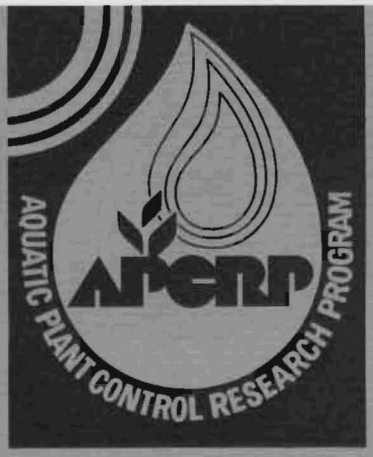

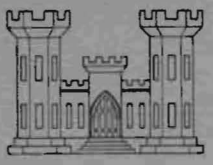

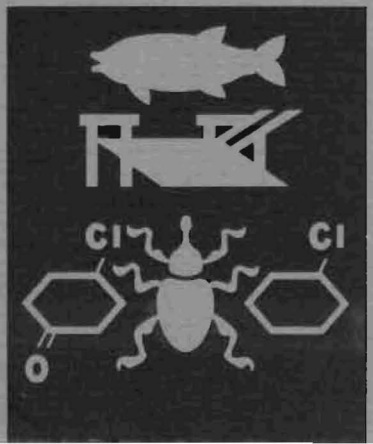

TECHNICAL REPORT A-77-2

A FIELD TEST OF SELECTED INSECTS AND PATHOGENS FOR CONTROL OF WATERHYACINTHS

Report 1

PRELIMINARY RESULTS FOR THE 1975-76 SEASON

by Eugene E. Addor

Mobility and Environmental Systems Laboratory
U. S. Army Engineer Waterways Experiment Station
P. O. Box 631, Vicksburg, Miss. 39180

September 1977
Report 1 of a Series

Prepared for Office, Chief of Engineers, U. S. Army
Washington, D. C. 20314

Destroy this report when no longer needed. Do not return it to the originator.

Unclassified

SECURITY CLASSIFICATION OF THIS PAGE *(When Data Entered)*

REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER Technical Report A-77-2	2. GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER
4. TITLE *(and Subtitle)* A FIELD TEST OF SELECTED INSECTS AND PATHOGENS FOR CONTROL OF WATERHYACINTHS; Report 1, PRELIMINARY RESULTS FOR THE 1975-76 SEASON		5. TYPE OF REPORT & PERIOD COVERED Final report 6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) Eugene E. Addor		8. CONTRACT OR GRANT NUMBER(s)
9. PERFORMING ORGANIZATION NAME AND ADDRESS U. S. Army Engineer Waterways Experiment Station Mobility and Environmental Systems Laboratory P. O. Box 631, Vicksburg, Miss. 39180		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS
11. CONTROLLING OFFICE NAME AND ADDRESS Office, Chief of Engineers, U. S. Army Washington, D. C. 20314		12. REPORT DATE September 1977 13. NUMBER OF PAGES 54
14. MONITORING AGENCY NAME & ADDRESS *(if different from Controlling Office)*		15. SECURITY CLASS. *(of this report)* Unclassified 15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT *(of this Report)*

Approved for public release; distribution unlimited.

17. DISTRIBUTION STATEMENT *(of the abstract entered in Block 20, if different from Report)*

18. SUPPLEMENTARY NOTES

19. KEY WORDS *(Continue on reverse side if necessary and identify by block number)*

Aquatic plant control
Insects
Lake Concordia
Pathogens
Waterhyacinths

20. ABSTRACT *(Continue on reverse side if necessary and identify by block number)*

During the growing season of 1975, a field experiment was initiated on Lake Concordia, Louisiana, to test the potential effectiveness of selected organisms as control agents against waterhyacinths, Eichhornia crassipes (Mart.) Solms. Ninety-seven floating frames, each approximately 2 m square, were anchored on the lake in open water and planted with waterhyacinths collected from the hyacinth population that grows naturally near the shore of this lake. Sixty of these

(Continued)

DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 65 IS OBSOLETE

Unclassified

SECURITY CLASSIFICATION OF THIS PAGE *(When Data Entered)*

20. ABSTRACT (Continued).

frames (plots) were subsequently selected for treatment with various combinations of two insects (Arzama densa Walker and Neochetina eichhorniae Warner) and two fungi (Acremonium zonatum (Sawada) Gams and Cercospora rodmanii Conway) in a random block factorial arrangement with four replications per treatment, and four frames were designated as control (no-treatment) plots. The remaining 33 frames were anchored apart from the test frames to be used as spares if necessary. The control and treated plots were weighed at 2-week intervals throughout the growing season, with the intention that weight changes in the plants would be the primary criterion for judging treatment effects. Flowering stalks were also counted, and the plant heights were measured at each weighing date. In addition, observations were made from time to time on insect and pathogen populations on the plots during the season. This report describes the experiment, presents the collected data, and summarizes the observations. Statistical analyses and interpretation of the data are also presented. Preliminary indications are that significant reductions in the growth rate or total accumulation of waterhyacinth mass in the test plots were not achieved by any of the treatment combinations during the first season's tests, but the potential for some of the treatments to effect the desired control began to emerge during the second year of observations. Appendix A discusses the life systems of the control organisms; Appendix B discusses the flowering activity of the waterhyacinths.

PREFACE

The tests reported herein were initiated in June 1975 by personnel of the U. S. Army Engineer Waterways Experiment Station (WES), with assistance from the U. S. Department of Agriculture Biological Control Laboratories (BCL) at Gainesville, Florida, and from the University of Florida (UF) Department of Plant Pathology at Gainesville, Florida. The study was sponsored by the Aquatic Plant Control Research Program of the Directorate of Civil Works, Office, Chief of Engineers (OCE), who provided funds under Department of the Army Appropriation No. 96X3122, "Construction General." Mr. H. R. Hamilton was the OCE Technical Monitor. Persons directly responsible for the design and conduct of the tests were:

Mr. E. E. Addor, Botanist, WES
Mr. S. O. Shirley, Engineer Technician, WES
Mr. N. R. Spencer, Entomologist, BCL
Dr. T. E. Freeman, Plant Pathologist, UF
Dr. K. E. Conway, Plant Pathologist, UF
Dr. Ted Center, Entomologist, UF
Mr. Victor Chew, Statistician, UF

Messrs. W. N. Rushing, P. A. Smith, and J. H. Meeks and Ms. J. C. Jones of WES intermittently contributed to various phases of the tests.

The cooperation of personnel at the Louisiana Wildlife and Fisheries Commission Field Station at Lake Concordia is gratefully acknowledged, especially the permission to use the boat dock and storage facilities.

The work was conducted under the general supervision of Messrs. W. G. Shockley, Chief, Mobility and Environmental Systems Laboratory (MESL), and B. O. Benn, Chief, Environmental Systems Division, and under the direct supervision of Mr. J. L. Decell, Chief, Aquatic Plant Research Branch (APRB). This report was prepared by Mr. Addor, APRB, with assistance from Messrs. Spencer and Shirley, and Dr. Freeman.

COL G. H. Hilt, CE, and COL J. L. Cannon, CE, were Directors of the WES during the conduct of the study and preparation of the report. Mr. F. R. Brown was Technical Director.

CONTENTS

CONVERSION FACTORS, METRIC (SI) TO U. S. CUSTOMARY AND U. S. CUSTOMARY TO METRIC (SI) UNITS OF MEASUREMENT

Units of measurement used in this report can be converted as follows:

Multiply	By	To Obtain
Metric (SI) to U. S. Customary		
centimetres	0.3937007	inches
metres	3.280839	feet
litres	0.001	cubic metres
grams	0.002204622	pounds (mass)
kilograms	2.204622	pounds (mass)
U. S. Customary to Metric (SI)		
inches	2.54	centimetres
feet	0.3048	metres
yards	0.9144	metres
miles (U. S. statute)	1.609344	kilometres
acres	4046.856	square metres
gallons (U. S. liquid)	0.003785412	cubic metres
pounds (mass)	0.4535924	kilograms
Fahrenheit degrees	0.555	Celsius degrees or Kelvins*

* To obtain Celsius (C) temperature readings from Fahrenheit (F) readings, use the following formula: $C = 0.555(F - 32)$. To obtain Kelvin (K) readings, use: $K = 0.555(F + 459.67)$.

A FIELD TEST OF SELECTED INSECTS AND PATHOGENS FOR CONTROL OF WATERHYACINTHS

PRELIMINARY RESULTS FOR THE 1975-76 SEASON

PART I: INTRODUCTION

Background

1. "Biological control" (also called biocontrol) refers to the use of one or more kinds of organisms to stress a pestiferous population of other organisms, whether by physical destruction, direct consumption, parasitism, or pathogenicity. With biocontrol techniques, it may be necessary to re-establish the control agent periodically, but for reasons of economy, an agent is sought that will adapt to the local environment, become established as a permanent member of the local biota, and be capable of adjusting its population rapidly in response to surges in the growth of the pest population. A most important characteristic of the control agent is that it must not pose a threat to other species whose presence in the ecosystem is valued, and in particular, it must not pose a threat to any economic species in any area where it may be introduced.

2. During the growing season of 1975, an exploratory experiment was initiated on Lake Concordia, Louisiana, to test the effectiveness of selected pathogens and consumer insects in various combinations as control agents against waterhyacinths. The concept for the test of the use of these biological agents for the control of waterhyacinths is based on the premise that it will be possible to: (a) establish the control organisms as a permanent component of the local biota and that the organism will build up to an epidemic on the pest organism and reduce and maintain that population to a nonnoxious level; (b) reduce the pest population by other means, if the biological agent(s) cannot exert sufficient stress to control the target plant, such that the introduction of the control agent will prevent resurgence of the pest

plant; (c) artificially increase the population of the control agent to insure sufficient abundance to control the target plant if the agent will not establish itself as a concentrated component of the local biota; and (d) use two or more biological control agents concurrently with more effect than use of higher concentration of a single control agent, i.e. integrated biological control.

3. To be effective over extended periods, populations of the biological control agents must stabilize in the wild; therefore, it is not expected that control of the target plant will normally be accomplished in a single growing season. The field tests of candidate organisms were designed to allow time to demonstrate, first, whether the agents will establish a permanent population in the release area, and, second, whether that population will increase sufficiently so as to effect a significant reduction in the abundance of the target pest plant.

Purpose and Scope

Purpose

4. The purpose of the overall study is two-fold: (a) determine the ability of selected insects (Arzama densa Walker and Neochetina eichhorniae Warner,) and pathogens (Cercospora rodmanii Conway and Acremonium zonatum (Sawada) Gams), once established on plots of waterhyacinths, to maintain an effective population throughout more than one yearly cycle; and (b) determine the effect of the presence of the agents (singularly or in various combinations) on the growth of the waterhyacinths.

Scope

5. At the start of the waterhyacinth growing season (May 1975), 97 floating frames, each approximately 2 m* square, were anchored in open water on Lake Concordia, in Concordia Parish, Louisiana (Figure 1), and stocked with young healthy plants collected from the waterhyacinth

---

* A table of factors for converting metric (SI) units of measurement to U. S. customary units and U. S. customary units to metric (SI) units is presented on page 5.

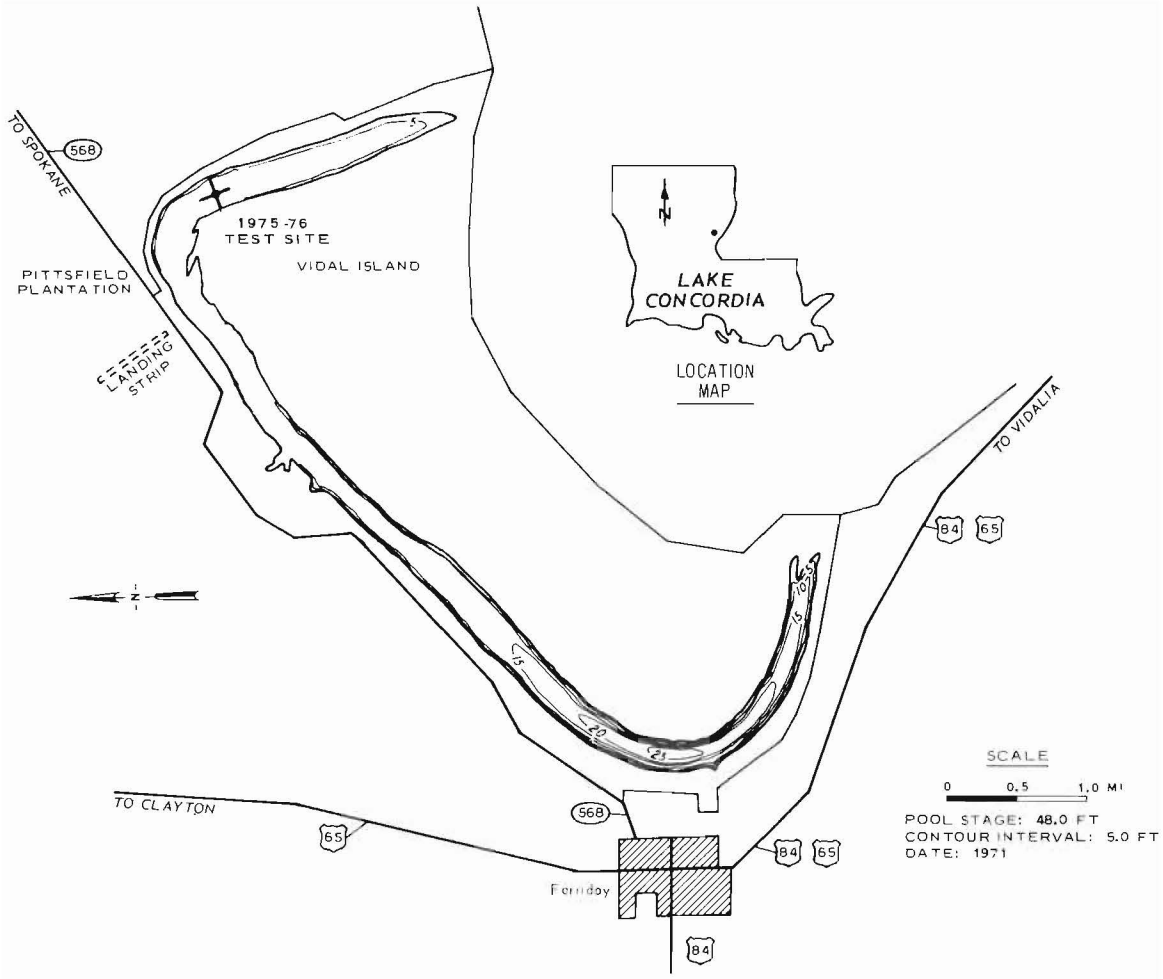

Figure 1. Location of Lake Concordia test site

population that grows naturally along the margins of this lake. When the plants were well established in the frames, 60 of the frames were selected arbitrarily for treatment with various combinations of the two insects and two fungi (see paragraph 4) in a random block factorial arrangement with four replications per treatment, and four were designated as control plots, i.e. plots to receive no treatment; and the remaining 33 frames were anchored apart from the test frames for use as spares if necessary. The plots were weighed at least twice before the test organisms were introduced, and at 2-week intervals throughout the growing season, with the intention that weight changes in the plants would be the primary criterion for judging treatment effect. However,

flowering stalks were also counted, and the plant heights were measured at each weighing date. The plots were also examined at each weighing and at various other times through the growing season for evidence of insect and pathogen activity. This report documents the first year's effort. Appendix A describes life systems of the control organisms used, and Appendix B discusses observations of the flowering of the waterhyacinths.

