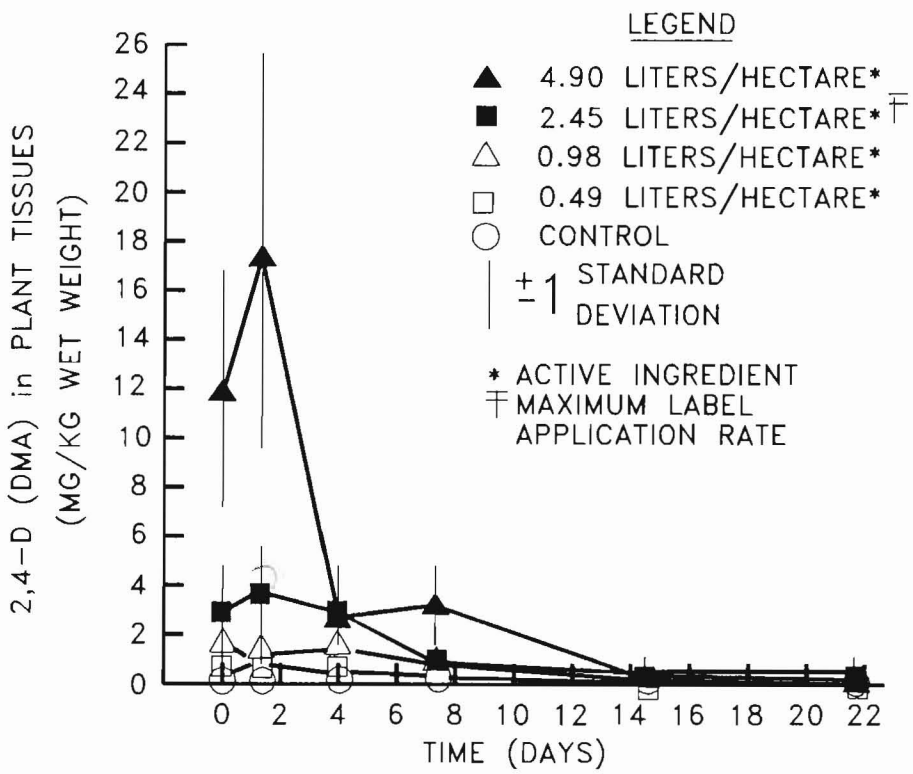

Figure 2. Measured 2,4-D (DMA) concentrations in waterhyacinth tissues during spray-exposure experiments

were not measured in sediment samples. The relationship between 2,4-D exposure and waterhyacinth mortality (Figure 4) indicates that the critical tissue concentration of 2,4-D in waterhyacinth tissues for a maximum mortality response is approximately 12 mg/kg wet weight. This value, the overall shape of the exposure/mortality curve, and an upward mortality bound of approximately

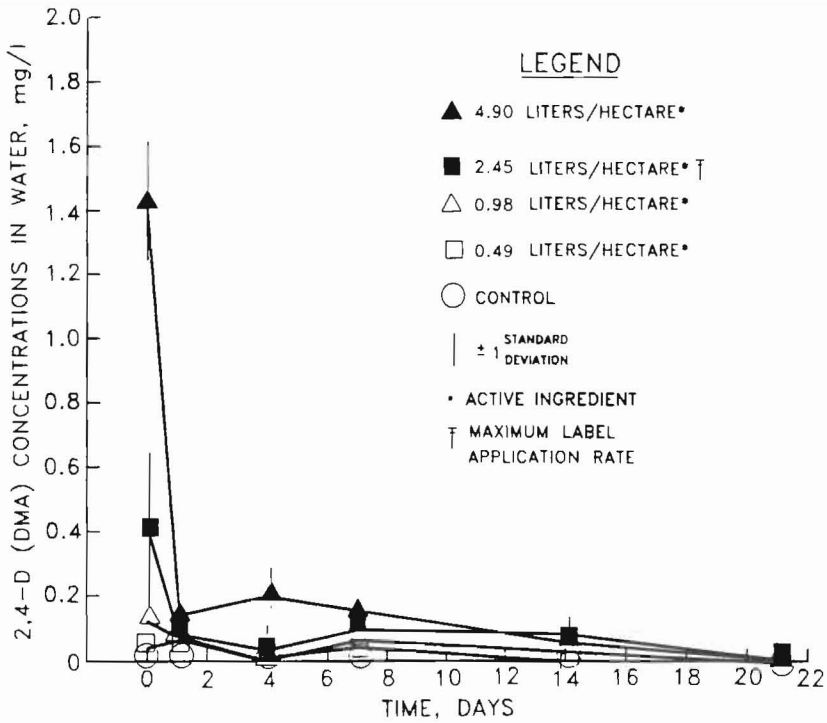

Figure 3. Measured 2,4-D (DMA) concentrations in water during spray-exposure experiments

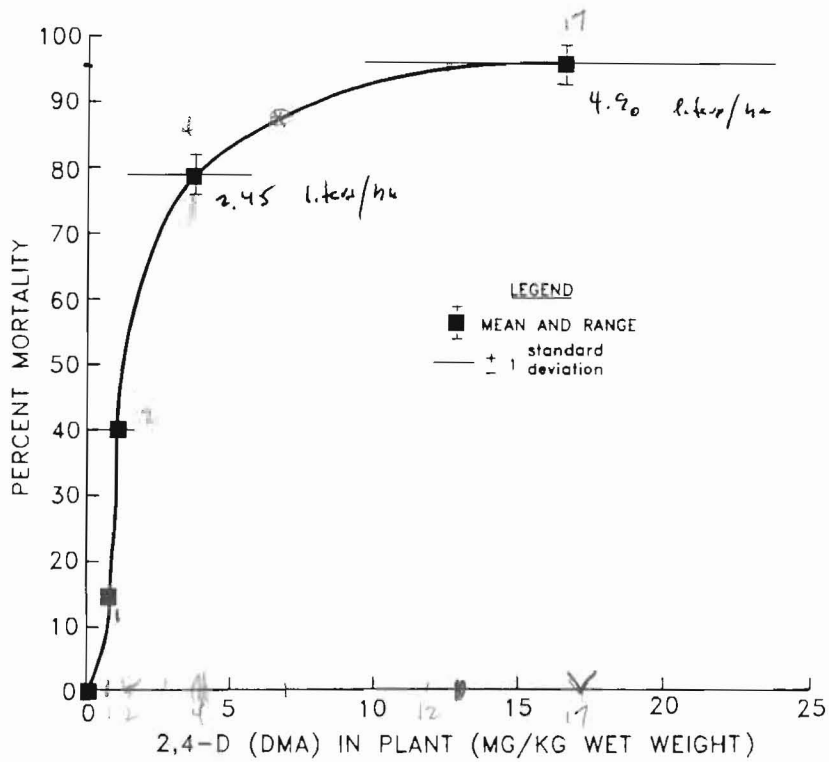

Figure 4. Relationship of waterhyacinth mortality to exposure to 2,4-D (DMA)

98 percent are three parameters needed to calibrate this fate/effects model for 2,4-D (DMA) application to waterhyacinth. One hundred-percent mortality was not observed during this experiment, even at twice the label application rates of this 2,4-D formulation. This apparently was caused from 2,4-D interception by mature plants which shielded smaller daughter plants from exposure to the herbicide spray.

The estimated half-life of 2,4-D is approximately 2.5 days. This value is probably somewhat inflated since all water samples were collected from the plant root zone (2 to 5 cm below the water surface) and complete mixing of 2,4-D within the tank water had probably not occurred when initial water samples were taken.

Figure 5 illustrates percent 2,4-D interception as a function of plant biomass. This relationship was determined by the following steps: (a) estimating the plant

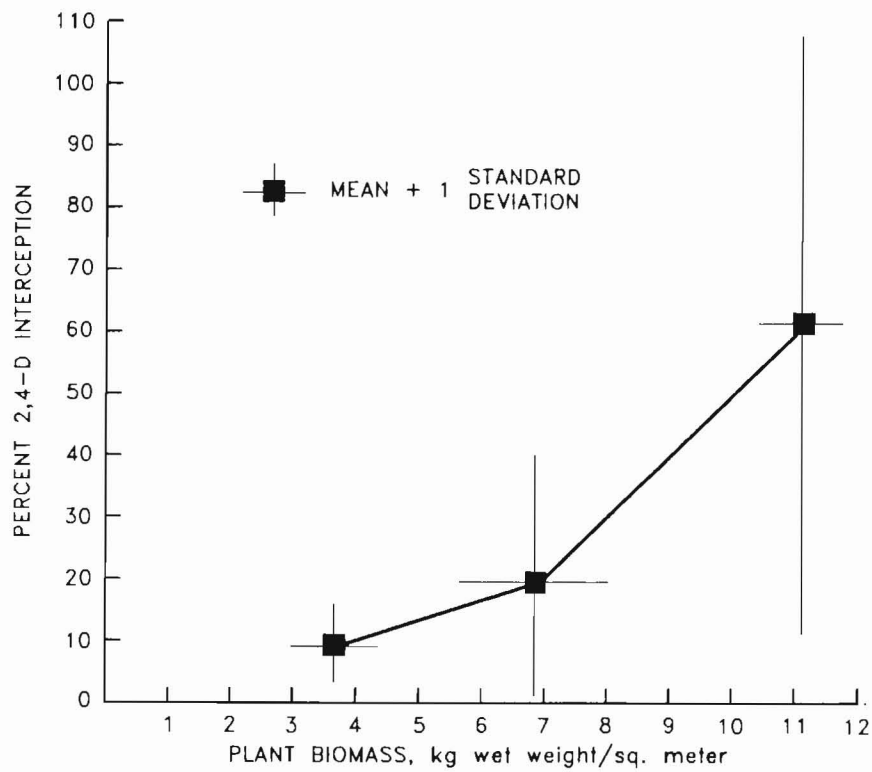

Figure 5. Interception of 2,4-D (DMA) by waterhyacinth as a function of plant density

biomass present in each tank (mean measured weight per plant times number of plants in each tank), (b) extrapolating the mass of 2,4-D present in plant tissues (2,4-D concentration in plant tissues times mass of plants), and (c) calculating the percent of 2,4-D present in plant tissues relative to the mass that was applied. Calculations were made using Day 1 data. Water concentrations of 2,4-D were not used for these calculations because incomplete mixing would tend to bias estimates. The maximum calculated 2,4-D interception percentage was

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61.8 percent ± 47.9 (standard deviation) at a plant biomass of 11.0 ± 0.62 kg plant wet weight per square meter.

Since the critical concentration of 2,4-D in tissues of waterhyacinth (12 mg/kg wet weight) occurred between one and two times maximum label application rates, it is important to determine whether this concentration is attainable under actual field conditions. Using the tested 2,4-D formulation, 12 mg 2,4-D/kg waterhyacinth wet weight is attainable at plant densities of 95 tons wet weight/acre (moderate to high density) if virtually 100 percent of the applied 2,4-D enters plant tissues. This may help to explain why repeat applications of 2,4-D are required to control waterhyacinth at high field densities.

COMPUTER SIMULATIONS

Figures 6-8 illustrate comparisons of computer simulation results with experimental observations made during Experiment III at the 2.45-ℓ/ha application rate. Parameters indicated above were used in these simulations. The computer model produced simulation results comparable with experimental observations of 2,4-D concentrations in plant tissues and water. Concentrations of 2,4-D in

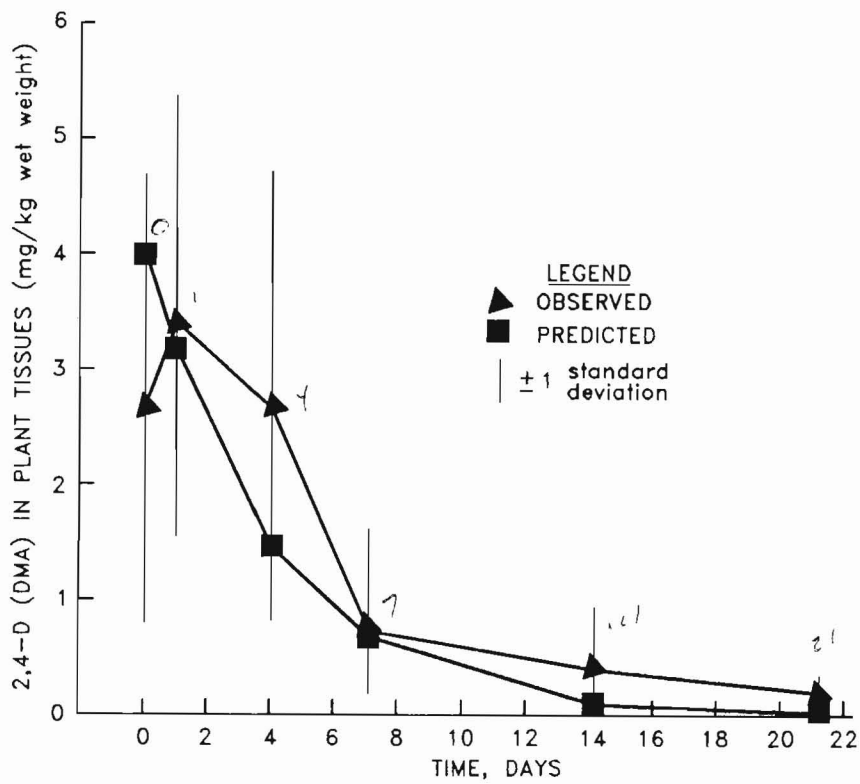

Figure 6. Comparison of computer simulation model predictions with experimental observations for 2,4-D (DMA) concentrations in waterhyacinth tissues. Comparisons are shown for an application rate of 2.45 ℓ/ha

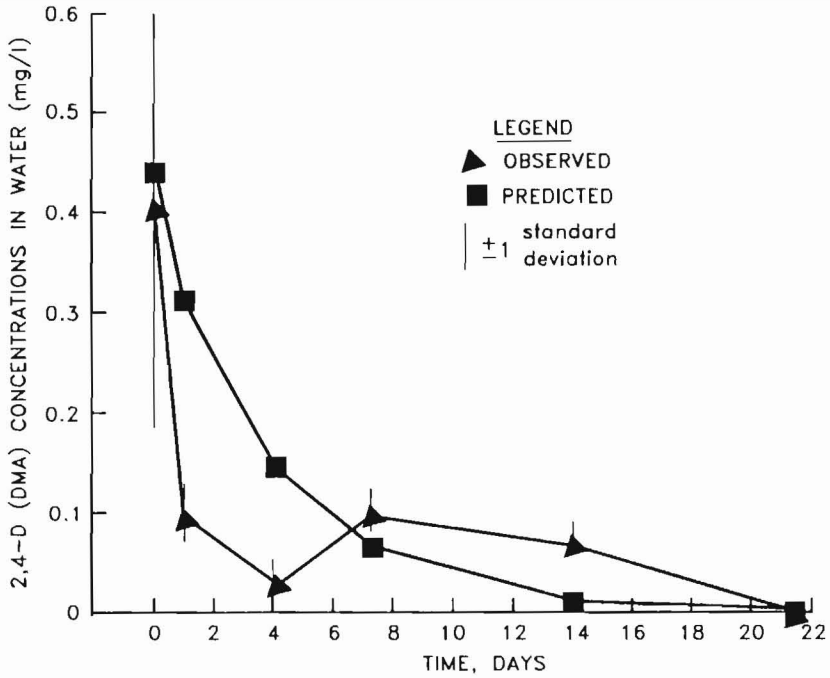

Figure 7. Comparison of computer simulation model predictions with experimental observations for 2,4-D (DMA) concentrations in water. Comparisons are shown for an application rate of 2.45 ℓ/ha

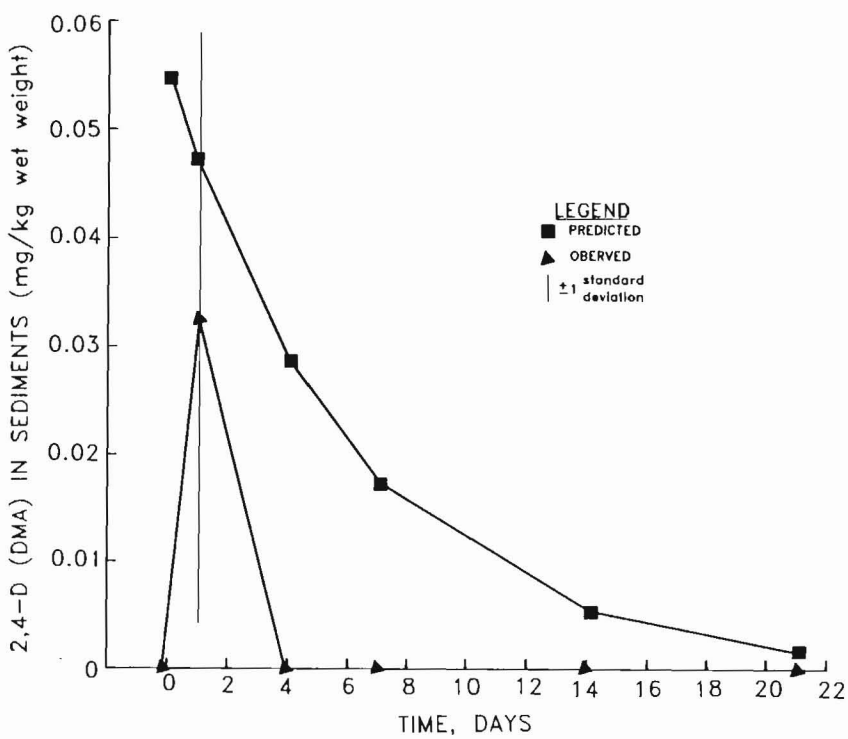

Figure 8. Comparison of computer simulation model predictions with experimental observations for 2,4-D (DMA) concentrations in sediments

sediments, however, were overestimated by the model. This may been due to an overestimation of the sediment partition coefficient, which was set to the maximum reported value of 0.25 (Reinert and Rodgers 1987).

CONCLUSIONS AND RECOMMENDATIONS

The primary route of exposure of waterhyacinth to 2,4-D appears to be via contact of the emergent portions of the plants with concentrated sprays. The concentration of 2,4-D in tissues of waterhyacinth required to elicit a maximum mortality response is approximately 12 mg/kg plant wet weight. This plant tissue concentration is attainable under actual field conditions of moderate to high plant density and label application rates. In this study, the maximum observed plant mortality was approximately 98 percent. The estimated half-life of 2,4-D in water was approximately 2.5 days. Plant biomass is an important factor in the interception of 2,4-D by emergent plant tissues. In the current version of the model, 2,4-D interception by waterhyacinth is simulated by an algorithm that is governed by the 2,4-D bioconcentration factor. Though calibration of the bioconcentration factor to a value of 10 resulted in an adequate representation of this relationship, improvements to this algorithm will be made in FY 89. Overall, the computer model produced simulations that compared well with experimental data. During FY 89, the model will be tested with data collected during a field application more representative of an operational treatment effort.

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Description and Update: An Improved Hydrilla Growth Model

by
Jean W. Wooten*

INTRODUCTION

An overview of the first-generation computer simulation model for dioecious hydrilla growth presented the concepts and algorithms to be used in construction of the working model (Wooten and Akbay 1988). At this stage of development, the dioecious hydrilla model provides for a maximum simulation time of 1 year and computes the biomass growth in a 1- by 1-m area with an assumed maximum depth of 4 m. Each three-dimensional column is subdivided into 1- by 1- by 0.10-m three-dimensional layers. The following information is requested from the user during model execution:

- The weather data to be used (code).
- Whether or not the plants overwinter essentially intact or "die back" (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 for stated ranges).
- Level of tuber production (code for minimum, average, or maximum).
- Extinction coefficient of the water (number, accessible from Secchi disk determinations).

The daily change in hydrilla biomass is computed for each layer, and the biomass in all layers is summed to obtain total hydrilla biomass within the square-metre water column. If winter dieback is indicated from user input, the total plant biomass to initiate the simulation is assumed to be distributed in the bottom layer of the water column on the first day of simulation. All other layers are assigned zero biomass. When net photosynthesis (i.e., gross photosynthesis - dark and photorespiration - mortality - tuber production + tuber germination) is negative, plants are assumed to be subject to biomass loss only from the standpoint of tuber production. When net photosynthesis is positive, the gain in biomass is assigned to the next zero layer upward toward the water surface. This process continues until

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all layers contain biomass. Following this occurrence, the daily amount of hydrilla biomass is distributed by the model so that layers within the top 0.5 m of the water column contain approximately 50 percent of the biomass (Haller and Sutton 1975). Net photosynthesis is calculated for each layer and added to or subtracted from the biomass in each. At the end of the growing season, presumably when mean air temperature for the day is less than or equal to 20° C, the biomass in the uppermost (toward the water surface) positive layer is daily added to that of the layer below until only the bottom layer contains hydrilla biomass. Thereafter, when net photosynthesis is negative, plants are assumed to be subject to biomass loss only by energy requirements expended in tuber production. If plants overwinter intact, plant biomass is assigned from the top layer to the bottom layer on the first and all subsequent days of the simulation. The model distributes the biomass within the layers in the same manner as described above when all layers contain biomass (i.e., 50 percent in the top 0.50 m of the water column).

The model calculates tuber production during Julian days when the mean air temperature is within the range (13° to 33° C) and photoperiod is within the range (10 to 13 hr). Tuber germination is assumed to occur when the mean air temperature is within the range (18° to 33° C) and photoperiod is greater than 13 hr.

SIMULATIONS COMPARED WITH LAKE CONWAY DATA

Field data treatment

Field data used in testing the accuracy of the algorithms and estimating the ability of the model to reflect biological reality were provided by the Florida Department of Natural Resources, Tallahassee, Florida. These data were the result of a Large-Scale Operations Management Test (LSOMT) of the use of the white amur for control of problem aquatic plants (Schardt, Nall, and Jubinsky 1982). As part of this LSOMT, baseline data (i.e., before release of white amur) were collected for 1 year (October 1976-September 1977) from five lake pools comprising the Lake Conway system.

Biomass data for hydrilla were extracted from information in the data base archive (magnetic tape) of the Lake Conway LSOMT. As stated in Schardt, Nall, and Jubinsky (1982), "Although *Hydrilla*, the target species of the study, was not a problem in Lake Conway...*Hydrilla* was a problem in the past, but had not recovered from a 1975 chemical treatment when the study began." Hence, hydrilla biomass values for the pools were low, and only Pool 4 data were sufficient to provide meaningful values in comparisons with results from model simulations. Means and 95-percent confidence intervals of the means were calculated for the 1977 data from Pool 4. Additionally, although the model was run for 1 year without impact from fish, the white amur was stocked in Lake Conway on September 9, 1977.

Model input values

Water depth was input as 3.0 m, the depth from which the greatest amounts of hydrilla biomass were collected during the LSOMT (Schardt, Nall, and Jubinsky 1982). The plants were assumed to die back during the winter, as was found by Bowes, Holaday, and Haller (1979) for Leon and Alachua Counties, Florida. Julian day (JD) 31 was used as the beginning day of the simulation. This day corresponds to the date of the first sample collected from Pool 4 during the LSOMT. Plant biomass for JD 31 was input as 0.0472 kg/m^2. Other values used in the model initialization, except tuber production, were derived from Kaleel (1981). The pH was 7.1 to 8.0 and, based on the low biomass present, the tuber production was selected to a minimum level. The extinction coefficient was input as 0.56, which represents a Secchi disk reading of 2.4 m.

Weather data were not available for Lake Conway. Consequently, a data file was generated from daily total solar energy and mean temperature data from Gainesville, Florida, for 1977 (Figures 1 and 2). Daily hours and minutes of

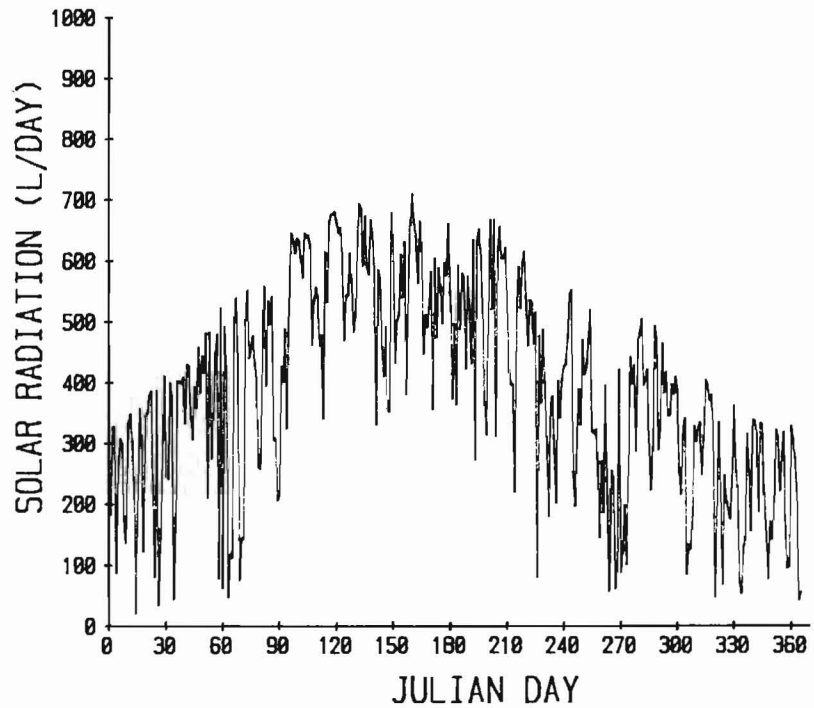

Figure 1. Solar radiation (Langleys/day) from Gainesville, Florida, 1977

daylight (photoperiod values) were derived for 1977 by interpolation for latitude 28.3° N from the ephemeris (Figure 3).