PART II: TEST AREA, PLOTS, AND EQUIPMENT

Test Area

6. Lake Concordia was chosen for these tests primarily because of two factors. First, tests to evaluate a CO_2 laser for control of waterhyacinths were conducted in this lake in 1973.[1] These tests generated a comprehensive data base on the plant growth and environmental factors that were deemed to be of possible use during these tests. Second, the lake is convenient to the U. S. Army Engineer Waterways Experiment Station (WES), being the nearest body of water of sufficient size to accommodate the test plots with a known naturalized population of waterhyacinths. It is an oxbow lake left by the Mississippi River in Concordia Parish, Louisiana (Figure 1), with its southernmost curve at the eastern edge of the town of Ferriday, Louisiana. It has a total length of approximately 10 miles. Its width is relatively uniform, not exceeding a few hundred yards at any place. The depth over most of its length lies between 5 and 15 ft at normal pool elevation of 48 ft mean sea level (msl) but drops below 25 ft at the southern curve; a bit north of midway on the long axis, there is a sinkhole that is more than 50 ft deep. Separation from the main river began at some early but uncertain date after 1776, but a connection with the main stream was retained at the lower end until the early 1930's.* A levee system constructed during the 1920's-30's closed this connection, and the lake now has no direct connection with the Mississippi River. The normal fluctuation of surface elevation is about 3-5 ft, with the inflow derived from direct surface runoff and groundwater.

7. The shoreline, especially on the westerly side, is spotted with permanently and seasonally occupied dwellings; but the general vicinity, including the shoreline, is used for agriculture, primarily for pastures.

---

* "Flood Control and Navigation Maps of the Mississippi River, Cairo to the Gulf of Mexico, 1933," Map No. 34; Mississippi River Commission, CE, U. S. Army, Vicksburg, Miss.

Cotton, soybeans, corn, and pecans are the major crops in the area. Bald cypress trees are abundant in the shallower portions of the lake, particularly at the northeastern tip and extending westward along the shoreline in a broad U-shaped area, comprising in all a few tens of acres. Waterhyacinths are abundant in this grove (Figure 2) and

Figure 2. Naturalized waterhyacinths at margin of cypress grove, northeast end of Lake Concordia, showing collection of plants for stocking the test frames[1]

are scattered elsewhere in small patches, but the plants usually do not extend noticeably onto the open waters. In early spring, however, small floatleaved plants are blown about, singly and in small clusters, over the entire lake surface.

8. The lake is near the northern limits of the waterhyacinth as a pest weed in the United States, but in the past hyacinths have grown extensively on the open waters of this lake. During some years, low winter temperatures and harsh wind (or wave) action have impeded plant growth on the open water. However, they grow quite well on the open water when confined in experimental plots, as has been amply demonstrated in earlier experiments conducted on the lake.[1] It is

understood that the extreme climatic conditions at this lake may influence the experiment in two important ways: first, it is a desirable condition for testing the climatic adaptability of the test organisms, but second, the brevity of the growing season may allow the organisms to effect a degree of control that may not be achieved under more equable climatic conditions.

Test Plots

9. The test plots are contained in frames designed to confine plants throughout the growing season. Each frame (Figure 3) is constructed of 10-cm-diam aluminum pipe welded into a square 1.8 m on a

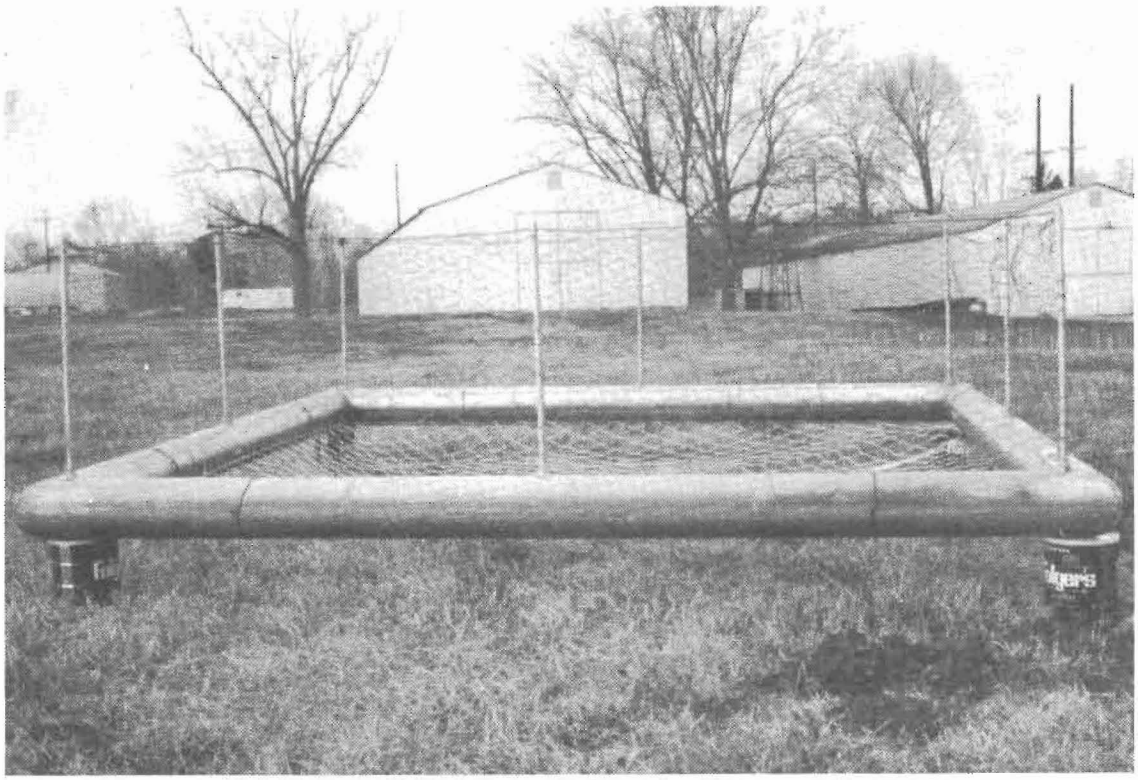

Figure 3. A plot frame used to confine waterhyacinths for the biocontrol tests

side, inside dimensions. A 0.61-m-high fence of 2.54-cm-mesh wire net is mounted on the frame, supported by upright metal rods welded onto the frame; this prevents the growing plants from falling over the edge of

the frame. A 5.8-cm-mesh nylon net is suspended slackly beneath the frame. This net prevents the plants from escaping from beneath the frame during periods of high wind or other violent wave action, as from passing boats. Each frame weighs between 26.5 and 27.7 kg.

Layout of the plots

10. The area selected for distribution of the test plots lies between the "arms" of the U-shaped cypress groves at the northeast end of the lake, where the naturalized waterhyacinth population is well developed within and adjacent to the cypress groves. Figure 4 shows the

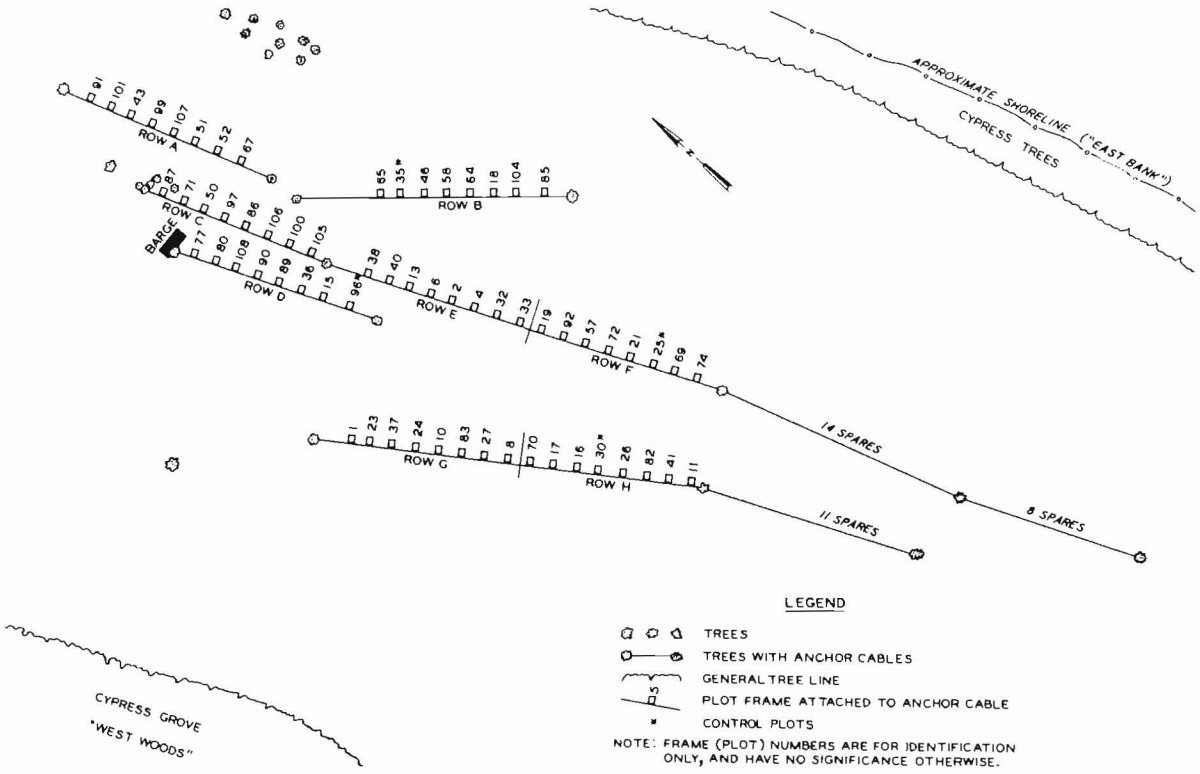

Figure 4. Arrangement of the plots in the test site, 1975

detailed layout of the plots in the site. A few cypress trees scattered in the otherwise open water provided suitable anchorage for stretched cables that held the plots in place. Eight floating frames were attached at 20-ft intervals, outside to outside, along each of eight lengths of cable; these were designated rows A-H. These rows were then towed to the test site and strung along four lines between suitable anchor trees so as to not block the lake, and so that no plot was

nearer than about 200 ft to the natural waterhyacinth population. These frames included the four control plots as well as the 60 frames to be treated.

11. In each case, the anchor cables were stretched between the anchor trees to prevent lateral drift, with the individual frames being attached to the anchor cables by a pair of short clip-on leashes to allow vertical movement with fluctuations of the lake level. These leashes also allowed a slight amount of independent lateral movement with respect to the 20-ft design spacing. Figure 5 is an aerial photograph of the test layout.

Figure 5. Aerial photograph of Lake Concordia test array, 1976. North toward top of photo. (Cf, Fig. 4. Slight differences from 1975 arrangement are collateral experiments using spare plots; the original test array is basically undisturbed.)

Stocking the frames

12. After being placed, the frames were stocked with healthy, vigorous young plants 15-20 cm tall, selected from the nearby natural population. Two workers in a boat selected desired plants, placed them into plastic wading pools carried in the boat, transported these plants to

the test area, and distributed them among the frames. The collection points within the natural population were selected arbitrarily, and each load was distributed by placing a few plants in each of several frames until each frame contained enough plants to cover the water surface approximately 100 percent without crowding. The stocking of all frames was completed on 28 May 1975. These plots were then left undisturbed until 23-24 June 1975, when the first weighings were conducted.

Weighing Apparatus

13. The weighing apparatus (Figure 6) was designed to allow the

Figure 6. Portable weighing apparatus

experimental plots to be weighed efficiently without excessive disturbance. It consists of an A-frame hoist mounted between two flat-bottomed boats, a lifting frame, an electric winch, and a load cell. The boats are separated sufficiently to let the lifting frame drop freely between them and are held in fixed relation to each other by a gangplank across

the sterns and by the A-frame supports fixed across the bows. The lifting frame is of sufficient size as to be freely slipped under the plot frame. The bottom of the lifting frame is covered with nylon netting to cushion and support the plants as the frame is hoisted from the water to be weighed. The winch is powered by a 12-v, d-c, reversible electric motor and has a lifting capacity of 681 kg. It is connected to the lifting frame through the load cell. The load cell output is translated to a differential analog amplifier with a digital voltmeter readout.

14. Weighing was accomplished by lowering the lifting frame into the water, moving the boats forward to position the lifting frame squarely under a plot frame, releasing the leashes from the anchor cable, and gently raising the lifting frame and plot until they cleared the water surface. The plants were allowed to drain for 1 min, and the weight was read directly from the digital voltmeter display. After being weighed, the plot was lowered into the water until it floated free from the lifting frame. The leashes were reconnected to the anchor cable, and the apparatus was backed away to leave the plot frame floating in its original position.

PART III: THE TEST PLAN

15. Four organisms, two insects and two fungi, were selected as the waterhyacinth control agents for this experiment. They were selected on the basis of their readiness for release into the field as determined by their performance in culture tests and required quarantine tests, and on the basis of a certain rationale for integrated biological control. In this part, this general rationale is discussed, followed by a brief discussion of the test organisms and their applications and of the experiment design. A more detailed description of the life cycles of the organisms is presented in Appendix A.