Simulation results compared with Pool 4 data

A comparison of simulation results with LSOMT baseline data is provided as Figure 4. For the comparison, mean biomass values for the LSOMT data are not

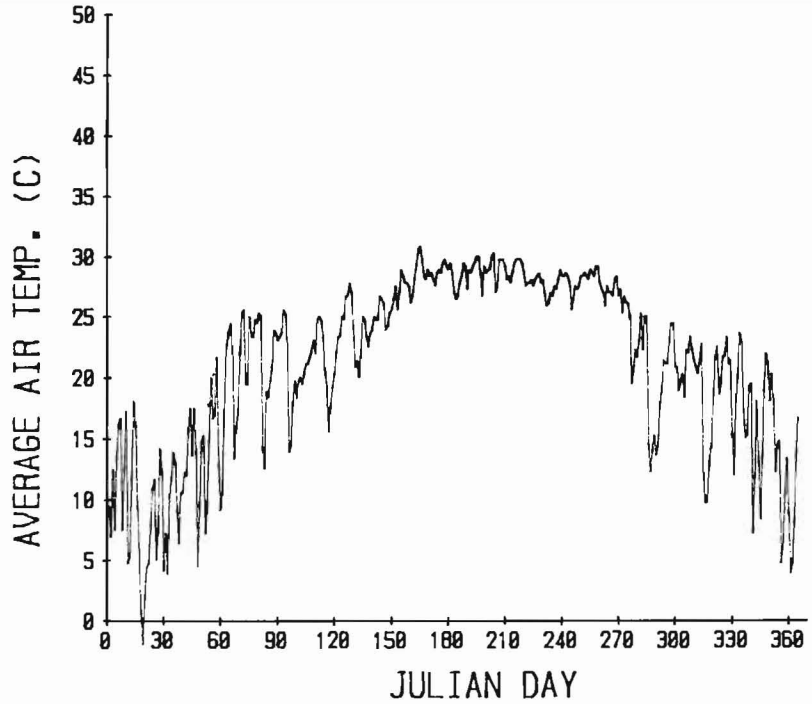

Figure 2. Average air temperatures (Celsius) from Gainesville, Florida, 1977. Values calculated from daily records of maximum and minimum temperatures

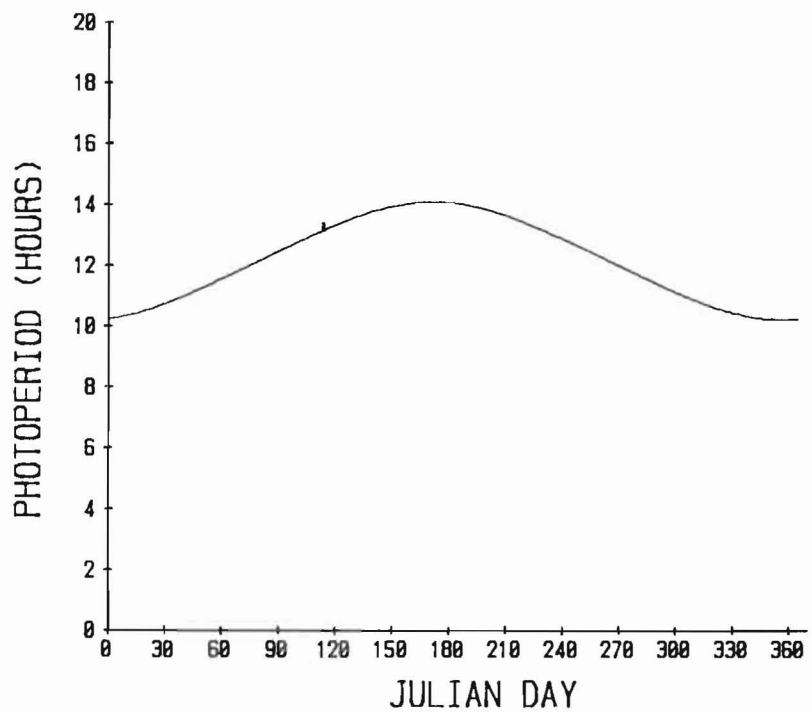

Figure 3. Photoperiod (hours:minutes) for Lake Conway, Florida, 1977. Values calculated by interpolation for latitude 28.3° N from the ephemeris

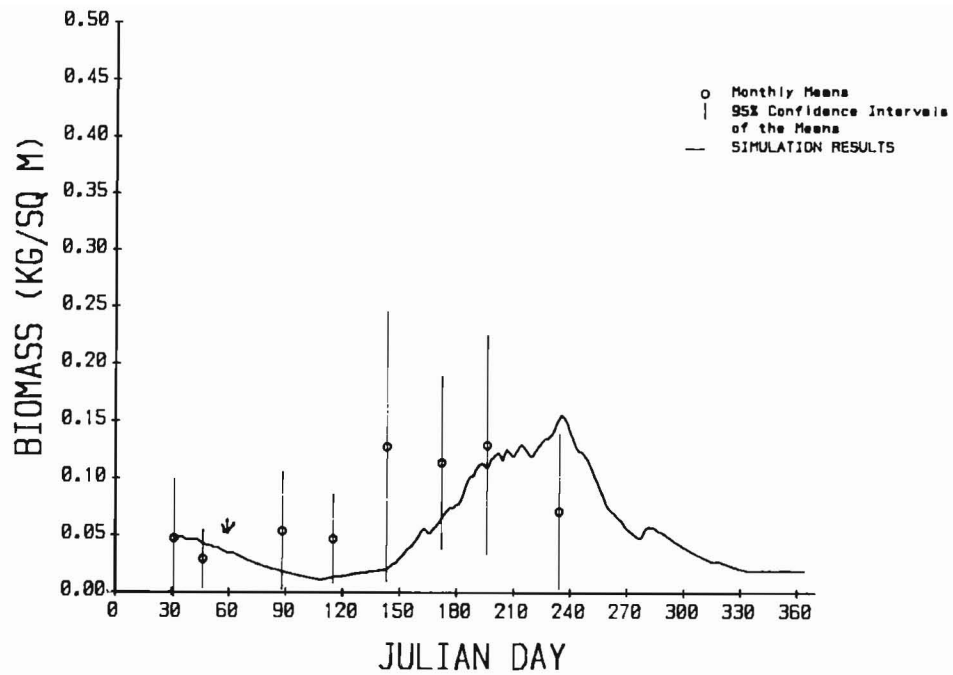

Figure 4. Comparison of *Hydrilla* biomass data collected from Lake Conway, 1977, with *Hydrilla* growth model simulation. Input values for the simulation are provided in the text

included for sampling dates after JD 252 since the introduction of white amur on that date (i.e., September 9) prevents valid comparison. In the simulation, biomass declined to 0.0106 kg/m^2 before net photosynthesis was positive on JD 133. Between JD 31 and JD 109, 13 tubers/m^2 were produced. Twelve tubers germinated between JD 132 and JD 235. All layers contained biomass on JD 165. Maximum biomass was 0.184 kg/m^2 on JD 237. Thirty-four tubers per square metre were produced between JD 236 and JD 365. Net photosynthesis became negative on JD 284. After JD 304, mean air temperatures were less than or equal to 20° C on most days.

Model simulation results were generally within the confidence intervals of the field data (Figure 4). There is some disagreement between the simulated and measured values, especially during the period JD 120 to JD 180. In general, the model generated peak biomass later in the year than was recorded during the field sampling. Since plant growth in the model is directly dependent on temperature and solar loading, it is possible that differences between simulated and measured biomass values resulted from differences between local meteorological conditions at the field site (Lake Conway) and those used in the simulation run. Further, the model was initiated with no underground propagules (tubers) present since the Lake Conway data did not include information on extant tuber density. It is probable that tubers were present in Lake Conway during the early 1977 months, since hydrilla was present in the lake during 1976. Planned modifications to the

model will include a method for estimating starting tuber densities in cases where this information is not available from measured data.

SUMMARY AND CONCLUSIONS

Based on preliminary comparisons with available field data, the hydrilla model appears to reflect biological reality. Improvements are needed with regard to tuber development and initial plant growth from the tubers. These modifications will be made during Fiscal Year 1989, and the model will be validated with field data. Additional algorithms will be developed to interface the model with a white amur growth model and also with the Waterways Experiment Station HARVEST model. When these two modifications are completed, further validation tests will be conducted.

DATA NEEDS FOR MODEL VALIDATION

Field experiments designed to provide data for dioecious hydrilla growth and reproduction are necessary to conduct further validation of this model. These data must be collected frequently throughout at least a 1-year span. Necessary parameters to be monitored include the following:

a. Biomass and its distribution within the water columns.

b. Daily weather and solar data from the field sites.

c. Water temperatures, pH, and Secchi disk readings through the water columns.

d. Photosynthetic and respiratory rates at various temperatures and extinction coefficients.

e. Plant mortality and sloughing.

f. Overwintering conditions.

g. Tuber production information.

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Prototype Digital Data Base for Aquatic Plant Simulation Models

by
M. Rose Kress,* John R. Ballard, Jr.,*
and E. May Causey*

INTRODUCTION

Each of the computer-aided simulation models discussed in this section of the proceedings requires various types of input data and produces results, all of which must be stored and organized. Data bases designed to support aquatic plant control simulation models must be able to accept, manage, analyze, and output information from many sources in various formats. One common thread running through these data, however, is that they a have a spatial component. These data have a locational aspect; the information was collected at or refers to a particular geographic location, which may be a specific region, a lake, or a location within a lake. We must be able to access, combine, modify, update, use, and output the data, always retaining this locational information. The user of simulation models will require data originally collected from maps, field surveys, published inventories, reports, remote sensors, and even results from other models.

The objective is to organize these data in such a manner that (a) the models have ready access to required input data, (b) information from one location is cross-referenced to all other information from that location, (c) data can be easily updated, and (d) new data can be easily added.

THE DATA BASE

Overlay concept

The digital data base can be conceived of as a series of registered map overlays with all tabular and attribute data tied to the map base by virtue of their geographic location. This continual reference to geographic location distinguishes the aquatic plant digital data base described here from simple arithmetic data arrays and lists of tabular data. Figure 1 illustrates the overlay concept and the digital data base interface with simulation models. The data base provides necessary input data to initiate and run the models, and it accepts model output for storage in a compatible format. The data base also contains information helpful in planning and evaluating aquatic plant operational activities. The goal of providing the data base and interfacing with the simulation models is to optimize use of available aquatic plant control measures for site-specific conditions.

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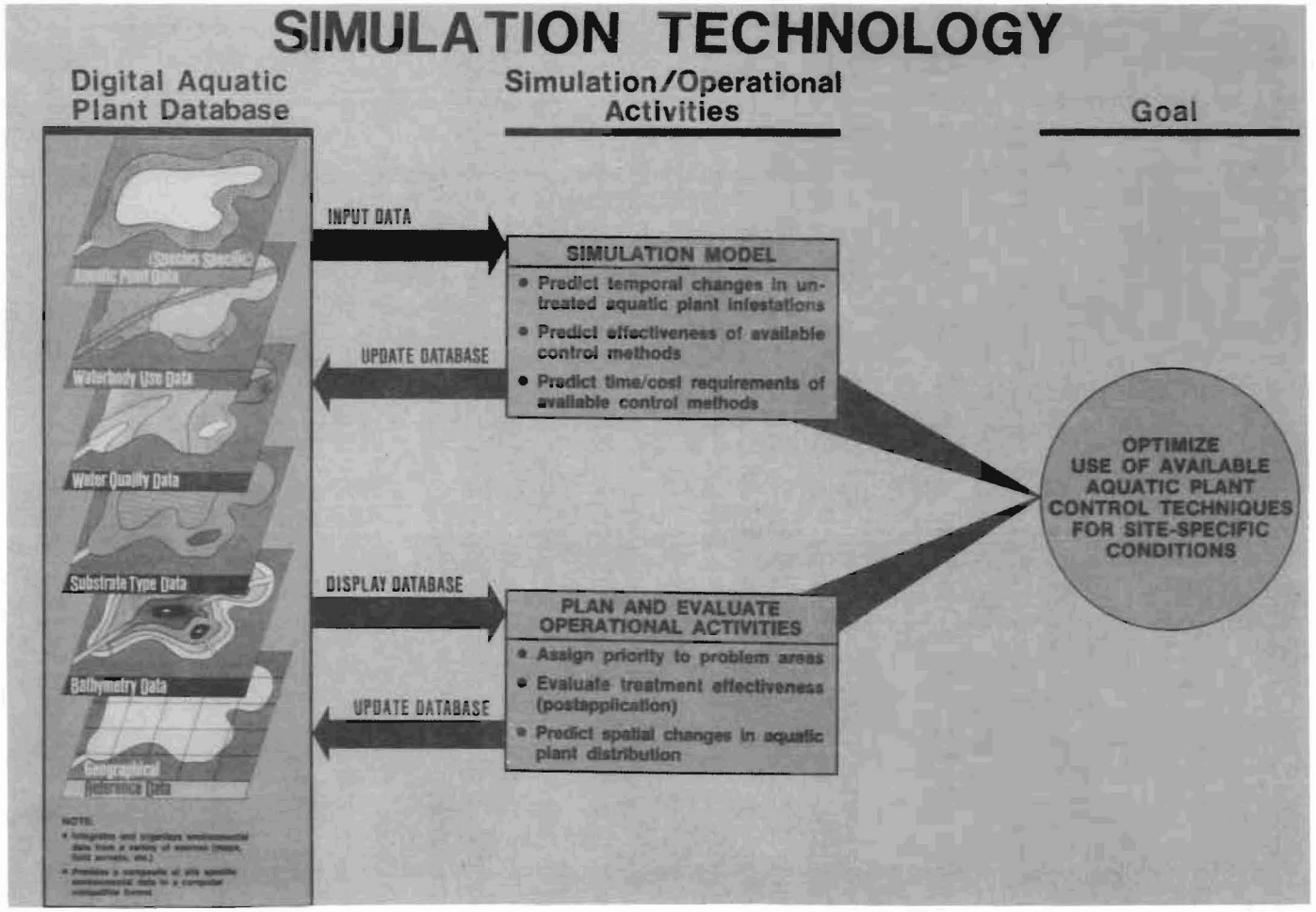

Figure 1. Interaction of aquatic plant digital data base and simulation activities

Data base contents

At a regional level the data base contains locational and descriptive information about water bodies, in addition to weather data such as air temperature and solar radiation.