Rationale for Integrated Biological Control

16. "Integrated control" is a general term used to refer to any combination of two or more procedures or agents applied together or alternately on a planned schedule to reduce the degree of infestation of a given pest organism. The general premise is that, although the population of the pest organism may withstand (or recuperate from) the stresses of one applied control measure, it will not survive the continued or extreme stresses imposed by the combination of measures.

17. "Integrated biological control" refers to the use of one or more kinds of organisms to stress a pestiferous population of other organisms. With biocontrol techniques, it may be necessary to reestablish the control agent periodically, but desirably, an agent is sought that will adapt to the local environment, become an established permanent member of the local biota, and will be capable of adjusting its population rapidly in response to surges in the growth of the pest population. A most important characteristic of the control agent is that it must not in any circumstance pose a threat to other species whose presence in the ecosystem is valued for any reason, and in particular, it must not pose a threat to any economic species in any area in which it may be introduced.

18. The organisms for the test plan were two consumer insects

(Arzama densa Walker, Neochetina eichhorniae Warner) and two pathogenic fungi (Acremonium zonatum (Sawada) Gams, Cercospora rodmanii Conway), introduced into the test plots in various prescribed combinations. Both fungi are native or naturalized on waterhyacinths, apparently throughout the range of that plant within the United States, but neither by itself appears to exert a debilitating pathogenic effect on the waterhyacinths anywhere under natural conditions.

19. The presumption for this test is that feeding by the insects will weaken the plants, thereby rendering them more susceptible to infection by the fungi; and in their movements over the plant surface, the insects will disperse the fungi spores and mycelium to the wound areas created by feeding, thereby increasing the rate of infestation by the fungi. The insects have life modes different from the fungi and attack different parts of the waterhyacinth plant, so that together their effect on the spread of the fungi and the intensity of the fungus infestation should be more extensive than either would have alone.

20. One insect, Arzama densa, is a moth with a large larva that bores extensively in the root crown and rhizome. The other insect, Neochetina eichhorniae, is a surface feeding weevil whose larva, though relatively small, is a borer of the petioles. One fungus (Cercospora) is a surface parasite, easily transported by the weevil as it feeds on the leaf surfaces; the other (Acremonium) is a vascular (i.e. internal) parasite with ready access to the vascular tissues through the feeding activity of the insect larvae. Thus, this combination of insects and fungi, plus various naturally occurring bacteria that may also infect the plants through the feeding wounds, is expected to result in a general debilitating effect, i.e. an epiphytotic, on the waterhyacinth population.

21. It should perhaps be noted that the fungi used in these tests are not specific to waterhyacinths, particularly Cercospora, which has a rather broad host range including several economic species. However, it is not seriously pathogenic on any economic species in areas in which it is now known to occur. Since the insects are specific to waterhyacinths during their entire life cycles, the danger of infestation of

crops and other plants by the fungi is not considered to be increased beyond their present normal potential.

Test Organism Preparation and Application

Insects

22. The Arzama used in this experiment were raised in a greenhouse at the U. S. Department of Agriculture (USDA) Laboratories at Gainesville, Florida. First instar larvae were collected from second-generation cultured eggs.* The larvae were placed in small twist-top jars, 50 per jar, with pieces of waterhyacinth leaf and stem. The jars were placed in chests with ice for transportation by auto to Lake Concordia. Release at the site was accomplished on 10 July 1975 by opening the jars and shaking and brushing the contents out over the plot. One jarful, i.e. 50 larvae, was released on each plot designated to receive this insect (32 plots in all).

23. The first release of Arzama was subsequently determined to be unsatisfactory. It is presumed that the larvae were lost in large numbers to predators (mostly spiders), or else fell or were washed off by a rain that occurred during the night after their release, and they were unable to swim to a plant. A second release was made on 13 August. The second batch of larvae, also first instars from second-generation cultured eggs, were placed in jars with segments of stem for transport to the test site, and release was accomplished by lifting the pieces of stem from the jars and placing them in leaf axils so that the larvae could bore from the stem pieces into the live stems. This release was considered successful.

24. Adult Neochetina were collected by USDA personnel from an existing population near Fort Lauderdale, Florida. The insects were placed in ice cream cartons as collected, 150 per carton, with fresh

---

* Complete genealogy as follows: wild eggs collected from field, larvae in laboratory, adults; eggs, larvae, adults (the "parent" (or F) generation); eggs (F_1 generation), larvae to test plots.

pieces of waterhyacinth plant. The filled cartons were placed in a chest with ice for transportation by auto to Lake Concordia. Release was accomplished on 10 July 1975 by opening the cartons and gently shaking or brushing the insects out over the centers of the plots. One cartonful, i.e. 150 adult insects, was released on each plot designated for this treatment (32 plots in all). The first introduction was considered entirely successful, and no further releases were made.

Fungi

25. The inoculum for both fungi was obtained from cultures prepared by the Plant Pathology Department, University of Florida. The fungi were cultured in shallow pans on liquid potato dextrose broth supplemented with 0.5 percent yeast extract. The mycelium was harvested from these culture pans, packaged in plastic bags, and placed in refrigerated containers (of the type used to transport blood plasma) for transport by auto to Lake Concordia. At the Louisiana Wildlife and Fisheries Station at Lake Concordia, the mycelium (for the species separately) was ground up in a commercial blender (approximately 4-ℓ capacity) with enough water to produce a thick, homogeneous soup. This soup was then portioned into the tank (70-ℓ capacity) of a powered sprayer with a controllable nozzle and diluted to a specified quantity as described below.

26. For Cercospora, an amount of soup equal to 80 g per plot was put into the tank and diluted with an amount of water sufficient to produce 1 ℓ of mix per plot, or 32 ℓ, and the nozzle was set to spray 1 ℓ in 15 sec. For Acremonium, the procedure was the same, except the proportion of undiluted inoculum was doubled to a distribution rate of 160 g per plot, or 5120 g, which was then diluted to 32 ℓ, or 1 ℓ per plot.

27. To reduce the possibility of uneven dosages due, for example, to possible settling of the mycelium particles in the sprayer tank, dripping from the plant as a result of oversaturation, etc., the species were applied to 32 plots alternately and in reversed sequence on two nights, according to the following schedule:

24 June	Cercospora:	1st to 32nd plot treated
	Acremonium:	1st to 32nd plot treated
25 June	Acremonium:	32nd to 1st plot treated
	Cercospora:	32nd to 1st plot treated

Experiment Design

Treatment combinations

28. As stated previously, two insects and two fungi were tested in various combinations. A 2^4 factorial experiment in an 8 by 8 quasi-latin square was selected[2] that provided four replicates of each of 16 possible combinations of treatments, including the controls, i.e. no treatment, distributed so that each combination occurred once in every pair of consecutive rows and in every pair of columns as shown below:

Row Pair	Row	Treatment							
I	A	c	abcd	b	ad	a	bd	abc	cd
	B	abd	0	bcd	bc	acd	ac	d	ab
II	C	d	bc	a	abcd	b	cd	abd	ac
	D	bcd	ad	acd	bd	abc	ab	c	0
III	E	a	bd	c	ab	d	abcd	acd	bc
	F	abc	ac	abd	cd	bcd	0	b	ad
IV	G	b	ab	d	ac	c	ad	bcd	abcd
	H	acd	cd	abc	0	abd	bc	a	bd

Note: a = Arzama; b = Neochetina; c = Cercospora; d = Acremonium; and 0 = controls.

Field layouts

29. This plan was adopted for the present experiment, with the plots to be spaced not less than 20 ft in any direction. Because of (a) limited space at the test site, (b) limited arrangement of suitable anchorages for the floating frames, and (c) the requirement that the lake not be blocked to passage of recreational boats, the field layout was arranged as shown in Figure 4. The treatment combinations presented

above were placed on the plots according to the key below:

Treatment	Row·Frame			
0	B·35	D·96	F·25	H·30
a	A·107	C·50	E·38	H·41
ab	B·85	D·36	E·06	G·23
abc	A·52	D·89	F·19	H·16
abcd	A·101	C·97	E·04	G·08
abd	B·65	C·100	F·57	H·26
ac	B·18	C·105	F·92	G·24
acd	B·64	D·108	E·32	H·70
ad	A·99	D·89	F·74	G·83
b	A·43	C·86	F·69	G·01
bc	B·58	C·71	E·33	H·82
bcd	B·46	D·77	F·21	G·27
bd	A·51	D·90	E·40	H·11
c	A·91	D·51	E·13	G·10
cd	A·67	C·106	F·72	H·17
d	B·104	C·87	E·02	G·37

PART IV: DATA COLLECTION AND TABULATIONS

Data Collection

30. With the weighing apparatus described in paragraphs 13 and 14, the plants were weighed at 2-week intervals until 30 September 1975, and three times during the subsequent winter months. Plant height was measured and flowering activity observed at each weighing interval. Height was measured from the bottom of the lifting frame (nominal water surface) to the top of the plants. Flowering activity was observed by ocular means. Temperature and precipitation data were obtained from records maintained at the Ferriday, Louisiana, Fire Department. This section of the report will discuss the data collection and tabulation, with reference to Appendix B, which includes field observations on flowering recorded during the first year.

Data Tabulations

Cumulative plant mass (measured weight)

31. Table 1 lists, by designated treatment, the measured net weight of each plot, to the nearest 0.5 kg, at each weighing date. The measured net weight is the measured weight as indicated by the digital readout on the weighing device, less 571.5 kg, which is the average weight of the empty wet frames.

32. Sources of possible error in the weighing procedure (see paragraph 14) are (a) assumption of a constant weight for the empty wet frame (the measured weights actually ranged from 26.5 to 27.7 kg) (see paragraph 9), (b) variation in the amount of water adhering to the plants after draining for 1 min, and (c) error in estimating the stable point on the readout when the suspended frame is swayed by wind or by rocking of the boat. The cumulative effect of these errors did not exceed 3 kg; therefore, the error in the values in Table 1 should not exceed 10 percent at the beginning of the growing season, nor 3 percent when the net weight is in excess of 100 kg.

Maximum and average plant heights

33. Table 2 lists, by treatment, the measured height of the plants on each test plot at each weighing time. Each entry consists of two values: (a) the first value is maximum height, and (b) the second is average height. Maximum height is the measured height of the tallest single plant in the plot, whether leaf or flower stalk. Average height was determined by measuring the usually estimated average height of the plant mass in the frame. Both heights were measured from the bottom of the lifting frame while the plot was draining prior to weighing (see paragraph 14), hence include the thickness of the root mass as compacted by the weight of the plants during the measurement.

Temperature and precipitation data

34. Table 3 lists the daily minimum and maximum temperatures and the daily precipitation values for May 1975 through February 1976. These data were obtained from the Ferriday, Louisiana, Fire Department, approximately 5.5 miles southwest of the test site. The extreme minimum and maximum temperatures for each month are indicated by single asterisks (*). In addition, the numbers of days in each month on which the temperature dropped below 60°F or rose above 90°F are noted, and the total precipitation for the month is shown.

Flowering activity

35. Table B1 in Appendix B lists, by treatment, the number of open inflorescences on each plot at each weighing date. An open inflorescence is defined as a flower stalk with the flowers fully protruded from the sheath and beginning to expand or are fully expanded, or are beginning to wither but not conspicuously withered nor the stalk conspicuously geniculated. Four values are listed for each treatment. These four values are the number of open inflorescences on the four replicates, respectively, as shown in the column headed "Frame Number Sequence." For example, on 7 July, plot number 35, a control plot, contained 4 open inflorescences, plot 96 contained 3, plot 25 contained 3, etc. The interpretation of the observed flowering activity is discussed in Appendix B.

Field observations

36. Important data on the experiment were the narratives and other field notes recorded by the WES crew and cooperating scientists. In addition, photographs taken during each data collection interval showed the overall appearance of the plants as well as detailed activities of the agents. These data and photographs are on file at the WES. They were used extensively in preliminary interpretation of the measured data, described in the following part, and will be documented in a subsequent report on the tests.

PART V: RESULTS

37. As previously indicated in Part IV, the basic data collected during the first year of this study pertain to the plant growth as characterized by height and biomass measurements, as a function of time. Also, data on the amount and timing of flowering were recorded, and the presence and migration of the test control agents were observed periodically. These efforts resulted in considerable data, which were summarized as described in the previous section. As of this date (November 1976), the data collection is still under way and is expected to continue at least for an additional growing season, i.e. through November 1977 and perhaps until November 1978. The long-term monitoring of the experiment is necessary because the biological agents may require 2-3 years to build up and maintain a population that represents their full potential to control waterhyacinth growth. For this reason, it is considered premature to conduct an exhaustive analysis of all the data collected to date. It is, however, especially pertinent to answer the questions posed below, because completely negative answers would raise questions as to the benefits of continuing the experiment. These questions are:

a. Does the presence of the agents (singularly or in various combinations) affect the growth of the waterhyacinths?

b. Do the test organisms (Arzama densa Walker and Neochetina eichhorniae Warner) and fungi (Acremonium zonatum (Sawada) Gams and Cercospora rodmanii Conway), once established on plots of waterhyacinths, have the ability to maintain an effective population throughout more than one yearly cycle?

38. To arrive at an expedient answer to question a above, statistical tests, i.e. analysis of variance and Duncan's Multiple Range determination, were applied directly to the weight and height data listed in Tables 1 and 2.

Treatment Effects on Weight

One-way variance tests

39. The initial test performed was a one-way analysis of variance

on the total data base (Table 1) to see if there existed any significant differences in biomass among different treatments, including the controls. The tests were performed by means of a time-sharing library program "ANVA1" on file at the WES Automatic Data Processing Center. The data for the four replications at each treatment level were input; variances, means, and an F-ratio comparing differences among treatments were included in the output. The F-ratio for each measurement date was tested at only the 95 percent level of significance, because it was expected that, during the first season after the agents were applied, the effect would not be substantial. The test results for 95 percent level of significance would indicate trends, even though a strong difference among treatments might not be apparent. If no difference emerged, further analysis would be unproductive. The results of this analysis of variance showed that there was no significant difference in the results until 16 September 1975. For those combinations in which the differences were observed, Duncan's Multiple Range determinations were made to determine exactly which treatments resulted in significantly different biomass values. The results of the one-way analysis of variance for the biomass data collected through 22 October 1976 are summarized as follows:

Sampling Dates	Significance
23 Jun 75	Not significant
7 Jul 75	Not significant
21 Jul 75	Not significant
6 Aug 75	Not significant
19 Aug 75	Not significant
2 Sep 75	Not significant
16 Sep 75	Significant
20 Sep 75	Significant
22 Oct 75	Significant

Duncan's Multiple Range tests

40. As shown, no significant effects were observed until 16 September 1975; therefore, Duncan's Multiple Range determinations were

performed only on the data for 16 and 30 September and 22 October 1975 to see which of the treatments resulted in significantly different biomass values. The following key will relate "treatment" to agent combinations (see paragraphs 28 and 29) applied.