The most basic geographic information to be stored in the data base is the location of the water bodies. The example data base constructed is in the central Florida Lake Kissimmee area (Figure 2). The lake boundaries shown in Figure 2 were digitized from US Geological Survey (USGS) 1:24,000 series topographic maps. This data base covers an area approximately 70 by 40 km and contains 46 individual lakes with a total water surface area of 430 sq km (106,000 acres, 165 square miles). In addition, 17 canals and 11 rivers and creeks are included. Water body boundary and type are considered somewhat static once entered into the data base; they require little modification from year to year. However, the attributes of the water bodies relevant to aquatic plant control activities change seasonally.

The Florida Department of Natural Resources (DNR) conducts an annual survey of aquatic flora and publishes the results as a series of tables. The DNR survey results for 1987 were entered into the example data base by keying the information to the individual lakes. A basic question a model user may ask at the regional level is, which lakes were surveyed in 1987? And, which of those contain hydrilla? It is a simple matter to highlight for graphic display or create a computer listing of these lakes using the digital data base. Figure 3 indicates the lakes surveyed in 1987. Those lakes reporting hydrilla are shown in Figure 4.

Eventually, more complex computer models will be developed to simulate plant conditions for a particular water body or for specific locations within a water body. These models will require more detailed site-specific information about (a) substrate materials, (b) water quality, and (c) water body use.

The data base is openly structured, allowing these new types of data to be included and accessed by model users. In addition, model results can be stored into the data base for comparison with field observations, measurements, and historical data.

Data base organization

The basic organization of the digital data emphasizes that each geographically referenced element is identified and stored separately in the data base, providing the user with maximum flexibility in accessing data. Figure 5 illustrates a 12- by 12-km portion of northern Lake Kissimmee. The primary element of the data base is the water bodies, each with a unique numerical identifier. This allows the user to consider individual water bodies (i.e., rivers, creeks, canals) or several water bodies (i.e., all canals) as a group. Islands in the lakes are included in the data base in their correct locations.

Areas within lake boundaries indicated as marsh on the USGS 1:24,000 maps

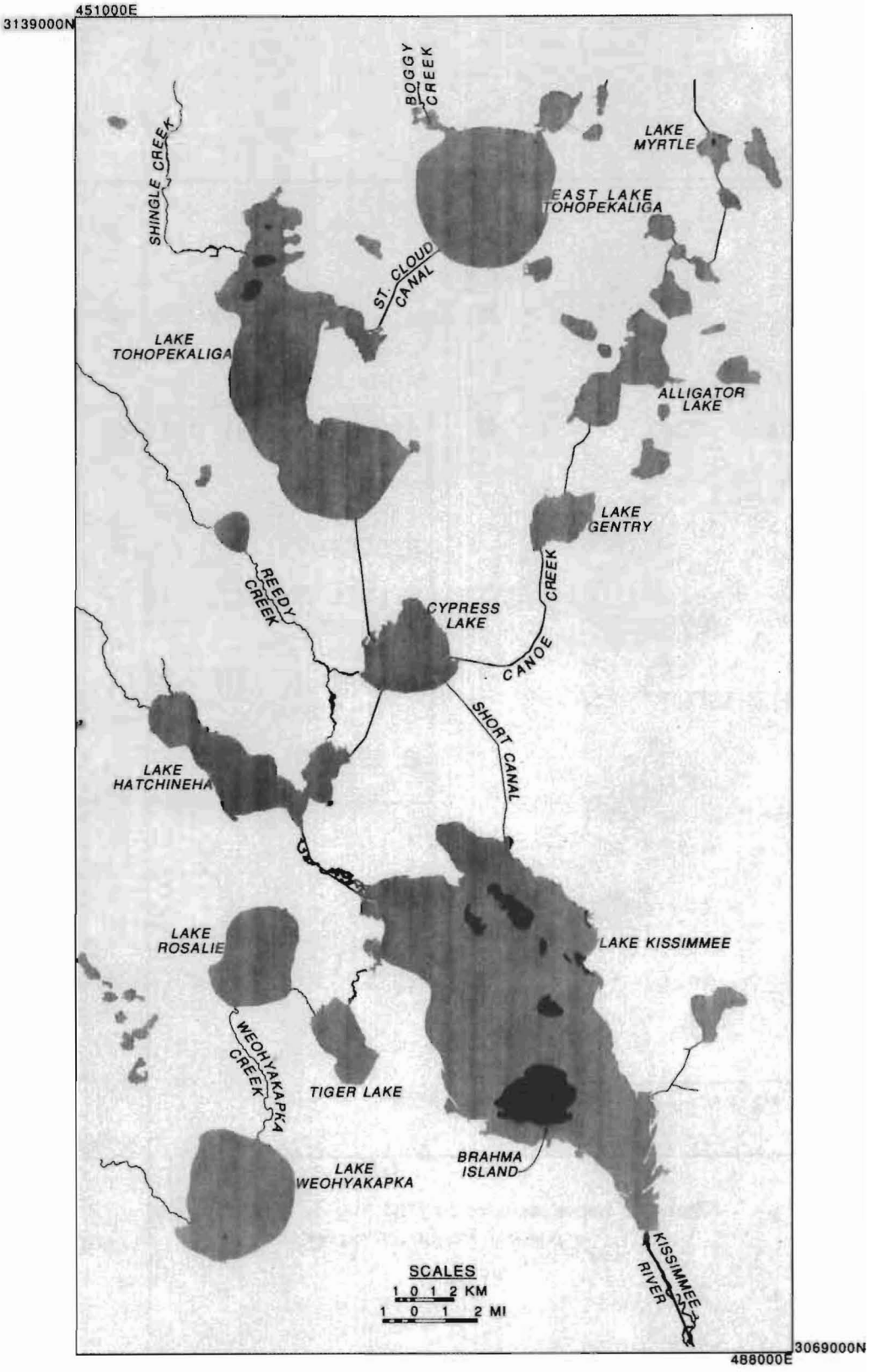

Figure 2. Aquatic plant digital data base study area

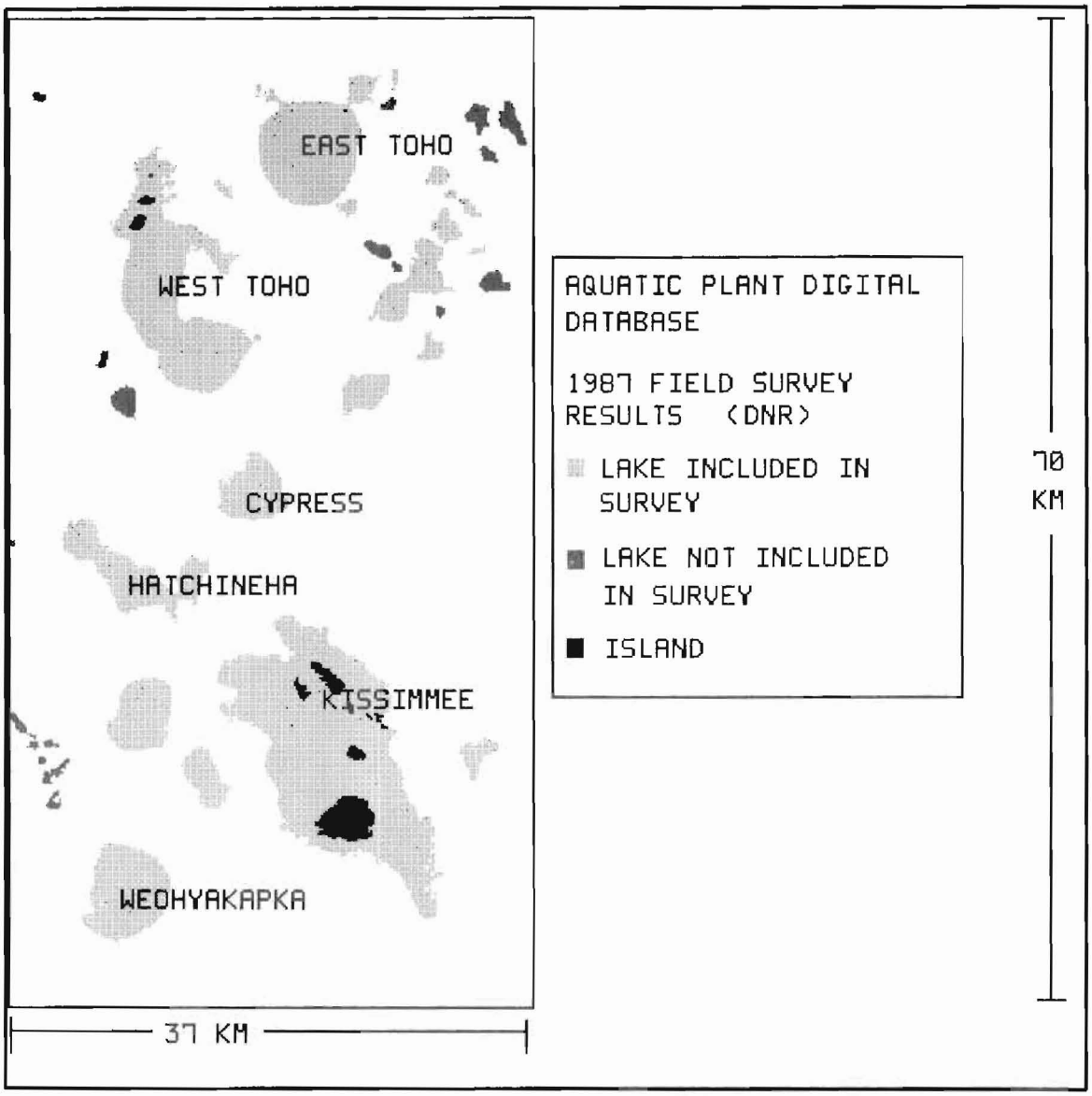

Figure 3. Lakes included in 1987 Florida Department of Natural Resources survey

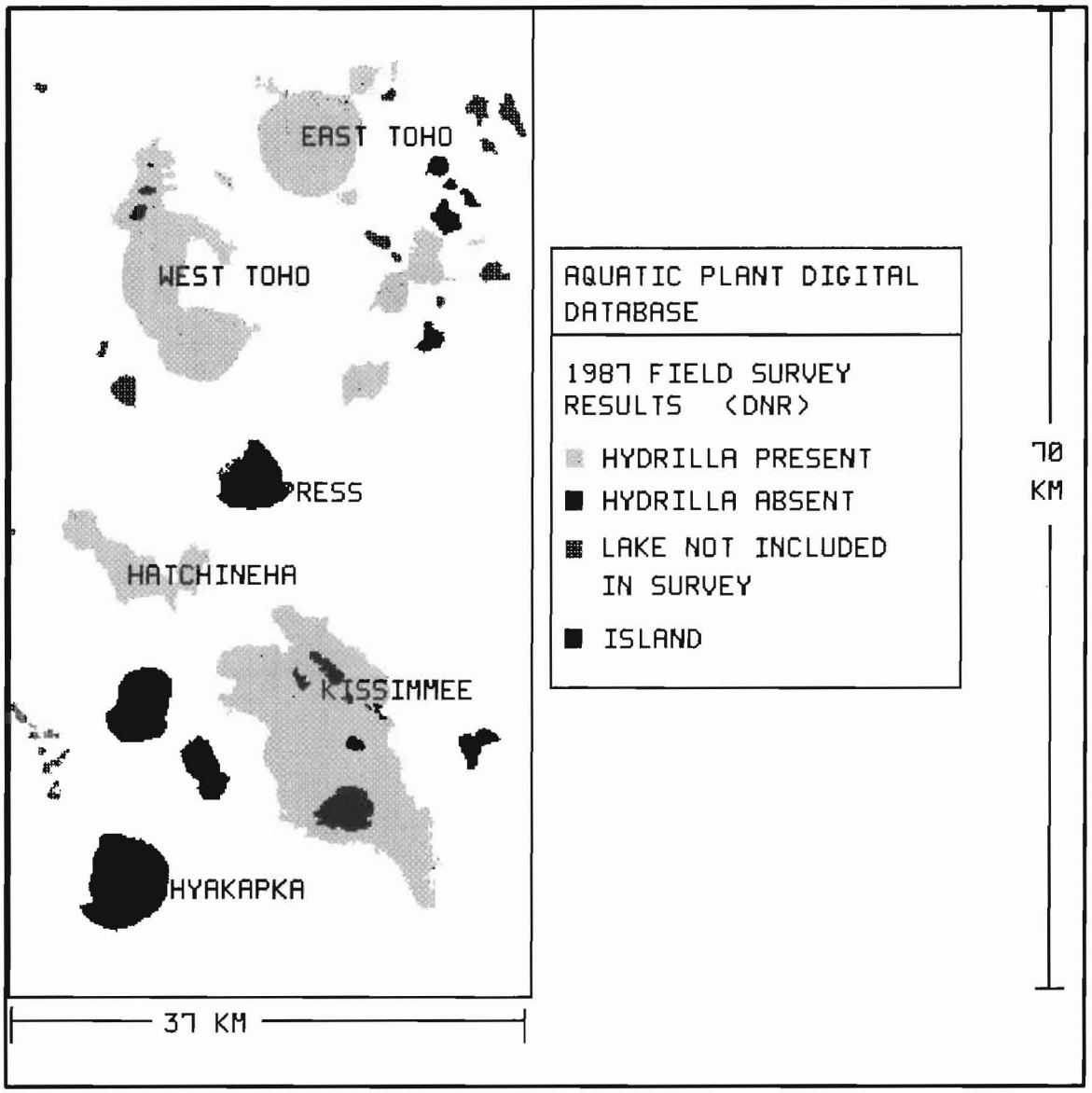

Figure 4. Lakes in which hydrilla was reported in the 1987 Florida Department of Natural Resources survey