Treatment	Agent Combination	Treatment	Agent Combination
1	0	9	c
2	a	10	cd
3	ab	11	ac
4	abc	12	d
5	abcd	13	ad
6	b	14	acd
7	bc	15	bcd
8	bd	16	abd

41. The convention from Reference 3 was used to summarize the results of the Duncan's Multiple Range tests. The convention and the results are discussed by way of example for the 16 September 1975 data:

Sampling Date	Treatments Resulting in Similar and Dissimilar Mean Biomass Values Treatment Code 5 15 14 4 7 16 8 11 3 6 10 9 13 2 12 1
16 Sep 75	

The treatment code shows the ranking by descending order of mean biomass for the various treatment results (Table 4), i.e. the left-hand number 5 indicates that treatment abcd, i.e. all agents applied, has the lowest mean biomass of all treatments, and the right-hand number 1 indicates that the control plot has the highest mean biomass. The first solid line compares the results of treatment 1 with all other treatments. It shows that there exists no significant difference between the control results and those of all treatments except 5 and 15.

Furthermore, treatment 14 and all treatments represented by the numbers to the right of 14 can be considered to be from the same population. The second and third solid lines compare the results of treatments 12 and 2, respectively, with all other treatments; and it is found that results of treatments 12 and 2 are also significantly different from those of 5 and 15. However, when the analysis starts with treatment 13, there is no significant difference in the results of treatments from 15 to 13. Finally, when the analysis begins with treatment 6, treatments 5 through 6 result in the same mean biomass. These same results can be presented more conveniently with only three lines on the chart:

Sampling Date	Treatments Resulting in Similar and Dissimilar Mean Biomass Values Treatment Code														
	5	15	14	4	7	16	8	11	3	6	10	9	13	2	12	1
	Groupings of similar mean biomass values															
16 Sep 75																

42. In this display, it should be noted that any two treatments not underscored by the same line have means that are significantly different; any two treatments underscored by the same line have means that are not significantly different. Thus, the difference in results for treatments 5 and 15 are significant when compared with the control results (treatment 1) and when compared with results of treatments 12 and 2. Results of treatment 5 are further significantly different from results of treatments 13, 9, and 10 for this sampling date. Furthermore, the solid lines indicate those treatments that resulted in significantly similar mean biomass values. In summary, for 16 September 1975 the greatest amount of biomass reduction resulted from the composite treatment with all agents (treatment 5). The next most effective treatment was combinations of Neochetina, Cercospora, and Acremonium (treatment 15).

43. Similar presentations for 30 September and 22 October 1975 biomass data are shown below:

Sampling Date	Treatments Resulting in Similar and Dissimilar Mean Biomass Values Treatment Code															
	5	15	14	4	16	7	3	8	11	6	10	9	13	2	12	1
30 Sep 75																

Sampling Date	Treatments Resulting in Similar and Dissimilar Mean Biomass Values Treatment Code															
	5	14	16	4	15	3	1	8	7	6	10	13	9	1	2	12
22 Oct 75																

Although the mean biomass changed slightly for the 30 September 1975 data, the results are essentially similar, i.e. treatments 5 and 15 resulted in mean biomass values that are significantly different from those of the controls and treatments 12 and 2, but the control results are similar to those of all other treatments. By 22 October 1975, the ranking order had changed considerably, and treatment 5 again had a significant difference in biomass values when compared with those of the controls and of treatments 12, 2, 9, 13, and 10. Results of treatments 14 and 16 were significantly different from those of treatments 12 and 2 but were not significantly different from those of the controls. Results of treatments 4 and 15 were significantly different only when compared with those of treatment 12. It should be noted, however, that treatment 5, i.e. all agents applied, still resulted in the smallest mean biomass values, but significant reductions were also found with treatments of Arzama, Cercospora, and Acremonium (treatment 14); Arzama, Neochetina, and Acremonium (treatment 16); Arzama, Neochetina, and Cercospora (treatment 4); and Neochetina, Cercospora, and Acremonium (treatment 15).

Treatment Effects on Height

One-way variance tests

44. The one-way analysis of variance was performed on the average

height data (Table 2), i.e. the second entry for each treatment and date (see paragraph 33). These test results show that the only significant difference between the heights of the treated and untreated plants occurred for the 16 September sampling, as summarized below:

Sampling Dates	Significance
23 Jun 75	Not significant
7 Jul 75	Not significant
21 Jul 75	Not significant
6 Aug 75	Not significant
19 Aug 75	Not significant
2 Sep 75	Not significant
16 Sep 75	Significant
30 Sep 75	Not significant
22 Oct 75	Not significant

Duncan's Multiple Range test

45. The summary of the Duncan's Multiple Range test conducted for the 16 September 1975 height samples is shown below. Ranking of treatments in descending order is presented in Table 5.

Sampling Date	Treatments Resulting in Similar and Dissimilar Mean Height Values Treatment Code														
	5	14	11	15	7	8	3	4	16	6	9	2	13	10	12	1
16 Sep 75																

The summary shows that treatment 5 results were significantly different when compared with those of the controls and treatments 12, 10, 13, 2, and 9. Results of treatments 14, 11, and 15 were significantly different when compared with those of the controls and treatment 12. In addition, results of treatment 7 were significantly different from those of treatment 12 but were not significant when compared with those of the controls. As was the case with the 16 September 1975 biomass data, treatment 5, i.e. all agents applied, had the most effect. Combinations of Arzama, Cercospora, and Acremonium (treatment 14) had the next largest

effect, and Arzama and Cercospora (treatment 11) and Neochetina, Cercospora, and Acremonium (treatment 15) follow.

Weight Versus Height

46. The preliminary analysis discussed in the previous paragraphs provide an affirmative answer to at least a portion of the first question posed in paragraph 37a, i.e., some combinations of the agents can adversely affect the growth of waterhyacinths and thus offer control potential. The statistical results, although informative, do not convey a clear physical picture of the effects of the treatments on the weight and height of the plants. Furthermore, the statistical analysis was conducted only on data collected through 22 October 1975. To further illustrate the effect of the treatments in this preliminary report, the average biomass and height values of the four replications of four treatments were plotted against time (June 1975 to September 1976) in Figures 7 and 8, respectively. As shown in the figures, the treatments are the control; Arzama; Arzama and Neochetina; Arzama, Neochetina, and Cercospora; and Arzama, Neochetina, Cercospora, and Acremonium. These four treatments are presented as examples of the trends in the data collected to date. A comparison of the effects of Arzama (Figure 7a) with those of each of the remaining three treatments indicates the difference in the effect of the Arzama used alone as compared with the effect of this agent in combination with 1, 2, and 3 of the additional agents. As expected, there tends to be a progressively larger difference between the treated plots and the controls as the number of agents increases. In general, these curves (especially the controls) show the characteristic steep growth of the hyacinths between June 1975 and September 1975. Then, a general decline in the biomass, regardless of treatment, occurred from September to May 1976, which is the accepted "beginning of the growing season." This decline is attributed to the natural decline in the biomass during the winter months. Worthy of note, however, is the indication from these curves that the increasing combination of agents has the effect of increasing the difference between the

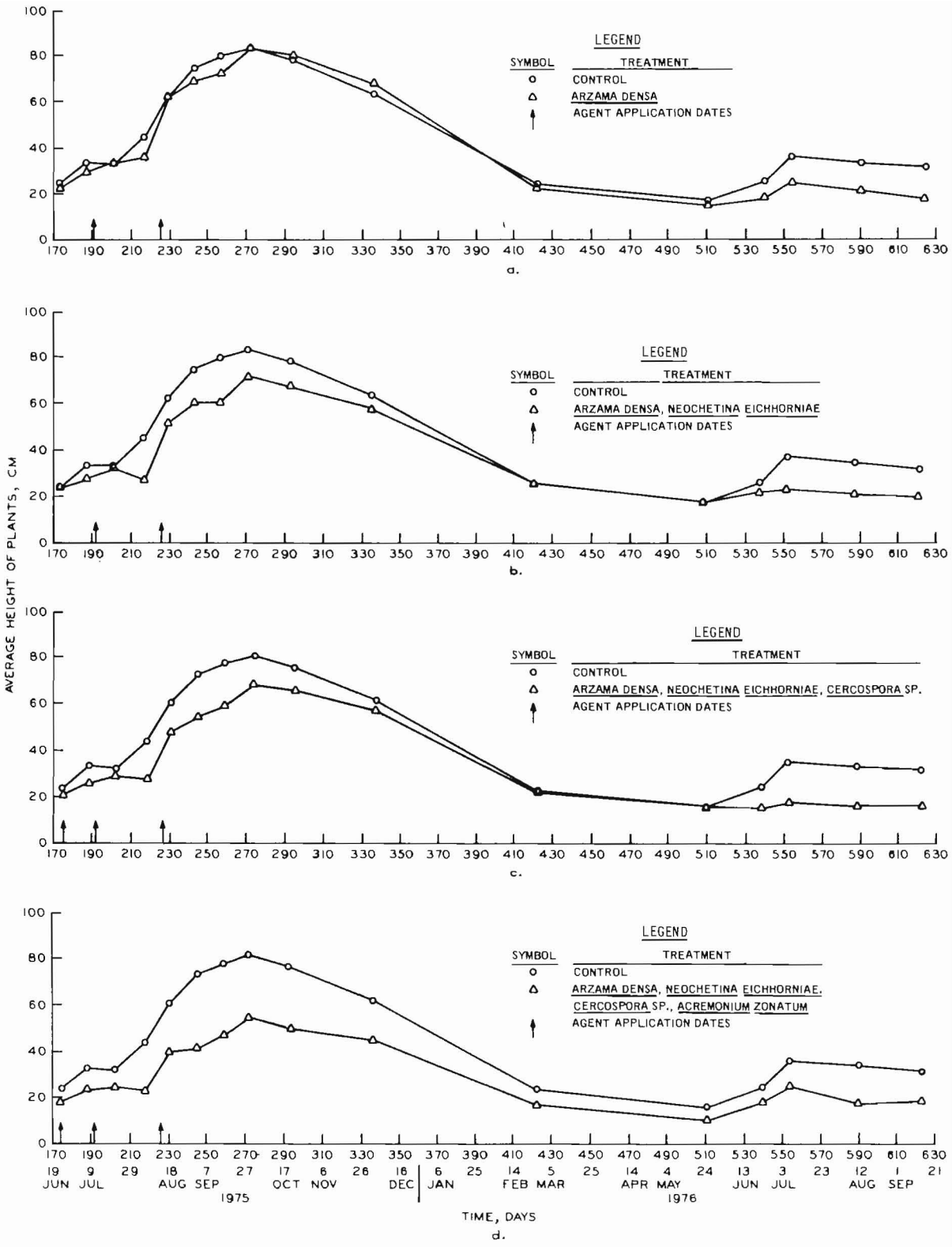

Figure 7. Average weight of plants versus time

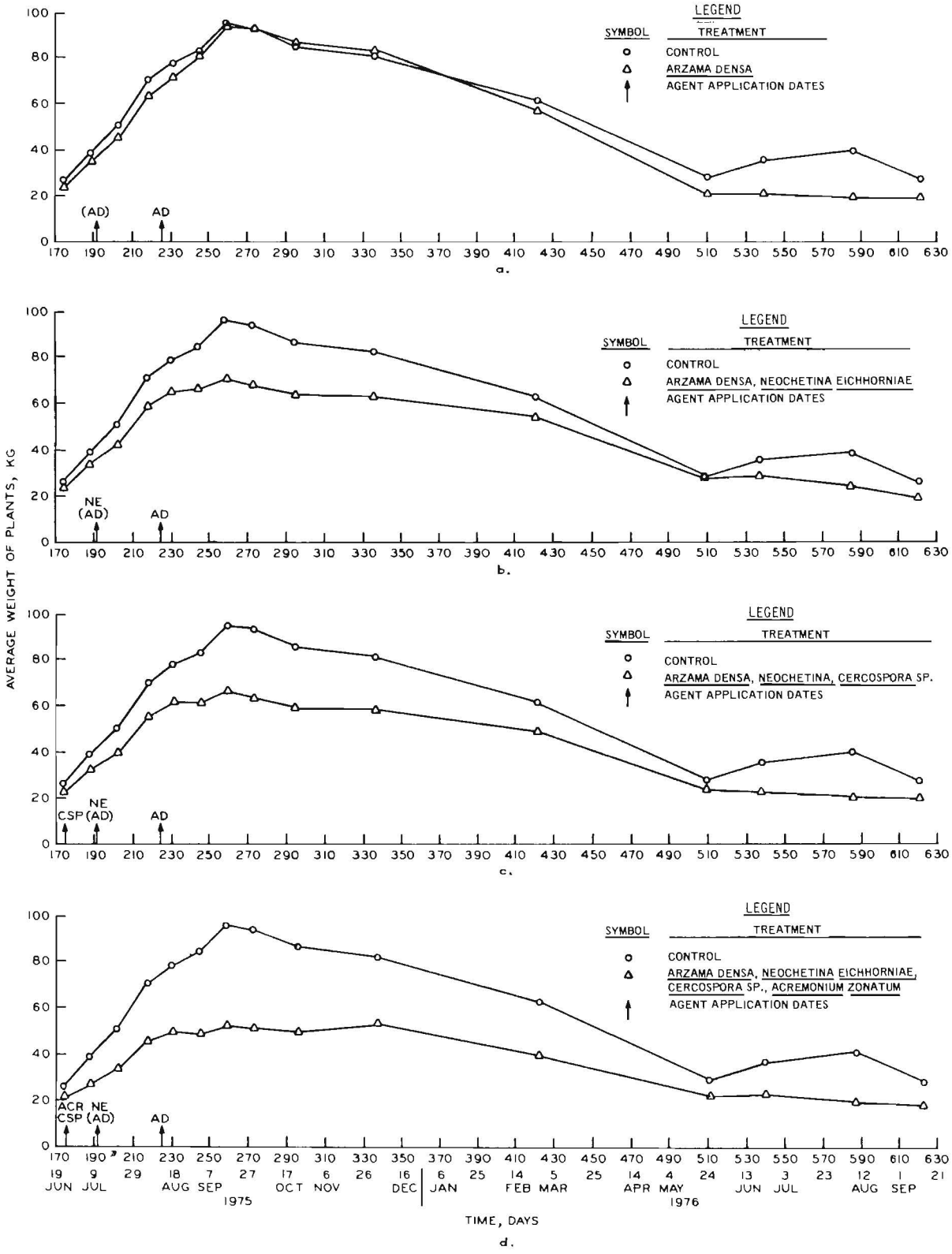

Figure 8. Average height of plants versus time

peak value of biomass for the treated plants when compared with that of the untreated (control) plants.