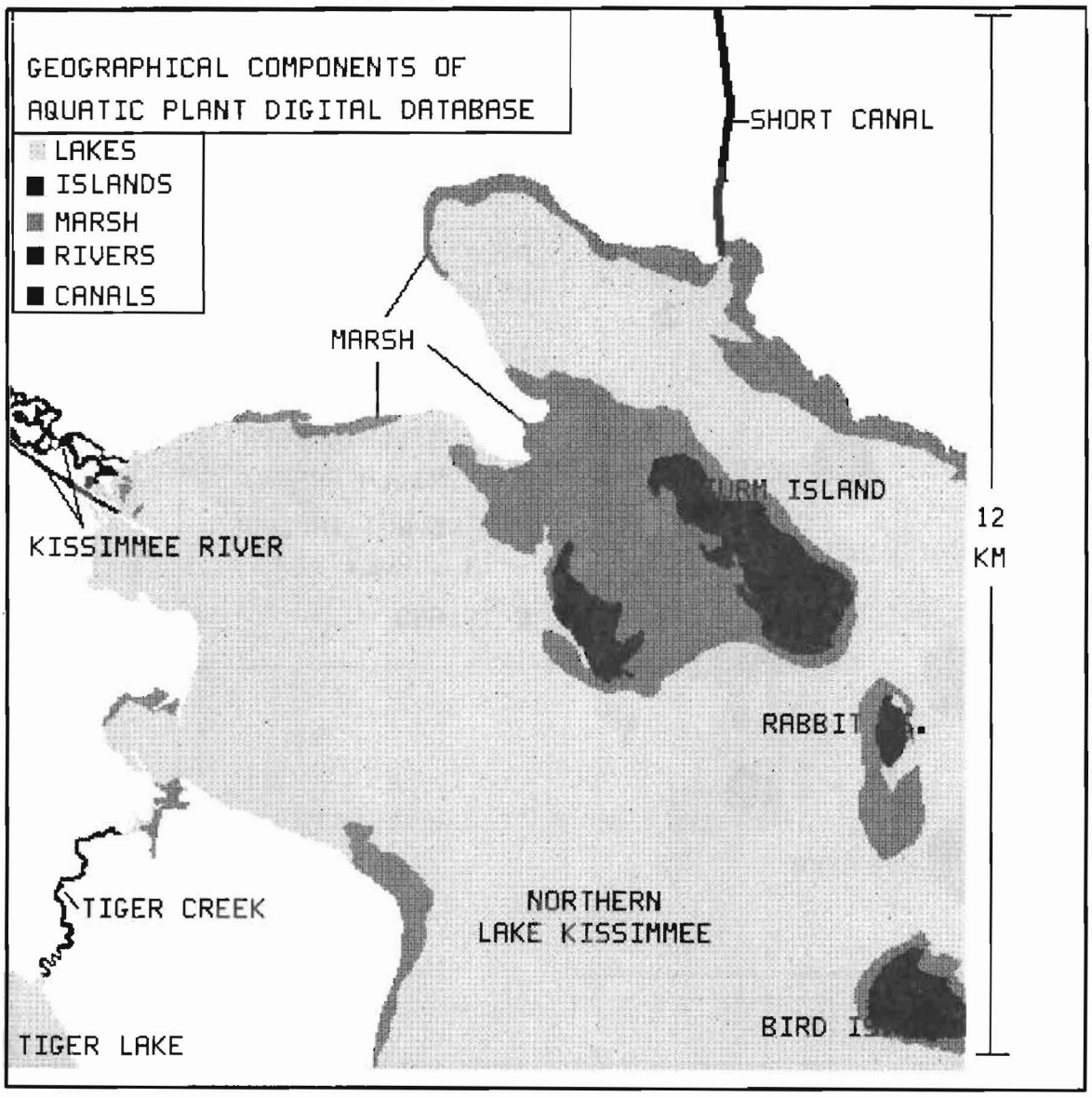

Figure 5. Geographical components of the aquatic plant digital data base

are also included as separate elements. At the site-specific level, the location of boat ramps, piers, potable water intake locations, etc., along with the relevant attributes of these man-made features (size, construction, age, and ownership), would become a part of the data base. This organization of uniquely maintained elements permits flexibility in combining, cross-referencing, overlaying, and extracting the particular piece of information required. The geographic location tag associated with each item of information provides the overall organizational structure of the data base.

Simulation models require water depth information. Figure 6 illustrates the lake bottom elevations for Lake Kissimmee. As the elevation of the water level changes, the water depth can be routinely calculated for all locations in the lake from a few water surface elevation measurements.

Data base update

Another important advantage of a digital information data base is that periodic additions, deletions, and updates can be made easily. For instance, the actual distribution of aquatic plant-infested areas may change dramatically from year to year. Figure 7 shows a satellite view of northern Lake Kissimmee from a 500-km altitude. Taken December 17, 1987, the satellite image was collected by the Thematic Mapper (TM) sensor onboard the Landsat 5 satellite. The TM provides seven channels of digital multispectral data. Shown in Figure 7 is the near-infrared channel (0.76 to 0.90 μ) in which the aquatic plant-infested areas appear as bright tones inside the lake boundary because of high vegetation reflectance in this wavelength interval.

By combining the enhanced, processed satellite image with the digital data base already described, we are able to extract only those areas in the image corresponding to aquatic plant-infested areas, eliminating the shore areas, islands, and open water. The result is depicted in Figure 8.

Further processing of the TM satellite data provides a general classification of the aquatic plant-infested areas into the four categories shown in Figure 9: marsh - tree dominated; marsh - shrub dominated; emergent herbaceous; and submersed herbaceous. These data can then be used to derive acreages of each category type. With ground truth data, the user can develop species-specific classifications that will allow him to detect, map, inventory, and store the distribution of specific plant communities in the digital data base.

This process updates the data base using remote sensor data, provides a permanent record of lake conditions at the time of the satellite overpass, and offers information for large areas. Data of this type can provide insight on plant distribution patterns and other information.

Plant changes over time

An existing digital data base documenting aquatic plant infestations in the Potomac River was used to render the map product illustrated in Figure 10.