47. Figure 8 shows the average plant heights for the four example treatments previously discussed. The height data indicate the same general trends as exhibited by the plant biomass data. The plant height increased from June to mid-September 1975, reaching a peak at this time. From mid-September 1975 until late May 1976, there was a general reduction in plant height. As was the case with the plant weight, the decline is attributed to naturally occurring reductions associated with the winter months. Like the weight data, Figure 8a-d shows an increasing effect, i.e., a greater decrease in height corresponds to an increasing number of agents applied. These results further support the hypothesis that some combination of agents can be found that will serve as an effective biocontrol agent for waterhyacinths.

48. Further evidence can be seen in Figures 7 and 8. For example, from May 1976 through September 1976, a sharp reduction occurred in the slope of the growth curves for the treated plants when compared with the slope of the growth curves for the same time period in 1975. Also, a pronounced reduction is noted in the slope of the second-year May-September growth curves for the controls. Infestation of the control plots was observed during the field data collection as early as August 1975, and the pronounced slope reduction noted in the second-year May-September growth curves for the controls can be attributed to the accelerated growth and spread of the agents, after overwintering. This overwintering and subsequent accelerated spread of the control agents will be discussed later.

49. To further illustrate the effect of the selected treatments, the weight versus height was plotted by treatment for the period 23 June 1975 to 23 June 1976 (Figure 9). The arrows on the graphs trace the time history of the height-weight ratios. It is commonly accepted that with waterhyacinths, crowded growing conditions induce accelerated height growth. The five plots in Figure 9 illustrate the range of variation in the pattern shown by this test series over the period covered by the first 12 weighing dates. In every case, an obvious

trend of low slope is observed as the plants gain little height relative to weight gain, followed abruptly by a sharp increase in height gain relative to weight gain. In every case, the intercept between the two slope trends is approximately on the fourth point, corresponding in time to 6 August.

50. Regardless of treatment, during a period from 23 June through 21 July 1975 the growth increase was in the form of weight increase with only slight increase in height. From 21 July until 30 September, a fairly proportional increase was indicated in height and weight. After September 1975, height and weight declined fairly proportionally, with the resulting ratio on 23 June 1976 approaching the original ratio of 23 June 1975. The difference in the growth or increasing portion of these curves when compared with the declining portions of the curves is an indication of the normal seasonal cycle of the plants' growth. The overall shortening on these curves, or altered normal cycle, tends to be proportional to the number of agents working on the plants. This effect can be shown by the following tabulation:

Figure 9	Maximum Average Height, cm	Maximum Average Weight, kg
a	80	140
b	80	140
c	80	110
d	70	100
e	60	80

However, this appears most significant for the treatments using three and four agents (Figure 9d-e).

51. Evidently, regardless of stresses from whatever source, the plants continue to spread and amass weight until they are confined, i.e. until the frame is congested, at which time height growth is quickly assumed. Note, however, the slight convex curve suggested by the first three or four points on these graphs (Figure 9). This trend was found to be consistent throughout the 16 graphs for the 16 treatments in this test series.

52. The relation between crowding and height growth for this

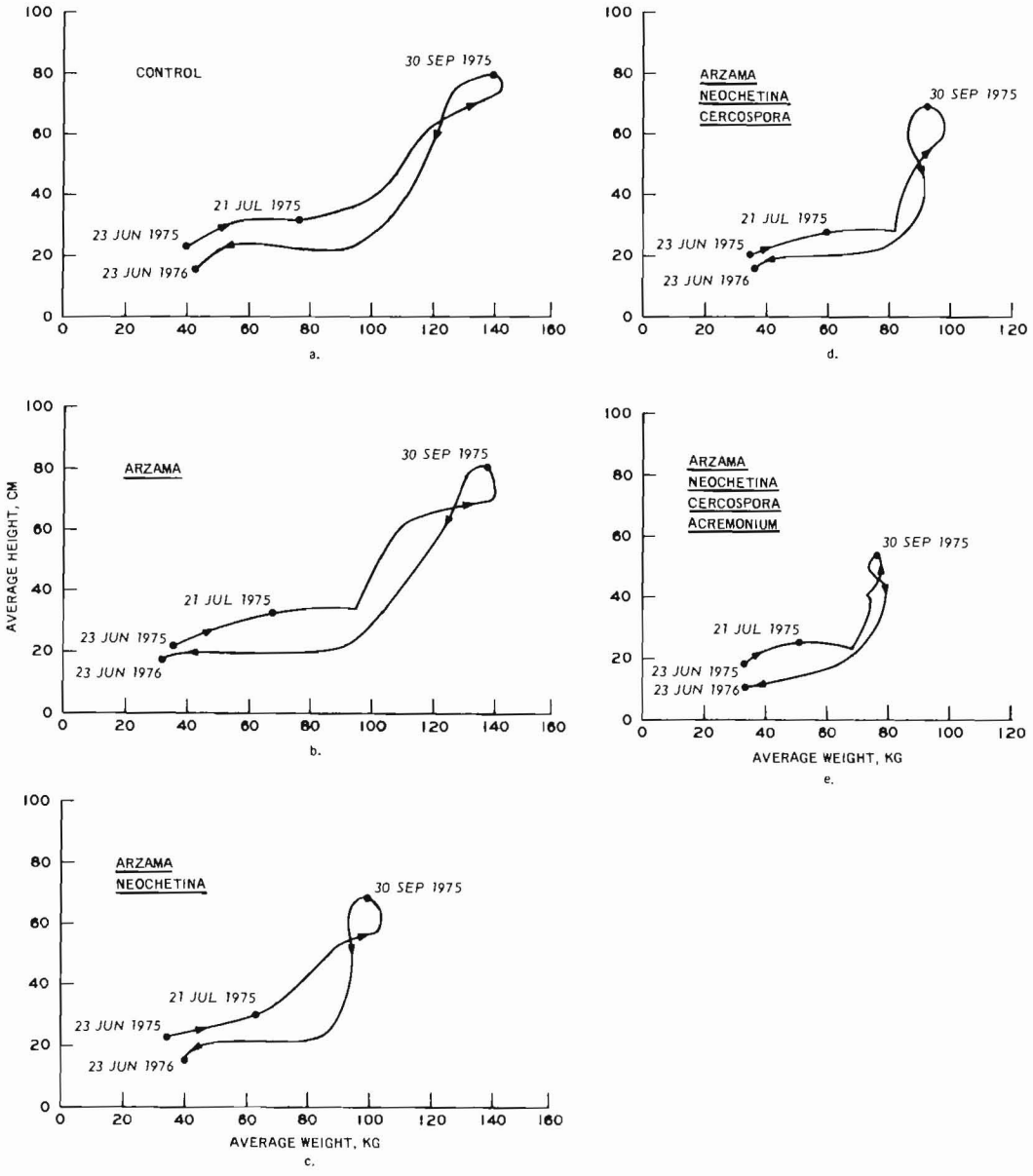

Figure 9. Weight and height of plants by treatment and time, 23 June 1975-23 June 1976

species is almost axiomatic, but it is surprising that the rapid height growth should be accompanied by so proportionally little weight gain. In effect, however, the phenomenon is consistent with the conclusions of Long and Smith[1] from their observations on growth of plants confined in these same frames for their experiments. They report that the peak biomass in their control plots occurred about 15 September, but that on a per plant basis, weight decline began in the latter part of July. They conclude,

> "The picture emerging is that the weights of individual plants tend to increase early in the growing season, reach a peak, and then decrease late in the season. The number of plants...increased drastically in all the control plots and the weights...[of these plots] increased as long as plants could reproduce unrestrictedly. At the end of the season, reproduction of the plants stopped or drastically slowed, and the average weight of an individual plant decreased, resulting in a slight decrease in biomass in the control plots."

53. This is consistent almost to the date with the results from the tests reported here, in which peak biomass (weight) was recorded for most plots on 16 September, followed by a continuing decline thereafter (Tables 2 and 3); whereas, the slight decline in height preceding the height growth spurt began with the 21 July observation on these plots.

Agent Persistence and Spread

Data presentation

54. Figure 10 summarizes the presence and migration of the insects and pathogens applied to the various test plots. Some further explanation of the legend in Figure 10 is needed.

55. The numbers enclosed by the square symbol represent the plot numbers. The normal treatment, i.e. the agent put on the plot, is represented by a column containing letters and dots located directly under the left-hand corner of each plot symbol. The letters a, b, c, and d are placed in the sequence shown in the lower part of the legend. If a dot is shown in the column instead of a letter, no treatment of the respective agent was placed on the plot. The observed treatment is

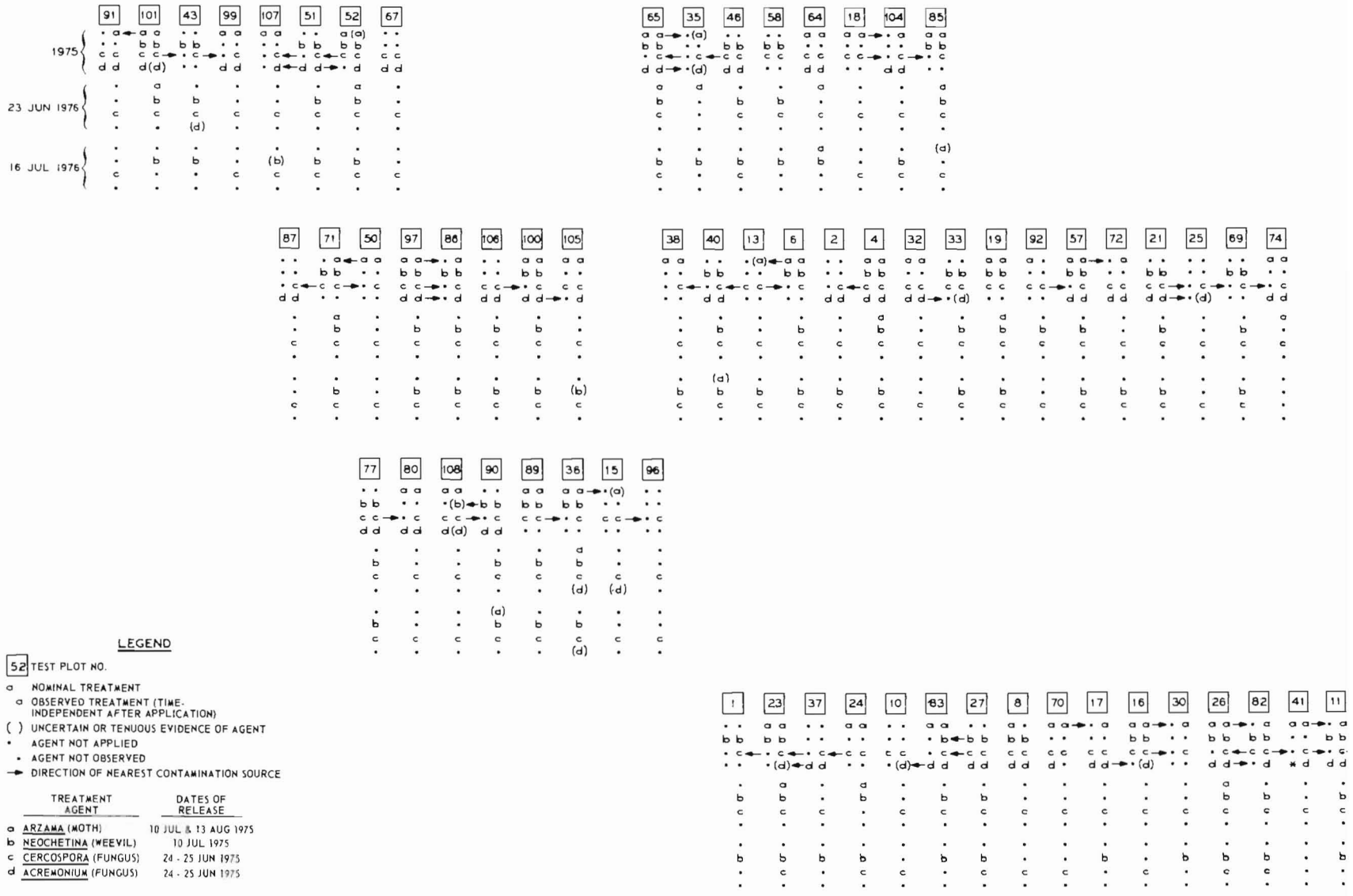

Figure 10. Chronology of insect and pathogen dynamics

what was actually reported to be on the plot and is designated in the same manner as described for the normal treatment, except the dot indicates that the respective agent was not observed. Three sets of treatment or observation symbols are shown under each plot in Figure 10, i.e., composite treatment and observation data for the entire season of 1975 irrespective of time of observation but exclusive of the dates of application, and observation data (observed organism presence) for the two dates in 1976. Because the agents were applied only in 1975, there are no treatment symbols corresponding to the 23 January and 16 July 1976 observations. Arrows between the columns for 1975 indicate the nearest potential source of infection when the observed treatment differs from the nominal treatment for a given plot. The direction of the arrow is arbitrary when a potential source of infection is equally near from either direction, i.e., when the next adjacent plot on either side has been treated with the contaminating agent.