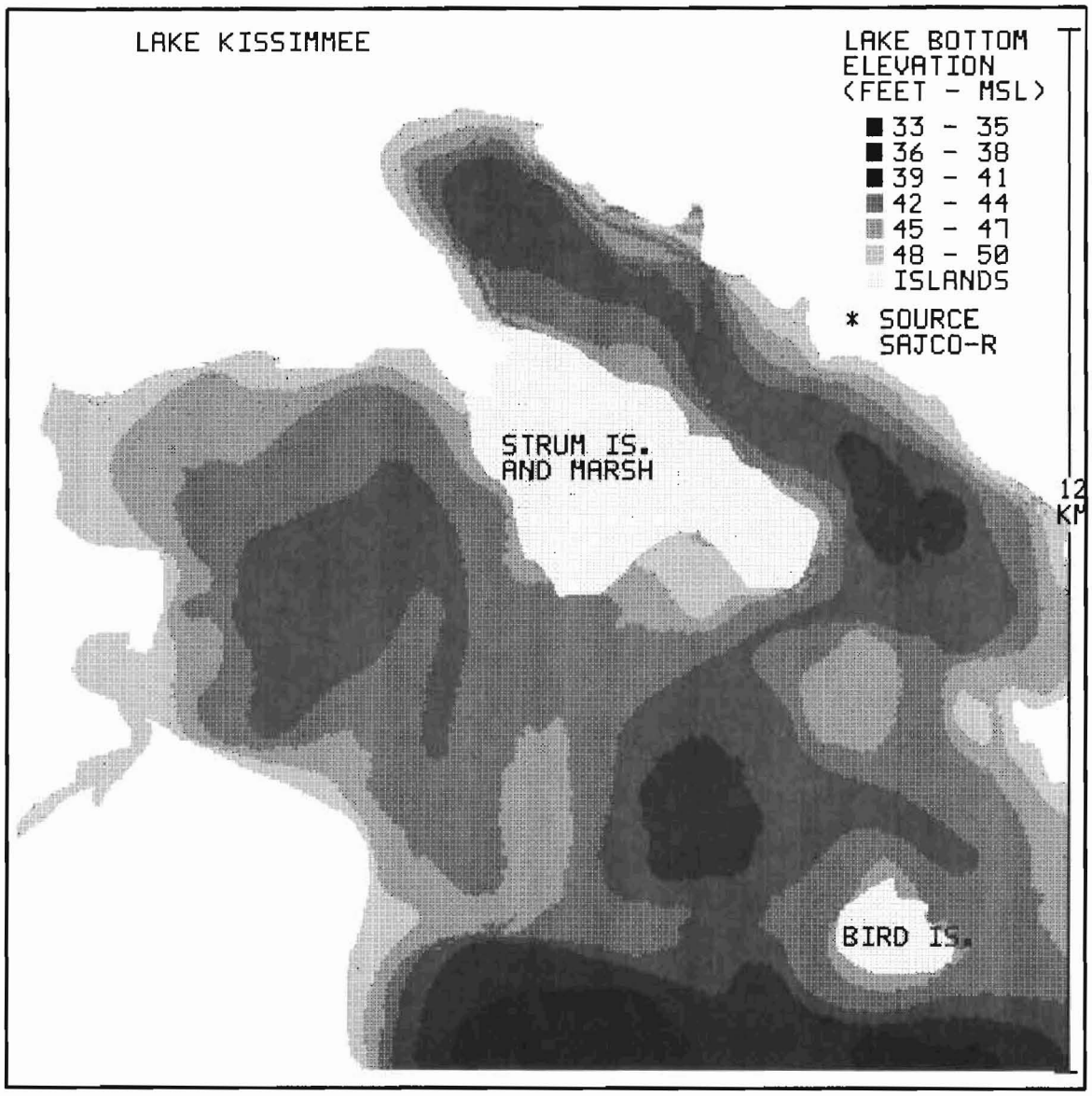

Figure 6. Lake bottom elevation of northern Lake Kissimmee

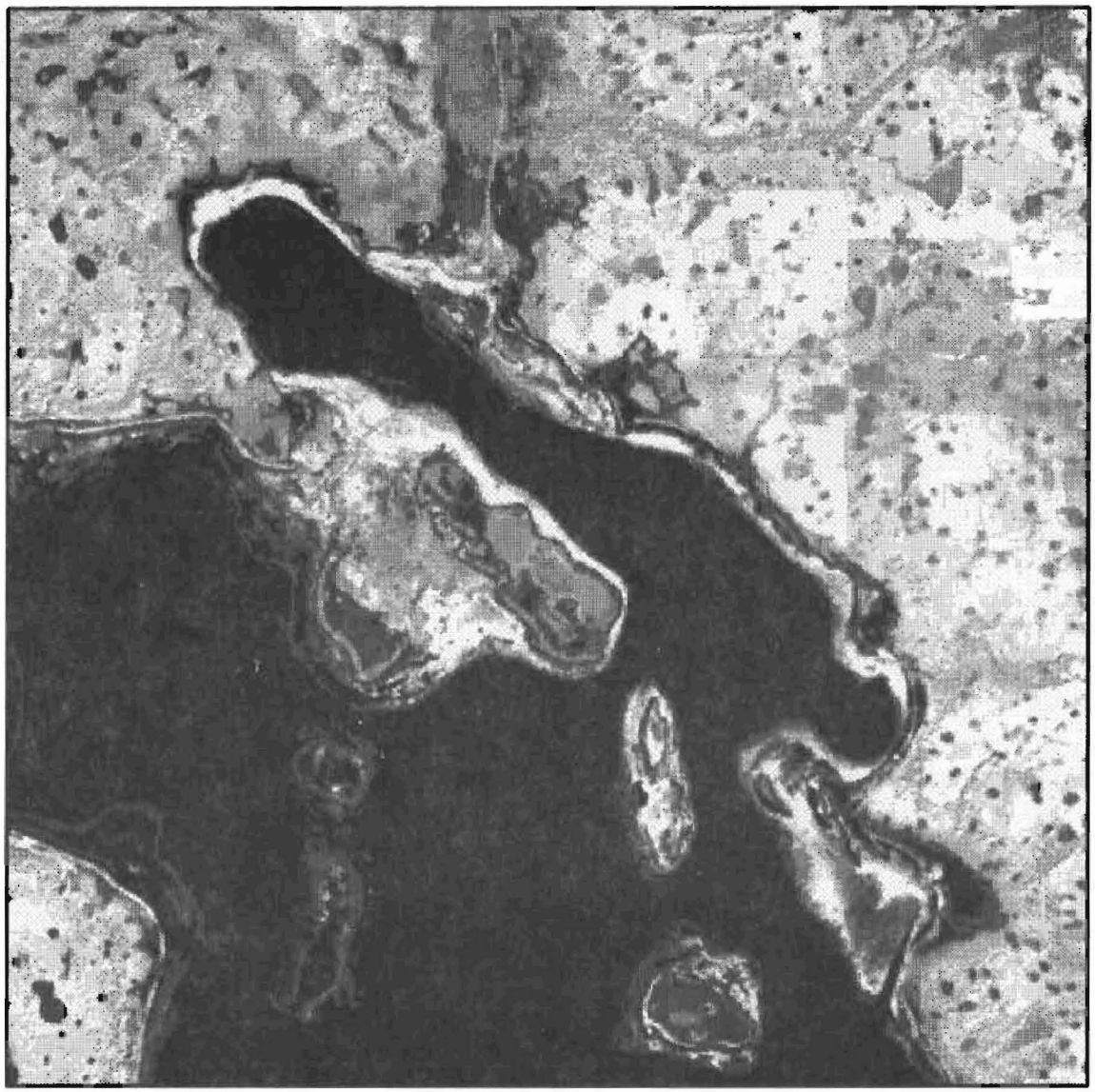

Figure 7. Satellite image of northern Lake Kissimmee

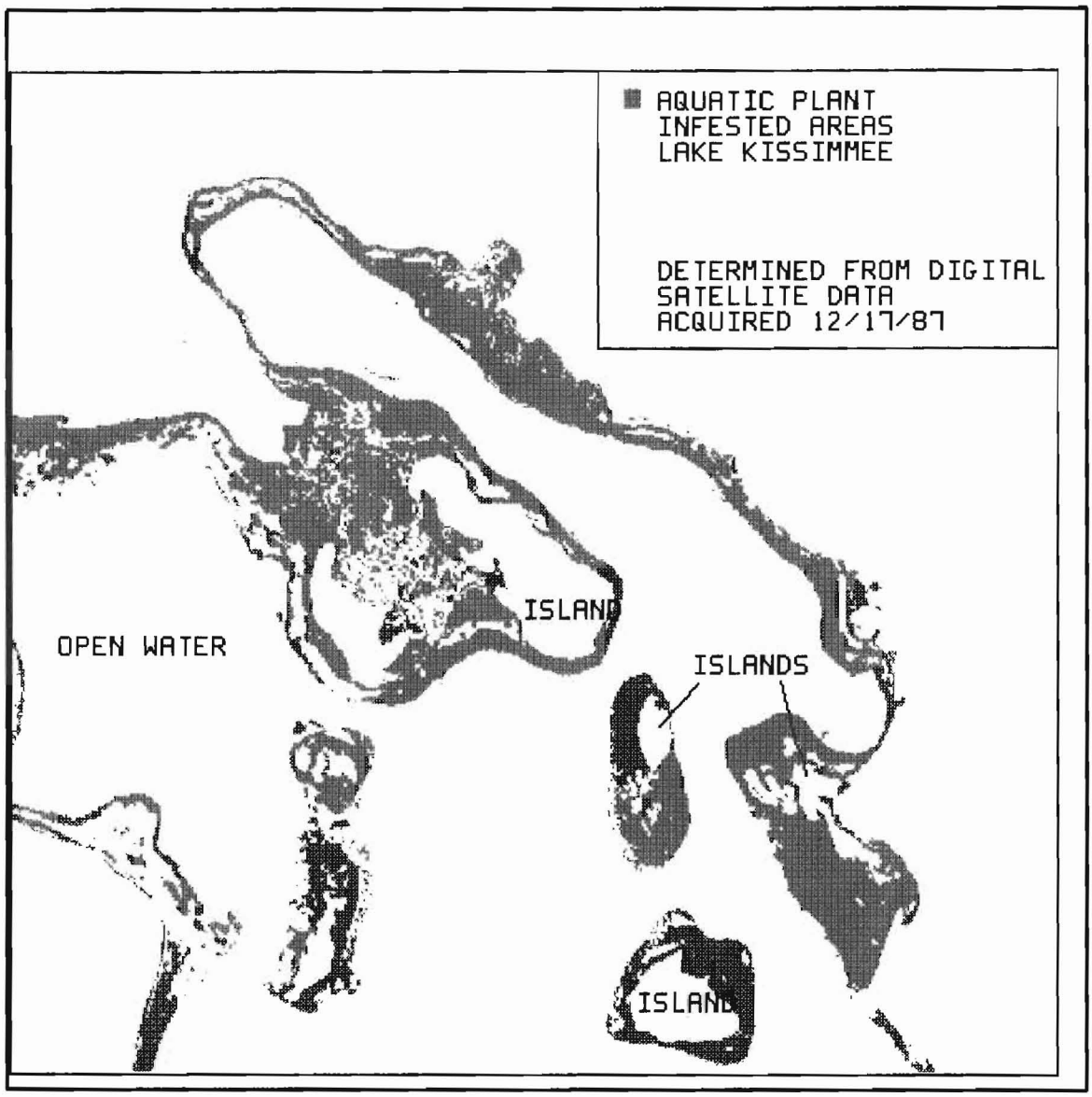

Figure 8. Aquatic plant-infested areas of northern Lake Kissimmee as determined from satellite data

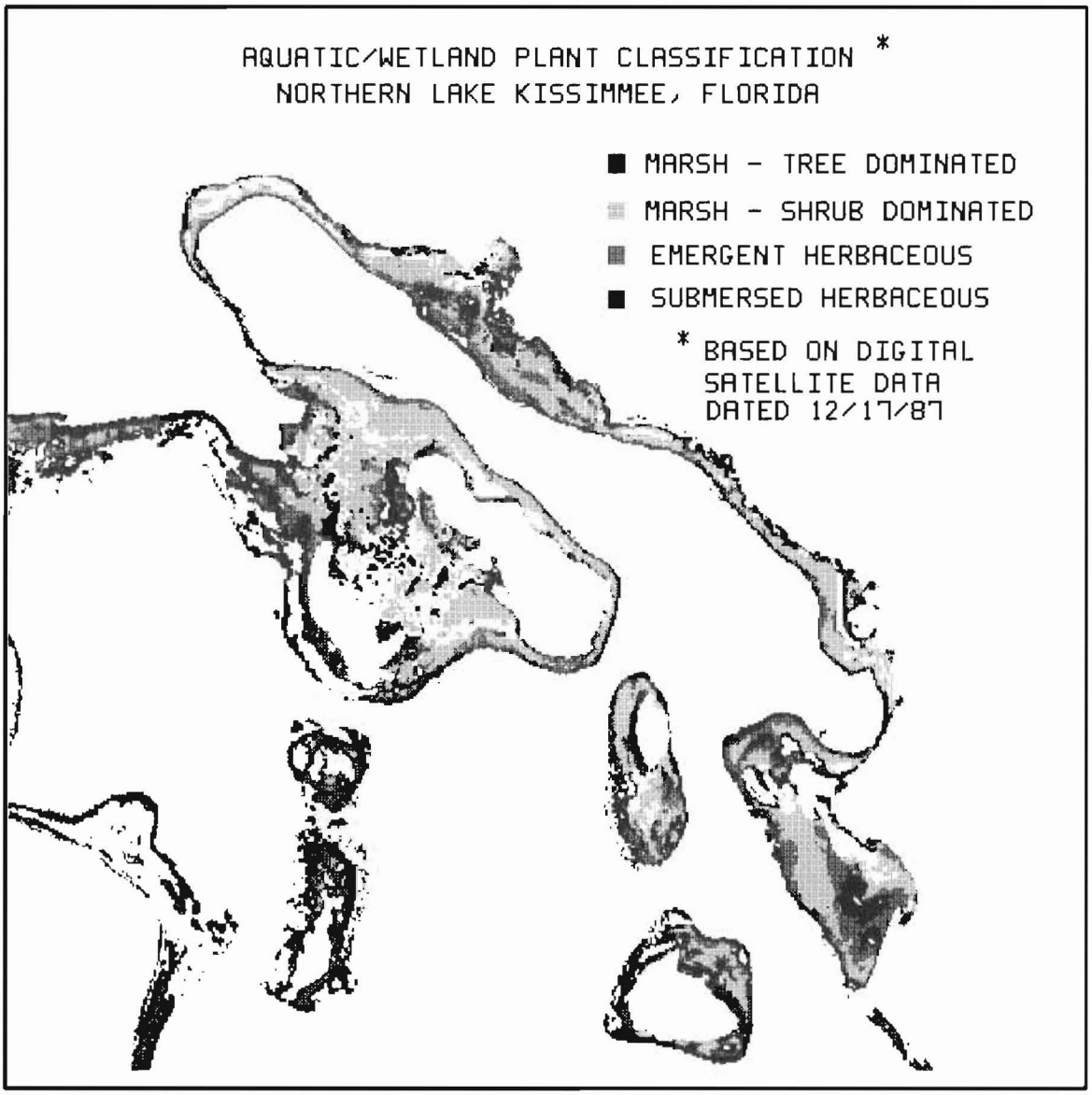

Figure 9. Aquatic/wetland plant classification of northern Lake Kissimmee determined from satellite data

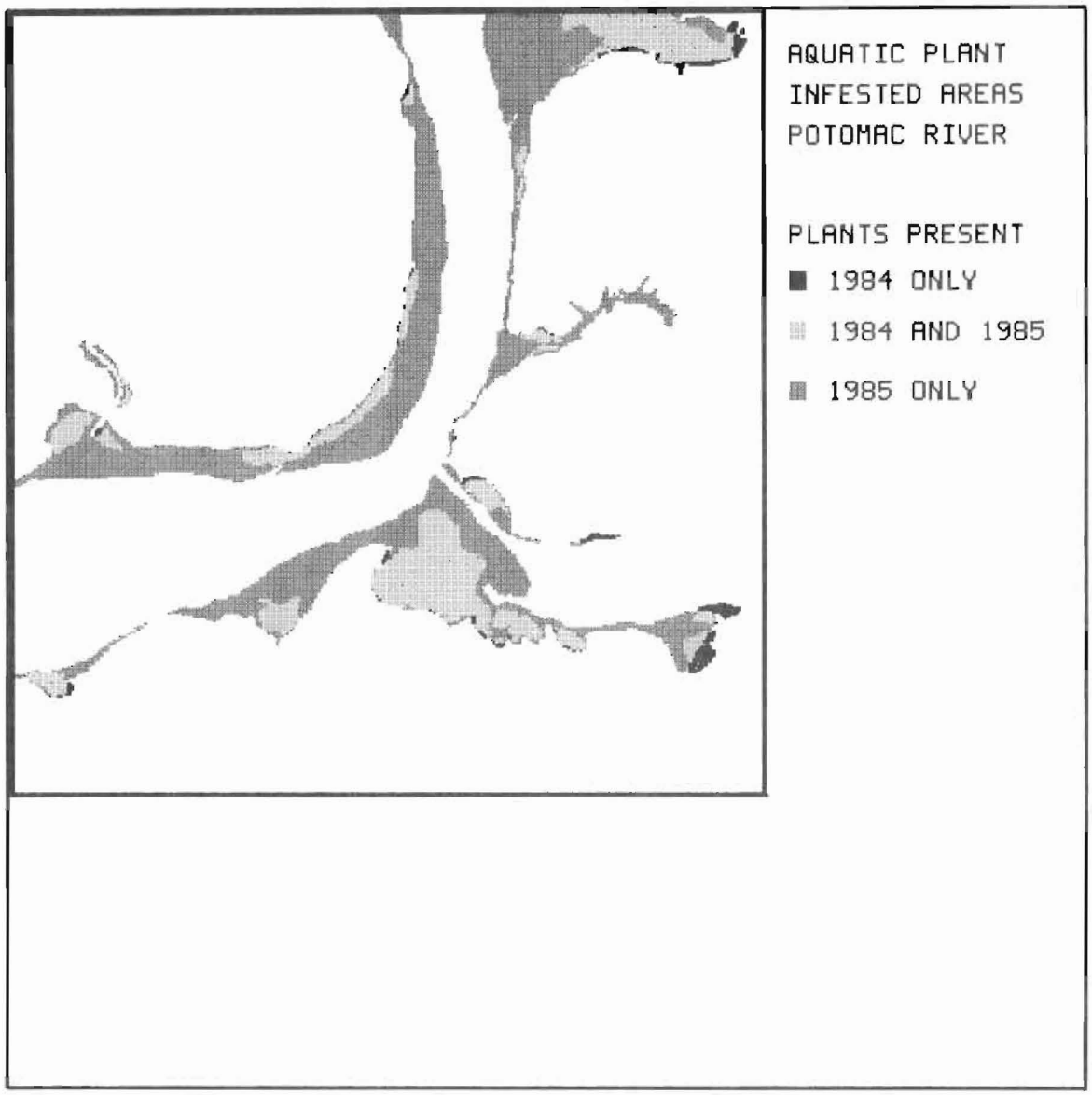

Figure 10. Change in aquatic plant-infested areas between 1984 and 1985 for a portion of the Potomac River

Two years of aquatic plant distribution data collected for the Mount Vernon, Virginia-Maryland, USGS quadrangle were combined to show the new infested areas between 1984 and 1985. The figure shows areas where plants were present in 1984, areas where plants were present in 1985, and areas where plants were present in 1985 but not 1984. Determining the total acreage of the increased plant-infested areas, a simple step using digital data, indicates an increase of 560 ha (1,400 acres) of aquatic plant-infested areas between 1984 and 1985 on the Mount Vernon quadrangle.

SUMMARY

In summary, the aquatic plant digital data base will:

a. Provide regional and site-specific information needed to initialize and run aquatic plant control simulation models for different geographic areas.

b. Include information needed to identify high-priority areas for treatment.

c. Include information relating to possible restrictions on operational control activities such as recreational activities and potable water intakes.

d. Include information useful in identifying lake locations favorable to aquatic plant colonization.

e. Serve as an effective mechanism to organize, store, manipulate, and retrieve complex data from many sources.

f. Generate a historical archive of water body and aquatic plant distribution data.

Finally, the well-structured digital format of the information data base will greatly facilitate technology transfer.

Aquatic Plant Control Operations Support Center

by
William C. Zattau*

INTRODUCTION

In October 1980, the Jacksonville District was designated by the Office, Chief of Engineers, as the Aquatic Plant Control Operations Support Center (APCOSC) 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 personnel assist other Corps elements and other Federal and state agencies in the planning and operational phases of aquatic plant control. The specific duties, relationships with other Corps aquatic plant control programs, and guidelines for utilization of the APCOSC are outlined in Engineer Regulation 1130-2-412.

FISCAL YEAR 1988 ACTIVITIES

The APCOSC responded to 128 requests for assistance during FY 88. A breakdown of these activities appears in Table 1. Figure 1 graphically indicates

Table 1
APCOSC Contacts, FY 1988

Type Assistance	*Corps OCE*	*Corps WES*	*Corps Div.*	*Corps Dist.*	*Other Fed.*	*Other Country*	*State/ Local*	*Industry*	*Private*	*Total*
Planning	6	2	3	9	1	0	3	0	2	26
Operations	7	4	7	22	6	0	15	1	1	63
Research	0	24	0	0	0	0	1	1	5	31
Training	0	0	0	8	0	0	0	0	0	8
Total	13	30	10	39	7	0	19	2	8	128

the types of information requested; Figure 2 provides a breakdown as to source of information requests. As would be expected, nearly 50 percent of the contacts were related to operational concerns, 35 percent of which were from Corps Districts. The total number of contacts increased by 60 percent over FY 87.

This year, since the Jacksonville District is serving as meeting host, our staff has participated in planning and organizing the 23rd Annual Meeting of the Aquatic Plant Control Research Program and the Lake Okeechobee field trip. The staff also organized an operations session at the Aquatic Plant Management Society meeting this year in New Orleans, Louisiana.

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

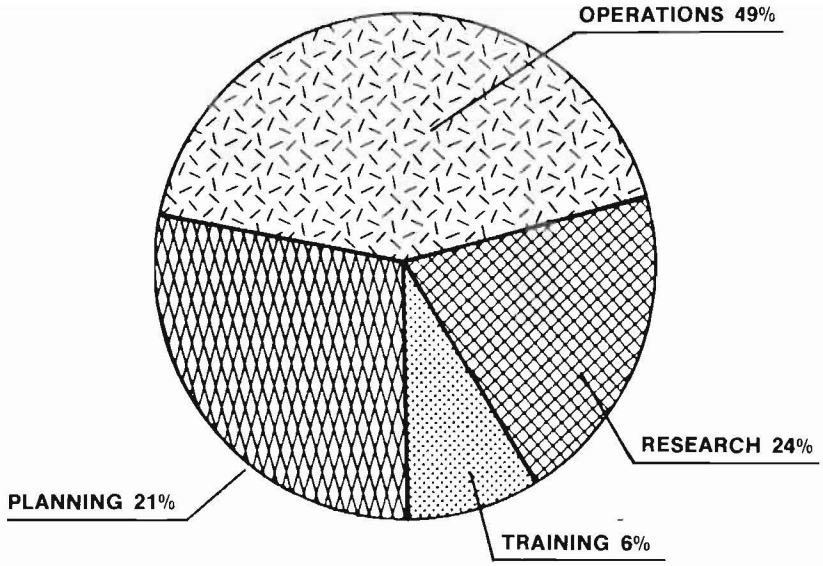

Figure 1. Types of information requested

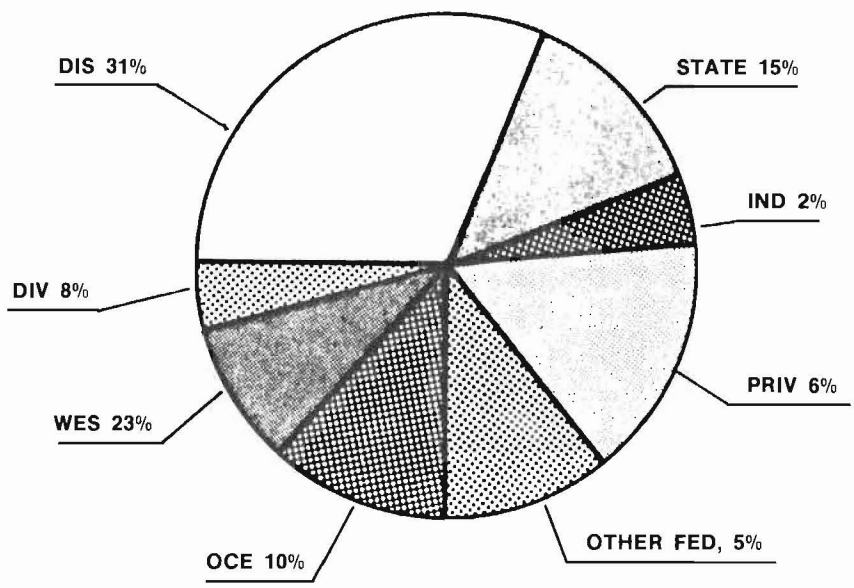

Figure 2. Breakdown of requests for information

The Center provided assistance for two Corps Districts that are in the process of establishing Aquatic Plant Control Programs. The Pesticide Applicator Training Course was taught by Center staff again this year.

The Center published four quarterly issues of the APCOSC Information Exchange Bulletin. Several articles were provided by Corps personnel in the audience, and I certainly appreciate those efforts. I request that those of you who have not yet participated in authoring an article contact me.

The second Annual Aquatic Plant Control Program District Survey will soon be distributed. This will once again detail aquatic plant management activities in 14 Corps District programs. This information was provided by Corps Operations personnel and was compiled by the Center.

In June, the APCOSC distributed approximately 80,000 alligatorweed flea beetles to seven state and five Federal agencies which had requested this annual service. The Center assisted in coordination of a demonstration at this meeting of a state-of-the-art computer-assisted aerial survey system for monitoring and documentation of aquatic plant populations.

CONCLUSION

The Center has once again had a busy and productive year. I hope that those of you in the audience will avail yourself of the services provided by the Center and, when possible, contact us with suggestions on how we can improve our support for Corps aquatic plant control activities.

Synopsis of the District/Division Aquatic Plant Management Operations Working Session

by
William C. Zattau*

The third annual Corps Division/District Working Session was held during the 23rd Annual Meeting of the Aquatic Plant Control Research Program. Thirty-seven individuals attended, representing Headquarters, US Army Corps of Engineers (HQUSACE), the Waterways Experiment Station (WES), six Corps Divisions, eleven Corps Districts, five state agencies, and industry.

Presentations were given by Clyde Gates, Little Rock District; Avis Kennedy, Nashville District; and John Emmerson, Pacific Ocean Division. Gates discussed the history of the aquatic plant problem in his District, stressing that no major problems currently exist and that the District is concentrating its efforts on monitoring. He mentioned that some sportsmen appear to be intentionally transplanting hydrilla and Eurasian watermilfoil in attempts to improve fisheries.

Kennedy discussed the appearance of Eurasian watermilfoil in Lake Barkley, Kentucky, and Nashville District's continuing management and monitoring efforts. She cited the adverse consequences of its likely spread upstream.

Emmerson provided an interesting presentation on emergent vegetation and its adverse contribution to a major flood event in Kawainui Marsh, Oahu. The Pacific Ocean Division is currently involved in determining the best strategy to prevent a reoccurrence.

Participants discussed the spread of aquatic plants by waterfowl and intentional spread by sportsmen. Several attendees expressed suspicion that fishermen were instrumental in causing new infestations in their Districts. The probability of educating these people as to the problems they were causing was determined to be low.

The possibility of convening a workshop to discuss the value of hydrilla and similar problem species in relation to fisheries and waterfowl was discussed. The information presented and compiled in such a workshop could be used in Corps public information meetings.

The problem of waterhyacinth spreading from wastewater treatment containment areas was discussed. The waterhyacinth is used for treatment operations by some cities and, due to the explosive growth of the plant, the population rapidly expands past the capacity of the containment area. Excess plants are sometimes inappropriately disposed of instead of destroyed. In response to this problem, the

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

Mid-South Chapter of the Aquatic Plant Management Society recently passed a resolution and forwarded it to the US Environmental Protection Agency and the State of Alabama permitting agencies, recommending they strongly enforce noxious plant containment and proper disposal of excess plants.

Carl Brown (HQUSACE) addressed the topic of Local Cooperative Agreements (LCA). He explained the basics of establishing a cost-shared Aquatic Plant Control Program. The first step is to conduct a reconnaissance study at 100-percent Federal cost to determine if there is a problem, and if so, if there is cause for Federal involvement, and then to determine if a favorable benefit-cost ratio exists.

If the reconnaissance indicates that a cooperative program is warranted, the District forwards this information to HQUSACE with a negotiated LCA for cost-shared, detailed environmental studies. After completion of these studies, a second LCA will be negotiated for the actual aquatic plant management operations.

If the second LCA can be used without change for subsequent years, it can be signed at the District Engineer level. If not, it must be approved by Washington. Alteration of the Annual Work Plan does not indicate that the LCA has to go back to Washington for reapproval. Only modifications to the basic LCA require that.

A discussion of what determines whether a research problem is of regional or national importance arose. If determined to be of national importance, it can be conducted at 100-percent Federal cost; if not, it must be cost-shared. Brown stated that this determination should be made by him and Lewis Decell, WES.

A spirited discussion centered around the topic of how local cooperators and Districts determine and document their respective administrative costs. Some state cooperators would like more opportunity to review and comment on District costs and the allowability of such costs. The subject of differences in state and Corps overheads charged to the program and their effect on operations was brought up.

A detailed explanation of the upcoming large-scale grass carp demonstration on Lake Marion in South Carolina was given.

The group decided that 2-1/2 to 3 hr was ample time for the Working Session. It was decided that having it early in the afternoon just after lunch was the preferred time.

Southwestern Division, Tulsa District

by
Loren M. Mason*

INTRODUCTION

Pat Mayse Lake, Texas, is the only Tulsa District lake experiencing an undesirable aquatic plant infestation of Eurasian watermilfoil (*Myriophyllum spicatum*), to date. Annual Control Programs have been conducted since 1983, with control emphasis placed on heavy infestations occurring in and around developed recreation shoreline areas.

A successful chemical control program was accomplished again during June 1988, when approximately 85 acres were treated with 18,000 lb of Aquathol-K to control watermilfoil infestations around recreational shoreline and designated swimming beach areas. With the successful demonstration that watermilfoil can be controlled effectively and safely in targeted areas on Pat Mayse Lake, with the use of Aquathol-K, the District initiated a test control program in May 1988 to determine the effectiveness of Sonar-SRP (fluridone).

EXPERIMENTAL PROGRAM

Because Pat Mayse Lake is the primary water supply source for the city of Paris, Texas, and label restrictions on Aquathol-K prohibit the use of treated waters for up to 14 days after treatment, our control program has had to be scheduled during a 2-week time frame in June of each year, during which the city of Paris, Texas, can go off-line from Pat Mayse to an alternate city lake.

To eliminate the restrictive 2-week time period associated with use of Aquathol-K, the District initiated an experimental chemical program on May 17, 1988, using a less restricted-use herbicide for potable water supplies. Sonar-SRP (fluridone) was applied in Forest Point Cove (see Figure 1). Approximately 1,200 lb of Sonar was applied to a 12-acre area.

A comprehensive water quality monitoring program was conducted in conjunction with the Sonar-SRP (fluridone) test program. Concentrations of fluridone were monitored through time in water, sediments, and fish tissue. Results of the program showed that Sonar-SRP was ineffective for control of the watermilfoil. Fluridone was not detected in water samples at any time during the treatment program. The only fluridone detected during the study period was in the sediment on the day of treatment and 7 days later.