Population dynamics

56. After a study of Figure 10, the following observations about the movements of the organisms during 1975 were made:

a. Arzama on 12 plots where it had not been applied.

b. Neochetina on two plots where it had not been applied.

c. Cercospora on 32 plots where it had not been applied (it is reported on nearly every plot).

d. Acremonium on 11 plots where it had not been applied.

e. No Arzama on two plots to which it had been applied (92 and 8). (This may be attributed to the "inherent" low vigor of these two plots.)

f. No Acremonium on one of the plots to which it had been applied (70).

57. In every case, except three cases for Cercospora, contamination could have occurred from an immediately adjacent plot. In fact, problems have been experienced with the leashes coming loose or breaking, allowing a plot to drift along the anchor cable to rest against its neighbor; therefore, other possible mechanisms for contamination, in addition to natural or self-dissemination, would be escaped plants drifting to their neighbors, or from natural vagrants drifting from

plot to plot. The surprise in this set of data is Neochetina, which established itself well during its first season and exhibited very little transmigration during that year, but which reappeared very early in the following spring and very quickly spread throughout the test plots (e.g. 16 July 1976). It also appeared extensively throughout the native waterhyacinth population on the lake early in the 1976 season.

58. Arzama appeared only sporadically in the first season, and indeed the first release on 10 July was declared a failure, so a second release was made on 13 August. Note that it reappeared sporadically in 1976, most often on plots with the taller plants (e.g. 101, 64, 85). Damage by Arzama is obvious this year (1976) on the native waterhyacinth population in the area but only on very tall, lush plants, or plants that were obviously lush at the time of attack. It does have a severe debilitating effect on such plants, however.

59. Note also the relation between Acremonium and Cercospora. Although Acremonium was never reported to be strong on any plot in 1975, it did at least establish itself on most plots to which it was applied and was still present on most of these plots on 16 September. At that time, Cercospora had not yet spread extensively and was not yet reported to be especially strong on any plot. But on 31 October, Acremonium was reported to be nearly extinct on most plots, while Cercospora was now very widespread and reported strong on most plots. Acremonium was reported tentatively on a few plots on 23 June 1976, early in the season, but its presence since that date has not been confirmed. Cercospora, by contrast, reappeared vigorously in the second season and, at the time of this report, is profuse throughout the test plots, as well as widespread on the native population of waterhyacinths in the area.

60. The Orthogalumna terebrantis (waterhyacinth mite) began to appear on the test plots on 20 August 1975 and, by the end of the season, was reported to be on every plot with Neochetina, but only on those. Tetranychus gloveri (spidermite) was discovered on several plots on 8 August 1975, but it was sprayed with an acaricide and was not reported on any plot after 20 August.

61. Orthogalumna has appeared again in the second season and is

distributed profusely over the test plots, but its specific association with Neochetina has not yet been examined. In September, Tetranychus had not yet reappeared on the test plots, though it is present on the native populations of waterhyacinths in the area. These are not shown on the chart.

62. The previous discussion provides a strong affirmative answer to the second question in paragraph 37b, for it appears reasonable to conclude that as a minimum Arzama, Neochetina, Cercospora, in some combination, are capable of establishing and sustaining a debilitating epiphytotic on waterhyacinths under the climatic and other environmental conditions of these tests.

Concluding Comments

63. The accumulated data are rather more complex than they originally were expected to be, consisting as they do of observations on several variables observed at several times. Obviously, the selection of the test design was based on the supposition that an analysis of variance and Duncan's Multiple Range tests would reveal the relative contribution of each agent combination to the demise of the waterhyacinths. However, the test agents have migrated to the controls, and this contamination makes a comparison of control and treatment meaningless. For this reason a more valid datum is needed to determine the effectiveness of the test agents, since the test provides for continuation for 1 or 2 years. Control data collected in 1974 at Lake Concordia during the evaluation of the effects of CO_2 laser irradiation on waterhyacinth growth[1] would probably be a good indication of expected plant growth in natural conditions. For example, the instantaneous growth rate (k) is 0.021645, which yields a daily increment factor (e^k) of 1.0219, a figure comparable to Bock's calculation[4] of 1.0217 for waterhyacinth growth at about the same time of the year.

PART VI: CONCLUSIONS AND RECOMMENDATIONS

Conclusions

64. As a result of the data collected during this reporting period and the preliminary analysis performed to date, the following conclusions were drawn:

a. The selected agents, once established on the plots of waterhyacinths, have maintained an effective population throughout more than one yearly cycle (paragraph 62).

b. The first-year effect of the agents' presence is to reduce the peak weight and height of the plants (Figures 7 and 8, respectively).

c. The optimum cause, i.e. the best combination of control agents, of the apparent reduction of the second-year growth rate from the first-year growth rate cannot be confirmed without additional data and systematic analysis of those data.

Recommendations

65. As a result of the effort conducted to date and the conclusions resulting therefrom, it is recommended that:

a. The experiment be continued for at least one more seasonal cycle to determine (1) the optimum cause of the apparent reduction of growth rate of the plants, and (2) the effective overwintering of the agents.

b. The analysis of the presently collected data be continued, and the results obtained from the nominal test design be reevaluated on the basis of the observations noting the cross-contamination of plots (Figure 10).

REFERENCES

1. Long, K. S. and Smith, P. A., "Aquatic Plant Control Program; Effects of CO_2 Laser on Waterhyacinth Growth," Technical Report 11, Nov 1975, U. S. Army Engineer Waterways Experiment Station, CE, Vicksburg, Miss.

2. Cochran, W. G. and Cox, G. M., Experimental Designs, 2d ed., Wiley, New York, 1957, p 328.

3. Duncan, D. B., "Multiple Range and Multiple F Test," Biometrics, Vol 11, 1955, pp 1-42.

4. Bock, J. H., An Ecological Study of *Eichhornia Crassipes* with Special Emphasis on Its Reproductive Biology, Ph. D. Dissertation, University of California, Berkeley, Calif., 1966.

5. Vogel, E. and Oliver, A. D., "Evaluation of *Arzama densa* as an Aid in the Control of Water Hyacinth in Louisiana," Journal, Economic Entomology, Vol 62, No. 1, 1969, pp 142-145.

6. Gordon, R. D. and Coulson, J. R., "Field Observations of Arthropods on Water Hyacinth," Aquatic Plant Control Program; Biological Control of Water Hyacinth with Insect Enemies, Technical Report 6, Appendix B, pp B1-B37, Jan 1974, U. S. Army Engineer Waterways Experiment Station, CE, Vicksburg, Miss.

7. Vogel, E. and Oliver, A. D., "Life History and Some Factors Affecting the Population of *Arzama densa* in Louisiana," Annals of the Entomological Society of America, Vol 62, No. 4, 1969, pp 749-752.

8. Center, T. D., The Potential of *Arzama densa* for the Control of Waterhyacinth, with Special Reference to the Ecology of Waterhyacinth (*Eichhornia crassipes* (Mart.)) Solms, Ph. D. Dissertation, University of Florida, Gainesville, Fla., 1976.

9. Spencer, N. R. et al., "Insect Enemies of Aquatic Weeds," Aquatic Plant Control Program; Biological Control of Water Hyacinth with Insect Enemies, Technical Report 6, Appendix D, pp D1-D21, Jan 1974, U. S. Army Engineer Waterways Experiment Station, CE, Vicksburg, Miss.

10. Perkins, D. D., "Biocontrol of Water Hyacinths," Aquatic Plant Control Program; Biological Control of Water Hyacinth with Insect Enemies, Technical Report 6, Appendix E, pp E1-E17, Jan 1974, U. S. Army Engineer Waterways Experiment Station, CE, Vicksburg, Miss.

11. Rintz, R. E., "A Zonal Leaf Spot of Waterhyacinth Caused by *Cephalosporium zonatum*," Hyacinth Control Journal, Vol II, Jun 1973, pp 41-44.

12. Charudattan, R. et al., "Studies on the Use of Plant Pathogens in Biological Control of Aquatic Weeds in Florida," Proceedings, EWRC 4th International Symposium on Aquatic Weeds, Vienna, 1974, pp 144-151; also published as Florida Agricultural Experiment Station Journal, Series No. 5443.

13. Freeman, T. E., Charudattan, R., and Conway, K., "Exploration, Pathogenicity, and Integrated Control," Aquatic Plant Control Program; Aquatic Weed Control with Plant Pathogens, Technical Report 8, Appendix I, pp I1-I9, Nov 1974, U. S. Army Engineer Waterways Experiment Station, CE, Vicksburg, Miss.

14. Gams, W., "Cephalosporiumartige Schimmelpilze (Hyphomycetes)," 1971, G. Fischer Verlag, Stuttgart, Germany.

15. Freeman, T. E. and Charudattan, R., "Occurrence of *Cercospora piaropi* on Waterhyacinth in Florida," Plant Disease Reporter, Vol 58, No. 3, Mar 1947, pp 277-278.

16. Chupp, C., A Monograph of the Fungus Genus *Cercospora*, Ithaca, N. Y., 1953 (cited by Freeman and Charudattan, Reference 15).

17. Thirumalcher, M. J. and Govindu, H. C., "Notes on Some Indian *Cercosporae*," Sydowia, Vol 8, 1954, pp 343-348.

18. Conway, K. E., "*Cercospora rodmanii*, a New Pathogen of Water Hyacinth with Biological Control Potential," Canadian Journal, Botany, Vol 54, No. 10, 1976, pp 1079-1083.

Table 1

Cumulative Plant Mass, kilograms

Treatment*	Frame No.	Date (Julian Day and Conventional Calendars), 1975-76										
		174 23 Jun	188 7 Jul	202 21 Jul	218 6 Aug	231 19 Aug	245 2 Sep	259 16 Sep	273 30 Sep	295 22 Oct	337 3 Dec	57 2 Feb
0	35	36.0	49.5	68.0	98.5	107.5	110.0	121.0	118.0	109.0	101.0	82.0
	96	34.5	60.0	77.0	108.0	123.0	137.0	151.0	146.0	134.0	133.5	98.5
	25	48.0	70.0	91.0	120.5	121.5	127.0	147.5	141.0	130.5	124.5	97.0
	30	39.0	53.5	67.0	92.0	111.0	125.0	147.5	150.0	136.0	125.0	92.5
a	107	30.0	43.0	58.5	87.0	104.5	121.5	134.0	133.0	124.0	115.0	67.0
	50	45.0	56.0	73.5	97.5	103.0	115.5	131.0	127.0	112.5	106.0	78.0
	38	38.5	53.0	67.0	95.5	106.0	116.0	139.5	136.5	134.0	132.5	95.5
	41	27.5	57.0	73.0	99.0	115.0	128.0	153.5	154.0	150.0	149.5	104.0
ab	85	32.0	55.0	72.5	103.0	114.0	114.0	114.5	109.0	101.0	100.0	84.5
	36	20.0	38.5	44.0	57.5	63.0	68.5	74.0	71.5	70.5	68.0	67.0
	6	48.0	62.0	78.5	102.0	110.0	110.0	121.5	115.5	104.5	102.0	82.0
	23	39.0	49.5	58.0	85.0	96.0	97.0	105.0	102.0	100.0	98.0	83.5
abc	52	25.0	32.5	44.0	65.0	177.5	78.0	81.0	83.5	81.5	81.0	53.5
	89	36.5	57.0	67.5	89.5	96.0	101.0	106.0	99.0	94.0	91.0	80.5
	19	39.0	54.5	63.0	90.5	100.0	104.0	114.0	106.5	97.0	95.0	85.0
	16	37.0	53.0	63.5	87.0	98.0	87.0	98.0	90.5	84.0	83.5	77.0
abcd	101	31.0	43.0	56.5	81.0	95.0	96.5	98.0	101.0	98.0	124.5	64.0
	97	45.0	55.0	69.0	93.0	98.0	95.0	102.5	102.0	97.5	96.5	80.5
	4	30.5	40.5	52.5	72.0	75.0	72.0	82.5	76.0	73.0	66.5	68.0
	8	19.0	22.0	22.0	25.5	25.5	24.0	24.5	24.0	21.0	24.0	22.0
b	43	28.0	36.0	48.5	71.0	86.0	93.0	95.0	95.5	94.5	94.5	63.0
	86	40.0	50.0	66.5	94.0	104.0	105.5	111.0	108.5	100.0	99.0	80.5
	69	37.0	43.0	59.0	81.5	95.0	98.5	110.0	100.0	96.0	94.0	86.5
	1	34.0	54.0	67.0	97.0	107.0	112.0	119.0	119.5	116.5	116.0	96.5
bc	58	35.5	48.0	62.5	90.0	95.0	93.0	99.5	98.0	100.0	98.5	86.0
	71	42.5	56.5	73.5	99.0	109.0	109.5	115.0	114.0	111.0	110.5	93.5
	33	37.0	47.5	57.5	81.0	88.0	90.5	102.0	100.0	98.0	95.0	85.5
	82	30.5	36.0	42.0	62.0	70.0	73.0	84.0	80.5	82.5	82.0	76.5
bd	51	30.0	42.5	58.0	84.5	95.5	96.0	93.0	95.0	94.5	94.0	60.5
	90	33.0	48.5	57.5	79.5	86.5	86.5	91.5	90.0	86.5	84.0	76.0
	40	38.0	46.5	60.5	82.0	90.5	94.0	106.0	103.5	100.0	98.0	85.5
	11	39.5	58.0	75.0	98.5	112.5	113.0	118.0	116.5	109.5	109.0	90.0
c	91	37.0	52.0	68.5	98.0	113.5	131.5	148.0	157.0	144.0	130.5	78.0
	15	23.0	34.5	37.0	52.0	58.0	70.5	85.0	85.0	83.5	81.5	69.0
	13	41.5	53.0	65.0	90.0	99.0	113.5	135.0	130.0	129.0	122.0	93.0
	10	39.5	50.0	57.0	82.5	95.5	111.5	134.0	137.0	129.0	129.0	96.0
cd	67	35.0	52.5	73.0	103.0	111.0	123.5	138.5	145.0	136.0	121.0	72.0
	106	48.0	59.5	80.5	111.5	121.0	133.5	156.0	157.5	143.5	134.0	103.0
	72	31.5	43.0	56.0	76.5	87.0	96.0	119.5	116.5	109.0	104.0	87.0
	17	33.0	33.0	36.0	49.5	56.0	67.0	82.5	81.0	76.5	76.5	69.5
ac	18	29.5	48.5	62.5	90.0	103.5	117.0	135.0	137.0	133.5	127.5	92.0
	105	47.0	65.5	82.5	112.0	115.5	127.0	144.5	141.0	126.0	116.0	94.0
	92	17.5	18.5	15.5	16.0	14.5	12.0	13.0	12.5	11.0	10.0	9.0
	24	44.0	56.0	63.5	89.0	94.5	108.5	122.0	121.0	113.5	113.0	87.0
d	104	31.5	54.5	76.5	108.0	119.0	128.0	144.0	149.0	145.0	137.0	106.0
	87	54.0	69.5	92.0	119.5	123.0	127.0	142.5	141.0	133.5	119.5	94.5
	2	41.0	53.5	67.5	96.5	106.5	117.0	139.5	133.0	129.5	125.0	94.0
	37	39.5	51.0	61.0	87.5	97.0	110.0	131.5	129.0	121.0	121.0	95.0
ad	99	37.0	52.0	72.0	101.0	117.0	125.0	136.5	140.5	124.0	107.0	62.5
	80	19.0	32.5	37.0	51.5	60.0	76.0	95.5	94.0	98.5	95.0	73.0
	74	50.0	57.0	81.0	114.5	123.0	134.5	156.5	149.0	137.0	126.5	97.0
	83	44.5	51.0	63.5	89.0	99.0	110.0	131.5	131.0	113.0	107.5	79.5
acd	64	39.0	64.0	85.0	118.5	123.5	129.5	144.0	139.0	132.0	124.0	99.0
	108	23.0	33.0	37.5	48.5	55.5	68.0	87.0	88.0	80.0	73.0	66.0
	32	36.5	48.0	58.5	77.0	83.0	91.0	109.0	101.0	92.0	89.5	74.0
	70	36.0	26.0	27.0	35.0	38.5	42.5	49.0	46.5	43.5	43.0	44.0
bcd	46	30.0	37.5	48.5	70.5	72.0	71.0	78.5	75.5	76.0	75.5	69.5
	77	43.5	51.0	64.0	91.0	97.5	100.0	105.0	109.0	107.5	100.0	91.0
	21	34.0	52.0	66.5	102.5	103.0	100.0	110.0	105.0	100.0	96.0	88.0
	27	35.0	41.0	45.0	63.5	67.5	71.0	79.0	77.0	76.5	76.0	73.5
abd	65	42.5	62.0	84.0	117.0	118.0	116.0	119.5	114.0	108.0	104.5	85.5
	100	43.0	56.0	77.0	105.5	117.0	115.0	122.5	114.5	104.5	104.0	85.5
	57	28.5	34.0	40.5	57.0	58.0	58.5	67.0	61.5	58.0	49.0	60.0
	26	38.0	56.0	71.0	91.0	93.5	88.5	95.5	89.0	82.5	82.0	75.0