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

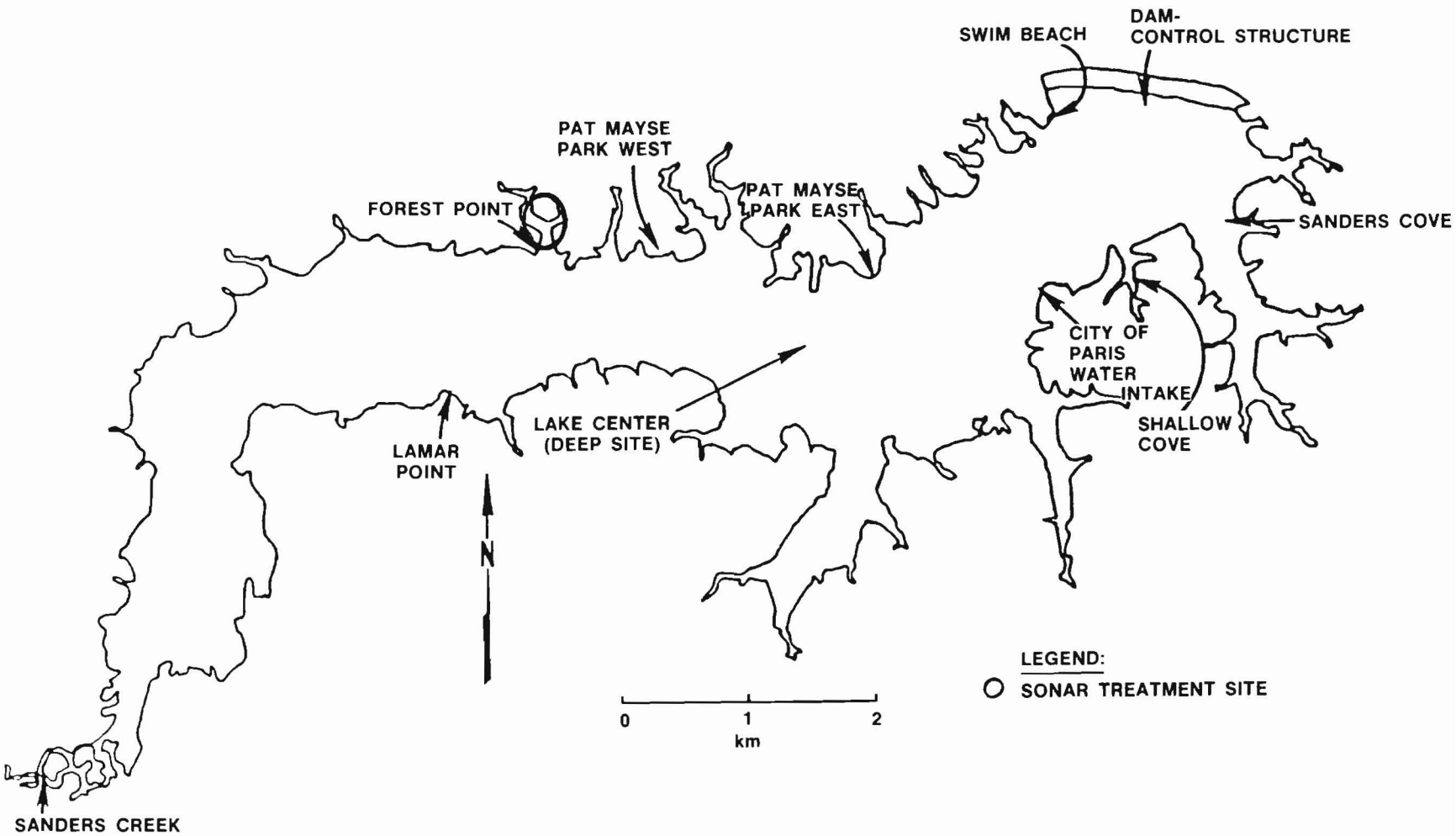

Figure 1. Pat Mayse Lake-Forest Point Cove (May 1988)

Although fluridone was present in the treated areas, there is a strong indication that some process, such as dispersion, dilution, photolysis, and perhaps biodegradation, may have been responsible for failure to detect fluridone in the water at any time during the study period.

Watermilfoil infestations in the treated area were not affected by the fluridone treatment, as biomass continued to increase steadily throughout the study period. This was also substantiated by visual observations throughout the summer and early fall of 1988.

The failure of fluridone to elicit mortality in the treated watermilfoil area was very disappointing, as well as somewhat unexpected. Although it is commonly accepted that fluridone is effective only when low concentrations remain in contact with target plants for a period of 14 to 21 days, it was expected that fluridone would work in the isolated and protected Forest Point Cove area. However, it now appears that the concentrations of fluridone, through time, may have been too low to elicit a mortality response. The reason for this is probably loss of fluridone from the treated area by dispersion and dilution.

To answer this question, a study was initiated by North Texas State University, Denton, Texas, to evaluate the cause for the failure of Sonar-SRP to work in the Forest Point Cove area. Results of that study will be available by January 1989, after which a decision will be made whether to try Sonar-SRP again.

CONCLUSION

Based upon results of the 1988 chemical programs for both Aquathol-K and Sonar-SRP, the District will evaluate the future use of both chemicals. If Sonar-SRP is eliminated from further consideration for use at Pat Mayse Lake, the District plans to submit, to the US Environmental Protection Agency and the State of Texas, all the water quality data obtained on Aquathol-K over the past 7 years, and request a Special Local Need exemption to the 14-day label restriction for use of lake waters after treatment.

Distribution of *Salvinia minima* in Louisiana

by
Glen N. Montz*

This paper was prepared to add to our knowledge of the aquatic flora of Louisiana. An earlier report (Montz 1985) revealed the spread of *Salvinia* in the state at that time.

Salvinia minima, an aquatic fern, was first reported in Louisiana by Landry (1981). He collected this floating fern in a canal near Franklin in St. Mary Parish in September 1980. The distribution of salvinia was reported by MacRoberts (1988) and includes Lafourche, Terrebonne, St. Mary, St. Martin, and Assumption Parishes. This writer has collected the species from 1980 to present and has documented the spread throughout the state. This paper will serve to document the distribution of salvinia in Louisiana at this time.

This species has spread throughout coastal Louisiana by means of the Gulf Intracoastal Waterway (GIWW). It has been found in most southern parishes and extends from near Lake Charles to Harvey, Louisiana. It is very abundant in St. Mary and Terrebonne Parishes and is well established in the state and continuing to spread.

This species will be difficult to eradicate, as it inhabits waterways, marshes, and swamps. It has the potential to become a troublesome, unwanted aquatic weed. Efforts by the US Army Corps of Engineers were begun in 1984 to control this species with the herbicide diquat. Between 1984 and 1988, 1,459 acres of infested waterways have been treated.

Tarver et al. (1986) note that "salvinia is believed to have been introduced into the United States from either Africa or South America. It is used by the aquarium industry and therefore sold throughout the United States. Florida contains the largest populations of salvinia, but its range covers much of the south and southeast. Dense infestations will shade out desirable submersed vegetation, although this is a rare occurrence. Salvinia is of little wildlife significance except for providing nesting habitat for aquatic insects and shade for fish."

Table 1 lists the collections of this species in the herbaria at Louisiana State University in Baton Rouge (LSU) and the University of Southwestern Louisiana (USL). Specimens collected by this writer were distributed to LSU, Tulane University, USL, Northeast Louisiana University, Southeastern Louisiana University, and Southern Methodist University. One specimen of *Salvinia auriculata*, collected by F. Mackaness in May 1941 from a Dillard University aquarium, was noted in the herbarium at Tulane University.

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*US Army Engineer District, New Orleans, Aquatic Growth Control Unit; New Orleans, Louisiana.

Table 1
Collections of Salvinia Noted in Louisiana Herbaria

Location (Parish/Area)	*Date*	*Investigator*	*Identifier*	*Herbarium*
Assumption				
Bayou L'Ourse	16 Sep 86	Montz	5445	LSU
Cameron				
Canal near Gibbstown	22 Nov 87	Rebertus	sn	LSU
Iberia				
Lake Fausse Pointe	2 Oct 86	Montz	5447	LSU
Lake Fausse Pointe	2 Oct 86	Montz	5448	LSU
Jefferson				
Fleming Canal	28 Oct 87	Montz	5494	LSU
GIWW near Bayou des Families	30 Oct 87	Montz	5495	LSU
GIWW at Crown Point	30 Oct 87	Montz	5496	LSU
Mississippi River at Harvey Lock	2 Nov 87	Montz	5498	LSU
Lafourche				
Canal in Delta Farms	30 Aug 84	Montz	5379	LSU
Canal in Delta Farms	9 Apr 87	Montz	5456	LSU
Bayou near Thibodaux	26 Jul 87	Givens	4980	LSU
Bayou des Allemands near Hwy 90	4 Jan 88	Montz	5503	LSU
St. Charles				
Bayou Gauche	15 Aug 88	Montz	5530	LSU
St. Martin				
Belle River	16 Sep 86	Montz	5446	LSU
St. Mary				
Canal near Franklin	6 Sep 80	Landry	7791	USL
Canal near Franklin	9 Sep 80	Montz	5101	LSU
Berwick Canal	19 Jul 84	Montz	5370	LSU
Canal near Bayou Sale	16 Nov 84	Montz	5399	LSU
Verdunville Canal	13 Feb 85	Montz	5400	LSU
Bayou Ramos	16 Sep 86	Montz	5444	LSU
Atchafalaya River Delta	28 Apr 88	Givens	5140	LSU
Terrebonne				
Bayou Copesaw	17 Oct 85	Montz	5432	LSU
Canal near Lake Theriot	15 Apr 87	Montz	5458	LSU

Observations of salvinia in waterways have been documented by many individuals. Several collections were made in a number of these waterways, but those listed in Table 2 are from competent observations. This information is given by parish and waterway, with acknowledgments to the individuals who shared this information. Table 2 gives waterways by parish where salvinia has been observed and/or collected.

The observations noted above were by Stewart Andrews, Albert Aucoin, Ray Blouin, Vernon Bunch, Rudy Champagne, Ernest Ginn, Florence Givens, Jarvis Guidry, Greg Guillot, Damian Harleaux, Charles Laird, Blake Landry, C. J. Landry, Garrie Landry, Wesley Law, Tommy Lee, Stacy Leonard, Alfred Lepine, Wayne Martin, Herschel Moll, Robert Price, Tom Self, Jack Terrebonne, Dan Tomei, Larry West, and Mike Wirsing. Within a short period of time, it can be expected that salvinia will be found in every southern parish in the state.

As of this date, collections of salvinia have been made in Assumption, Cameron, Iberia, Jefferson, Lafourche, St. Charles, St. Martin, St. Mary, and Terrebonne

Table 2
Observations/Collections of Salvinia in Louisiana

Assumption Parish	
Bay Natchez Gas Field Canals	Bayou L'Ourse
Bay Natchez	Bayou Corne
Belle River	Big Bayou Goddell
Bayou Pierre Part	East Lake Verret Gas and Oil Canals
Bayou Grobec	Grand Bayou
Bayou Magazile	Lake Verret
Bayou Sherman	
Bayou Cherami	
Iberia Parish	
Bayou Teche	Lake Fausse Pointe
Big Bayou Pigeon	Little Bayou Pigeon
Grand Lake	Loreauville Canal
GIWW	Old River
Hog Island	
Iberville Parish	
Big Bayou Pigeon	Upper Grand River
Lake Natchez	West Fork Bayou
PAWW	White Castle Oil Field Canals
Jefferson Parish	
GIWW between Crown Point and Harvey Lock	Harvey Canal
	Fleming Canal
Lafourche Parish	
Bayou des Allemands	Delta Farms Canals
Bayou des Amoreux	GIWW
Bayou L'Eau Bleau	Grand Bayou
Bayou Perrot	Hollywood Canal
Company Canal	Lake Long
St. Charles Parish	
Bayou des Allemands	Burchel Canal
Bayou Gauche	Grand Bayou
St. Martin Parish	
Bayou Long	Lake Palourde
Bayou Milhomme	Little Bayou Sorrel
Bayou Teche	Middle Fork Bayou
Belle River	Old River
Big Fork Bayou	PAWW
Duck Lake	Six Mile Lake
Flat Lake	West Fork Bayou
Grand Lake	West Lake Verret Oil and Gas Field Canals
Grassy Lake	

(Continued)

Note: GIWW = Gulf Intracoastal Waterway; PAWW = Port Allen Alternate Waterway.

Table 2 (Concluded)

St. Mary Parish	
East Gate Waterways	West Gate Waterways
Bar Pit	Bar Pit
Bayou Patterson	Bayou Blue
Little Bethel Bayou	Bear Bayou
Possum Bayou	Big Oaks Bayou
Simmons Bayou	Crow Bayou
Thompson Bayou	Log Bayou
Bayou Sale Oil and Gas Field Waterways	Grand Lake
Bayou Blue	Baldwin Canal
Bayou Chaffe	Bayou Ramos
Cutoff Bayou	Bayou Teche
East Over Bayou	GIWW
Hog Bayou	PAWW
Horseshoe Bayou	Hanson Canal
Leopard Bayou	Lake Fausse Pointe
Lone Oak Bayou	Lake Palourde
West Over Bayou	Mud Lake
Charenton Canal	Six Mile Lake
Flat Lake	Verdunville Canal
Franklin Canal	
Terrebonne Parish	
Bayou Black	Lake Hackberry
Bayou Carencro	Lake Hatch
Bayou Chene	Lake Penchant
Bayou Copasaw	Lake Theriot
Bayou Penchant	Little Horn Bayou
Big Horn Bayou	Orange Grove Oil and Gas Field Canals
GIWW	Shell Oil Field Canals
Hutch Canal	Turtle Bayou
Lake Carencro	70 Mile Canal
Lake Gasha	
Vermilion Parish	
In marsh, junction Hwy 82 and old GIWW, south of Cow Island	

Parishes. Observations with no collections have been made in Iberville and Vermilion Parishes.

Results of these collections and observations were placed on quadrangle maps and planimetered. As of September 1988, there are 3,927 acres of salvinia in Louisiana.

Salvinia mats have been noted to get 8 to 10 in. thick in waterways in East and West Gate in St. Mary Parish. Garrie Landry noted that it was very abundant in a *Sagittaria* marsh in Vermilion Parish. It is abundant in the Bayou Copasaw and Lake Hackberry areas of Terrebonne Parish and Bayou L'Ourse in Assumption Parish. Salvinia noted in most other waterways has been infrequent to scarce.

ACKNOWLEDGMENTS

Appreciation is extended to the following individuals because, without their assistance, this paper would not have been possible. Florence Givens (LSU); Garrie Landry (USL); and Stewart Andrews, Albert Aucoin, Ray Blouin, Vernon Bunch, Frank Cali, Rudy Champagne, Ernest Ginn, Jarvis Guidry, Greg Guillot, Damian Harleaux, Charles Laird, Blake Landry, C. J. Landry, Wesley Law, Tommy Lee, Stacy Leonard, Alfred Lepine, Wayne Martin, Herschel Moll, Robert Price, Tom Self, Jack Terrebonne, Dan Tomei, Larry West, and Mike Wirsing (Corps of Engineers).

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