* 0 = no treatment (controls); a = Arzama; b = Neochetina; c = Cercospora; and d = Acremonium.

Table 2

Plant Height, centimetres (Maximum/Average)

Treatment*	Frame No.	Date (Julian Day and Conventional Calendars), 1975-76										
		174 23 Jun	188 7 Jul	202 21 Jul	218 6 Aug	231 19 Aug	245 2 Sep	259 16 Sep	273 30 Sep	295 22 Oct	337 3 Dec	57 2 Feb
0	35	43/22	68/28	72/33	103/40	111/52	113/72	112/70	103/88	100/68	68/58	35/18
	96	42/23	67/29	80/31	110/40	102/64	117/77	119/86	112/84	110/75	81/65	48/25
	25	50/26	70/40	90/34	106/51	118/63	118/77	112/74	103/80	100/78	75/57	38/23
	30	42/22	57/32	87/28	92/40	99/60	117/61	114/79	110/78	105/80	80/63	37/23
a	107	36/18	46/20	70/28	91/24	104/52	113/69	110/70	112/83	106/74	74/66	30/18
	50	48/28	60/28	76/34	96/40	106/63	106/63	107/64	97/76	95/75	52/68	30/17
	38	34/20	54/31	77/38	94/41	104/62	116/68	112/67	106/80	104/84	80/64	28/23
	41	44/20	61/35	74/30	97/32	114/62	116/67	118/80	114/84	110/77	83/63	33/23
ab	85	40/25	57/29	76/34	94/22	98/60	104/65	90/61	90/74	83/70	72/58	38/24
	36	26/21	49/21	37/18	57/12	75/38	80/46	78/55	77/62	80/53	68/47	25/19
	6	50/27	64/35	80/36	99/49	94/60	98/63	96/62	89/74	84/70	74/57	35/23
	23	34/18	55/22	67/32	80/16	86/39	92/55	89/55	85/67	83/66	68/57	35/23
abc	52	27/14	36/16	46/21	63/19	80/37	82/39	85/51	78/67	81/61	68/58	35/24
	89	29/24	62/25	72/33	87/33	94/53	93/66	87/60	84/67	83/65	67/57	40/23
	19	54/24	64/30	72/31	86/23	88/52	92/56	99/62	80/73	86/72	73/58	28/20
	16	44/20	59/31	72/27	77/32	80/47	85/54	94/62	88/64	80/62	67/52	25/17
abcd	101	22/14	42/22	55/26	73/20	82/48	81/43	89/58	87/71	83/63	70/61	37/17
	97	39/26	68/33	72/34	81/35	100/56	98/65	98/65	90/75	83/67	74/59	35/20
	4	44/19	46/24	54/27	67/26	74/41	89/46	88/50	75/58	75/58	68/48	30/19
	8	21/13	31/13	19/10	37/09	28/12	28/08	28/12	25/10	23/08	12/07	10/06
b	43	36/16	41/20	47/23	73/21	84/33	90/51	88/54	86/76	84/67	75/62	30/22
	86	38/20	60/26	70/45	81/24	94/61	98/67	99/68	88/73	82/65	75/60	38/23
	69	31/15	50/27	59/27	77/38	96/50	94/53	103/69	90/70	87/70	75/58	85/24
	1	39/18	60/28	68/32	85/20	97/53	93/72	90/66	83/69	87/66	76/62	35/22
bc	58	38/20	62/29	72/32	82/29	94/51	91/65	89/61	84/70	85/63	76/63	37/24
	71	52/29	70/36	80/39	90/36	97/60	93/62	93/60	87/75	85/68	75/60	30/20
	33	46/21	69/25	67/28	82/18	90/38	89/62	94/59	87/70	86/72	72/57	27/24
	82	30/16	57/18	43/15	75/18	75/48	81/50	90/44	85/66	84/65	66/54	27/18
bd	51	37/21	42/25	54/19	70/18	78/41	87/52	91/52	87/63	81/62	71/59	27/20
	90	26/20	56/23	62/25	78/30	79/46	87/58	86/57	87/65	87/65	70/53	35/23
	40	43/22	59/27	77/48	86/31	92/51	96/67	94/56	90/75	90/72	79/62	37/20
	11	49/21	68/33	74/34	94/48	99/56	94/58	98/66	90/70	89/73	76/62	32/22
c	91	30/19	51/29	75/29	102/37	108/56	110/74	110/72	110/93	105/85	74/63	35/17
	15	27/19	34/15	34/18	70/10	78/30	95/52	96/58	90/68	83/63	65/52	37/18
	13	48/25	60/33	87/32	102/43	103/50	114/72	118/80	104/85	103/78	80/61	36/25
	10	42/22	60/30	65/30	92/20	110/50	102/62	116/70	108/83	105/76	83/66	38/24
cd	67	44/25	53/29	69/31	107/20	110/52	112/69	113/80	105/87	104/74	80/66	42/24
	106	44/26	67/31	76/37	101/34	107/59	118/74	120/75	113/86	100/82	80/64	42/25
	72	32/18	48/27	67/22	86/22	96/53	109/65	104/69	97/80	102/75	77/61	43/25
	17	24/17	44/25	48/12	75/16	85/33	84/46	96/66	93/65	84/62	60/43	37/17
ac	18	53/20	58/26	72/33	96/34	104/55	111/62	115/70	109/84	105/75	82/67	32/20
	105	53/28	71/39	88/38	104/41	120/65	116/67	115/72	106/86	105/78	76/59	38/23
	92	19/14	19/10	15/09	12/07	18/09	18/07	13/09	12/06	11/05	10/05	03/03
	24	42/20	64/28	80/32	98/36	107/54	111/61	107/63	100/78	96/72	76/60	33/18
d	104	41/23	57/28	72/36	98/49	110/63	118/73	114/82	104/86	100/74	78/67	30/18
	87	57/35	86/38	90/44	110/40	114/68	116/72	116/72	105/85	100/78	76/58	34/17
	2	43/19	61/28	89/39	97/36	96/43	114/67	117/87	110/85	100/80	80/64	30/18
	37	42/20	56/23	73/26	96/14	102/53	110/70	114/73	110/82	107/74	77/61	43/23
ad	99	55/20	52/18	76/37	94/32	109/59	107/61	105/77	102/89	95/66	72/63	30/12
	80	22/12	36/13	37/12	62/15	69/19	86/44	105/65	90/70	93/70	71/58	33/18
	74	39/24	69/32	77/33	109/46	99/63	116/65	120/78	107/83	102/74	80/63	42/22
	83	40/20	62/30	76/31	90/38	103/54	104/64	102/66	102/75	100/73	73/58	43/22
acd	64	52/25	67/33	80/33	105/48	118/60	116/80	113/74	110/88	105/72	80/68	43/24
	108	26/17	42/18	44/13	59/14	67/28	78/42	89/48	87/66	88/60	65/54	37/19
	32	47/24	57/28	66/25	84/33	90/48	99/60	112/66	95/75	95/73	71/49	30/20
	70	32/14	37/16	36/10	43/12	43/28	56/31	69/24	63/33	55/30	50/22	16/08
bcd	46	25/16	35/21	48/17	72/20	74/42	76/45	80/50	83/66	80/62	63/50	37/15
	77	38/25	60/27	67/23	89/28	93/47	91/56	98/63	85/70	87/68	77/62	76/24
	21	42/24	61/34	70/28	86/27	92/56	88/58	93/62	88/72	86/73	73/59	35/25
	27	31/18	54/23	56/19	70/16	77/39	79/47	87/48	80/60	82/62	65/49	34/22
abd	65	50/30	72/30	78/40	94/36	97/61	93/64	97/65	88/70	85/68	76/62	32/24
	100	52/27	70/30	74/23	91/47	97/58	96/62	97/65	88/70	94/70	72/59	37/22
	57	23/14	36/13	38/15	48/17	70/28	70/36	80/54	75/50	75/55	55/42	25/14
	26	44/24	65/33	74/28	88/39	88/50	86/53	87/56	87/62	84/65	70/54	25/17

* 0 = no treatment (controls); a = Arzama; b = Neochetina; c = Cercospora; and d = Acremonium.

975	December 1975			January 1976			February 1976		
Precipitation in.	Temperature °F, min	Temperature °F, max	Precipitation in.	Temperature °F, min	Temperature °F, max	Precipitation in.	Temperature °F, min	Temperature °F, max	Precipitation in.
--	30	50	0.8	42	65	--	38	54	1.8
--	32	50	--	50	68	--	36	58	--
--	35	57	--	39	45	1.1	40	63	--
0.6	39	64	--	26	34	--	52	68	--
0.7	50	65	--	24	39	--	60	69	--
1.1	59	60	--	39	55	--	39	45	0.8
0.5	47	55	0.1	53	60	0.7	32*	43	--
--	46	50	--	20*	28	--	32*	52	--
--	40	52	--	20*	38	--	41	66	--
--	37	57	--	32	56	--	55	70	--
--	43	65	--	50	60	--	57	68	--
--	46	67	--	50	62	0.2	56	70	--
--	50	80*	--	61	70*	--	56	69	--
--	60	72	--	40	54	0.9	58	69	--
--	62	73	--	32	50	--	58	70	--
--	41	63	0.7	43	53	--	62	72	--
--	44	46	0.6	30	41	--	61	74	--
--	26	30	--	31	57	--	56	72	0.6
--	23*	41	--	39	60	--	46	79*	--
0.4	34	50	--	40	46	--	50	73	--
--	32	40	--	31	53	0.2	60	64	1.3
--	33	41	--	35	55	--	40	48	--
--	34	47	--	38	61	--	36	70	--
--	33	44	--	54	64	--	40	62	--
--	46	47	1.9	60	60	0.5	46	68	--
0.3	40	46	0.4	40	40	1.6	52	67	--
0.7	35	54	--	28	41	--	50	72	--
--	43	62	--	26	49	--	49	72	--
0.2	52	61	0.3	37	46	--	50	73	--
--	45	45	0.4	40	65	--			
	46	52	--	41	46	--			
4.5	29	0	5.2	29	0	5.2	25	0	4.5

Table 4

Ranking of Treatments by Mean Biomass Values

16 Sep 1975			30 Sep 1975			22 Oct 1975		
Treatment Code	Rank*	Mean Biomass kg	Treatment Code	Rank	Mean Biomass kg	Treatment Code	Rank	Mean Biomass kg
1	16	141.750	1	16	138.500	1	16	132.300
12	15	139.425	12	15	138.125	2	15	130.100
2	14	139.350	2	14	137.725	1	14	127.325
13	13	129.925	13	13	128.425	9	13	121.325
9	12	125.275	9	12	127.275	13	12	117.925
10	11	123.950	10	11	125.000	10	11	116.125
6	10	108.650	6	10	105.825	6	10	101.700
3	9	103.725	11	9	102.850	7	9	97.925
11	8	103.475	8	8	101.225	8	8	97.575
8	7	102.150	3	7	99.500	11	7	96.075
16	6	101.100	7	6	98.250	3	6	93.925
7	5	100.050	16	5	94.800	15	5	90.025
4	4	99.775	4	4	94.775	4	4	89.125
14	3	97.100	14	3	93.650	16	3	88.150
15	2	93.125	15	2	91.675	14	2	86.750
5	1	76.900	5	1	75.675	5	1	72.400

* 1-16; most to least effective.

Table 5

Ranking of Treatments by

Mean Height Values

16 Sep 1975		
Treatment Code	Rank*	Mean, cm
1	16	77.25
12	15	77.25
10	14	72.50
13	13	71.50
2	12	70.25
9	11	70.00
6	10	64.25
16	9	60.00
4	8	58.75
3	7	58.25
8	6	57.75
7	5	56.00
15	4	55.75
11	3	53.50
14	2	53.00
5	1	46.25

* 1-16; most to least effective.

APPENDIX A: LIFE SYSTEMS OF ORGANISMS USED AS CONTROL AGENTS

1. This appendix presents a description of the life systems of the organisms being used in this experiment. Also, some background experiences with the organisms are included.

Arzama densa Walker

2. Arzama densa is a noctuid moth (family Noctuidae*) whose larva is a stem and root borer of certain aquatic plants, but it is not known to attack any cultivated plant species. It was apparently established in the United States before waterhyacinths were reported naturalized in this country and is presumed to be native. It feeds extensively on pickerelweed (Pontederia cordata L.), a native aquatic plant closely related to waterhyacinths and presumably its principal host in the United States prior to introduction of waterhyacinths. It is commonly abundant in both Louisiana[5]** and in Florida.[6]

3. The life cycle of A. densa and its potential effectiveness as a control agent for waterhyacinths were studied by Vogel and Oliver.[5,7] They concluded[7] that it would have significant potential for this purpose if biotic factors (insects and diseases) that reduce its field populations could be reduced so that populations of greater than normal density could develop. Furthermore, they suggested that natural field populations might be supplemented with laboratory-raised larvae if a satisfactory method for raising could be devised.

4. Work on methods for raising and transplanting Arzama densa was begun in 1974[8] and has continued to the present. The techniques that have been developed to date are inefficient in terms of numbers of insects successfully transplanted in relation to the numbers of eggs

---

* The family Noctuidae includes the armyworms, cutworms, and their allies, many of which are serious pests of economic plants important in agriculture, horticulture, and forestry. Most of the species are host-specific.

** Raised numbers refer to correspondingly numbered items in the references at the end of the main text.

started and the effort required. Nonetheless, successful transplants have been made for experimental purposes, and a relatively large number of insects were raised and, with two attempts, were successfully transplanted to the test plots used in this experiment. Large Arzama larvae were present in the test plots during late winter following the first growing season of the tests.

Neochetina spp. (Waterhyacinth Weevil)

5. Two closely related species of weevil, Neochetina bruchi Hustache and N. eichhorniae Warner, have been introduced into the United States from Trinidad and Argentina as potential agents for control of waterhyacinths. The history of discovery, introduction, and quarantine tests of these insects is reviewed by Spencer et al.[9] and Perkins.[10] N. bruchi was known as a consumer of waterhyacinths in South America and was undergoing specificity tests in Argentina when N. eichhorniae was discovered as a cohort in the test populations.

6. Tests with isolates of the newly discovered species revealed greater specificity of this species for Eichhornia crassipes than had been shown by N. bruchi. The latter will nibble various species of plants in the pickerelweed family (Pontederiaceae), including pickerelweed (Pontederia cordata), Reussia spp., and other species of Eichhornia,[9] but it exhibits a strong preference for E. crassipes. N. eichhorniae appears to be specific to E. crassipes.[10] It was concluded from the specificity studies that a close evolutionary tie exists between the insect genus Neochetina and the plant family Pontederiaceae,[10] with N. bruchi being the more general feeder, N. affinis being restricted to E. azurea, and N. eichhorniae being restricted to E. crassipes.

7. Both N. bruchi and N. eichhorniae have been introduced into the United States under quarantine by the USDA Biological Research Laboratories and have undergone the required specificity tests; and, with permission from the appropriate controlling authorities, both have been released in selected areas in Louisiana and Florida. Post-release observations on the release sites to date indicate that further studies

with these species as candidate control agents are warranted. Only N. eichhorniae were used in this experiment.

8. The adults of these insects feed on the leaves of the waterhyacinth and deposit their eggs in the leaf petioles. The larvae tunnel in the stems and basal portions of the plant and pupate within a cocoon among the fine hair-like roots of the plants.[9] Because of the specialized pupal cell formed in the floating root mass peculiar to E. crassipes, the insects can complete their life cycle only on that species. Finally, neither adult nor larva is able to survive but a short while in the absence of very high atmospheric humidity.[10]

9. Their potential effectiveness as control agents for waterhyacinths in the United States derives from three aspects of feeding behavior:[10] (a) larval feeding and tunneling, (b) adult feeding, and (c) bacterial and fungal decay associated with the feeding wounds. These effects should be enhanced with the higher population densities that are expected to occur in the United States in the absence of parasites and competitors that tend to suppress populations in their native Argentina. The effect of bacterial and fungal decay of the waterhyacinths should be enhanced in proportion to the excessive feeding. In particular, the effect of decay associated with feeding by the insects should be greatly enhanced with pathogenic fungi deliberately inoculated into the plant populations in conjunction with the introduced Neochetina population.

Acremonium zonatum (Sawada) Gams

10. For the present purpose, Acremonium zonatum is the organism studied by Rintz[11] and by Charudattan et al.[12] Specifically, for purposes of this test, the name applies to a strain or a mixture of strains isolated from material found occurring naturally in Florida and maintained in culture at the Florida Agricultural Experiment Station.[12,13] When studied by Rintz, it was known as Cephalosporium zonatum Sawada, which he took to include certain other species of Cephalosporium previously described as parasitic on various species of plants in the

tropics around the world. This interpretation was accepted by Charudattan et al. The included species are C. eichhorniae Padwick, C. zonatum Sawada, and C. fici Tims & Olive. Subsequent to Rintz's study, however, it was found that the genus Cephalosporium had been restudied and reassigned by Gams[14] to the genus Acremonium so that C. zonatum Sawada became Acremonium zonatum (Sawada) Gams.

11. This fungus attacks the surface tissues of the leaf and petiole of the waterhyacinth, producing lesions that gradually enlarge and coalesce, and ultimately destroys the functional capacity of the leaf.[11] The descriptive epithet zonatum derives from the characteristic alternating concentric zones of dark- and light-brown coloration that develop as the lesion enlarges.

12. As understood in its broader sense (i.e., collectively all of the species considered here to be synonymous with it, as described above), A. zonatum is potentially pathogenic on a wide assortment of plant species, including several economic crop species, but under natural conditions in North America, it apparently attacks only the fig;[12] on that species, it apparently is not seriously pathogenic.[13]

13. Though the disease is quite virulent on leaves of waterhyacinths,[12] Rintz did not consider it to be a likely candidate for pathogenic control of waterhyacinths unless it could be artificially cultured and applied at abnormally high concentrations. Field trials with cultured material from the Florida isolates have shown promising pathogenicity.

Cercospora piaropi Tharp

14. This fungus causes a leaf-spot disease on waterhyacinths. It is apparently a widespread, well-established native or naturalized disease of waterhyacinths in the United States and is specific to waterhyacinths. Originally, the fungus was described by Tharp from waterhyacinth in Texas in 1914[15] and was known only from that location until 1954, when found on waterhyacinths in India as reported by Chupp,[16] and Thirumalacher and Govindu,[17] as cited by Freeman and Charudattan.[15]

Its presence in the United States was recently reconfirmed,[12] and it was discovered occurring naturally on waterhyacinths in the vicinity of Gainesville, Florida, in 1972.[15] The paucity of reports of its occurrence in the past derives apparently not from its rarity but from a general lack of interest in the parasites of waterhyacinths.[15] The specific epithet piaropi derives from the generic name Piaropus Raf.,* which was applied to waterhyacinths in North America at the time Tharp reported this fungus. Piaropus Raf. has since been declared indistinct, and the waterhyacinths in North America are now reassigned to Eichhornia Kunth.

15. In general, Cercospora piaropi is a disease of limited pathogenicity, but Freeman and Charudattan concluded that the host specificity generally exhibited by species of Cercospora qualified this species as a potential biocontrol agent for waterhyacinths, provided a method could be developed for inducing an artificial epidemic of damaging proportions. Investigations along this line were continued, using strains of the fungus cultured from material collected in the vicinity of Gainesville.[15] From these cultures, a form of the fungus was discovered that appears to be both highly specific to waterhyacinth and highly virulent on it. This form was subsequently determined to be specifically distinct and was described by Conway[18] as C. rodmannii sp. n. For the test reported herein, it was anticipated that the insects, Neochetina in particular, would induce such an "artificial epidemic" by disseminating the fungus spores over the surface of the plant and by creating wounds as entry sites for the germinating spores.

16. As with Acremonium, specificity of the insects for waterhyacinths will minimize the danger of transferring the fungus spores to other plant species (particularly economic species) in any vicinity in which the fungus may be used in conjunction with the insects, but the specificity of C. rodmanii to waterhyacinth makes this consideration less important for this species than for Acremonium.

---

* Apparently from Piaroa, a South American Indian tribe inhabiting the region of the Orinoco River.

APPENDIX B: FLOWERING ACTIVITY OF WATERHYACINTHS

1. In general, flowering (Table B1) was reported to be profuse on 23 June 1975 and, according to the recorded data, continued so through 6 August. No flowers were observed on 19 August, but flowers were observed sporadically thereafter until 12 October and presumably continued so until frost. This pattern is consistent with the observations reported by Long and Smith.[1] Their data for 1974 show a profusion of flowering on the test plots at the 24-25 June and 11 July observations, with sporadic flowering thereafter.

2. Any deviations from the designated treatment series that may affect the results of the experiments with respect to plant mass accumulation must, of course, also be accounted for in the interpretation of the flowering data. Though it would be erroneous to assume that a one-to-one correlation or a cause-effect relation exists between plot weight and flowering activity, observation suggests that an inverse relation probably exists; that is, lush plants bloom profusely, less vigorous plants bloom sporadically, and stunted plants bloom little or not at all.

3. An uncritical tentative evaluation of the flower data for the test (Table B1), without consideration of unsuccessful introduction of agents, contaminations, or other extra-experimental effects, suggests the following results: all plots treated with Neochetina (and coincidentally with Orthogalumna), in the absence of other designated treatments, exhibited somewhat suppressed late-season flowering relative to the controls, and the plots treated with both Neochetina and Cercospora exhibited greater suppression of late-season flowers. None of the other treatment combinations appear to have had a noticeable suppressive effect on flowering, but some plots designated for Arzama appear to have exhibited somewhat enhanced late-season flowering, relative to the controls. These observations, however, must not be taken uncritically.

Table B1

Flowering Activity, Number of Open Inflorescences

Treatment Code	Frame No. Sequence				1975 23 Jun				7 Jul*				21 Jul				6 Aug				19 Aug				2 Sep				16 Sep				30 Sep				22 Oct				3 Dec
0	35	96	25	30	2	3	8	6	4	3	3	2	3	1	4	0	7	0	4	2	0	0	0	0	0	1	0	0	0	2	0	1	1	0	1	0	1	0	0	1	None
a	107	50	38	41	1	7	4	2	1	2	6	1	2	2	0	1	3	1	8	2	0	0	0	0	0	4	1	0	0	0	0	0	1	1	0	0	0	1	2	3	
ab	85	36	6	23	5	0	3	1	2	0	5	1	4	0	0	0	2	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	
abc	52	89	19	16	1	3	8	6	0	6	0	1	2	3	9	2	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
abcd	101	97	4	8	0	10	3	0	0	3	0	0	2	3	1	0	6	1	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
b	43	86	64	1	1	10	1	3	1	0	0	2	2	0	0	1	0	3	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	
bc	58	71	33	82	1	5	4	1	3	4	0	0	11	6	12	0	0	2	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	
bd	51	90	40	11	2	2	6	3	1	1	2	9	1	0	3	1	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	
c	91	15	13	10	0	0	10	2	4	0	1	1	5	2	8	2	5	0	1	0	0	0	0	0	0	1	0	1	0	4	2	1	1	0	1	2	0	1	4	1	
cd	67	106	72	17	3	9	0	0	8	6	0	0	2	0	4	2	3	1	2	0	0	0	0	0	0	0	1	0	0	0	1	6	1	1	3	1	0	1	1	1	
ac	18	105	92	24	4	11	0	5	0	3	0	0	11	12	0	1	3	0	0	1	0	0	0	0	2	0	0	0	2	0	0	1	4	1	0	1	3	1	0	1	
d	104	87	2	37	3	12	8	6	1	4	6	1	3	3	1	0	3	2	3	5	0	0	0	0	1	0	0	0	0	0	1	0	0	0	1	0	0	3	3	1	
ad	99	80	74	83	0	0	1	8	2	0	3	1	5	0	3	0	3	1	2	1	0	0	0	0	0	1	0	0	0	9	0	4	0	1	2	1	0	1	0	1	
acd	64	108	32	70	2	1	4	2	4	0	0	1	2	1	2	0	1	0	3	0	0	0	0	0	0	1	0	2	0	8	4	12	1	2	1	3	2	0	0	0	
bcd	46	77	21	27	0	2	6	3	1	3	2	0	5	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
abd	65	100	57	26	2	8	2	5	0	5	1	2	2	2	0	0	0	2	2	3	0	0	0	0	0	0	0	1	0	0	2	0	0	0	0	0	0	0	0	0	

Summary:

- abc, abcd, bc, bcd, ab — Suppressed late inflorescence
- b — Somewhat suppressed
- a, acd — Greatly enhanced late inflorescence
- c, cd — Somewhat enchanced late inflorescence

* Field inspection on 1 July 1975; no flowers available for photographs.

In accordance with letter from DAEN-RDC, DAEN-ASI dated 22 July 1977, Subject: Facsimile Catalog Cards for Laboratory Technical Publications, a facsimile catalog card in Library of Congress MARC format is reproduced below.

Addor, Eugene E
A field test of selected insects and pathogens for control of waterhyacinths; Report 1: Preliminary results for the 1975-76 season / by Eugene E. Addor. Vicksburg, Miss. : U. S. Waterways Experiment Station, 1977.
44, [12]p. : ill. ; 27 cm. (Technical report - U. S. Army Engineer Waterways Experiment Station ; A-77-2, Report 1)
Prepared for Office, Chief of Engineers, U. S. Army, Washington, D. C.
Bibliography: p. 43-44.

1. Aquatic plant control. 2. Insects. 3. Lake Concordia. 4. Pathogens. 5. Water hyacinths. I. United States. Army. Corps of Engineers. II. Series: United States. Waterways Experiment Station, Vicksburg, Miss. Technical report ; A-77-2, Report 1.
TA7.W34 no.A-77-2 Report 1