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EVALUATION OF THE INFECTIVITY OF A CERCOSPORA RODMANII FORMULAT--ETC(U)
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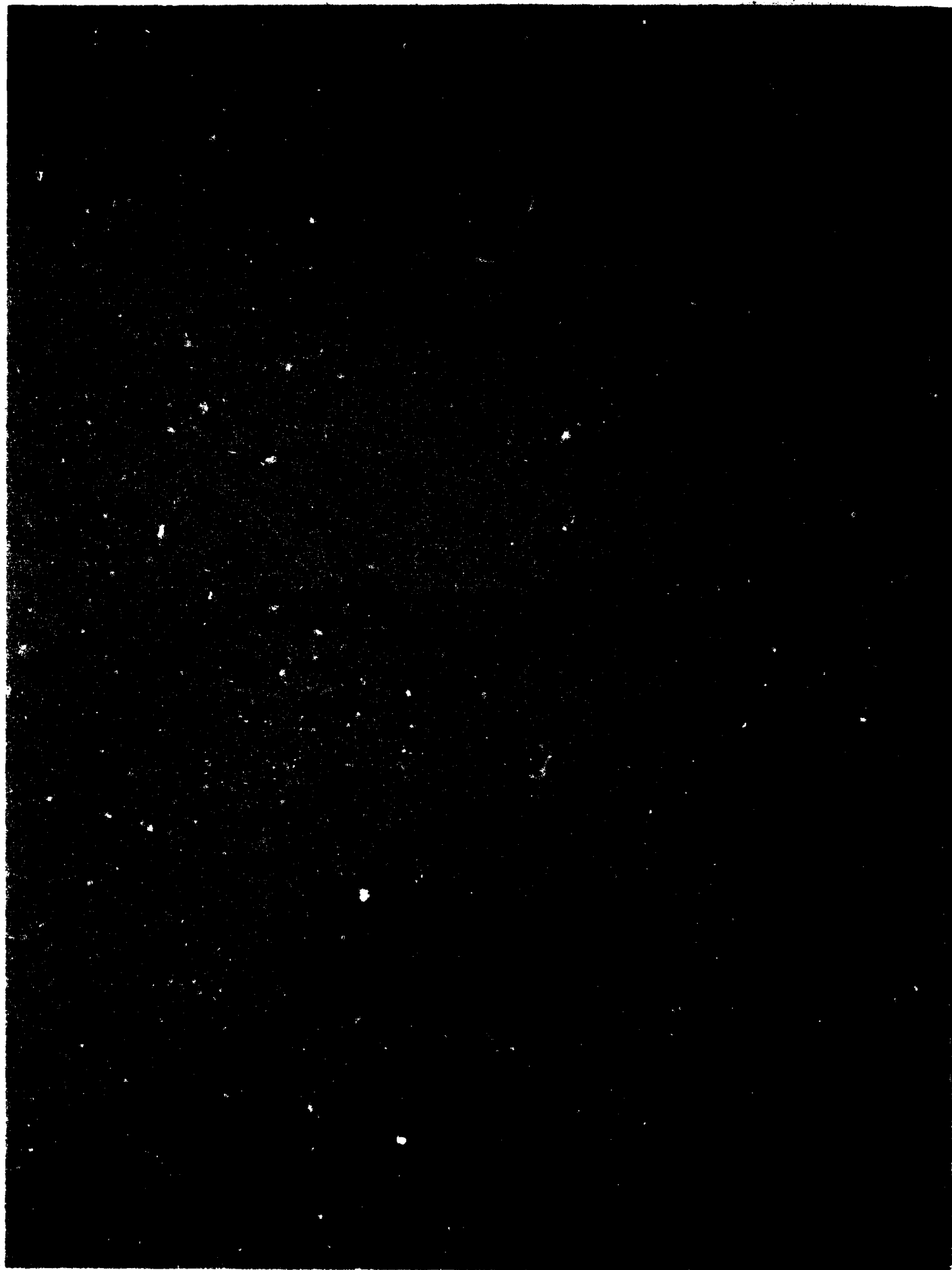
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20. ABSTRACT (Continued).

low-pressure impeller pump at 20 to 25 psi. In the fall study, a C. rodmanii formulation was applied to test plots in a roadside canal in Louisiana at a rate of 5 g (5×10^4 Colony Forming Units) per square metre. Cercospora rodmanii in the formulation proved to be infectious on the test plants; based upon observed infectivity in field plots 7 weeks after application of the formulation, it was concluded that there was no significant difference in infectivity when using a high-pressure piston pump system at 150 psi or a low-pressure impeller pump system at 25 psi.

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PREFACE

Funds for the study described herein were provided to the Aquatic Plant Control Research Program (APCRP) through Department of the Army Appropriation No. 96X3123, "Operations and Maintenance General," by the U. S. Army Engineer District, New Orleans.

This document describes a study to evaluate equipment for the large-scale application of the fungal pathogen Cercospora rodmanii Conway as part of the Large-Scale Operations Management Test (LSOMT) of insects and pathogens for the control of waterhyacinth in Louisiana. Dr. D. R. Sanders, Sr., was the team leader for the LSOMT. Mr. R. F. Theriot was responsible for the LSOMT field application tests, and Mr. E. A. Theriot was responsible for the equipment evaluation study.

This report was prepared by Messrs. E. A. Theriot and R. F. Theriot and Dr. Sanders of the Wetland and Terrestrial Habitat Group (WTHG), Environmental Resources Division (ERD), Environmental Laboratory (EL), U. S. Army Engineer Waterways Experiment Station (WES). Mr. S. O. Shirley (WTHG) assisted in establishing the test plots and in the collection of data. Messrs. James Manning and Don Lee, Louisiana Department of Wildlife and Fisheries, provided the application equipment and valuable technical assistance for this study. Abbott Laboratories, Inc., provided the C. rodmanii formulation evaluated in the study.

All phases of this study were conducted under the direct supervision of Dr. H. K. Smith, WTHG, and under the general supervision of Dr. C. J. Kirby, Jr., Chief, ERD, and Dr. J. Harrison, Chief, EL. Manager of the APCRP at the WES was Mr. J. L. Decell.

Commanders and Directors of the WES during the performance of the research and preparation of the report were COL J. L. Cannon, CE, and COL N. P. Conover, CE. Technical Director was Mr. F. R. Brown.

This report should be cited as follows:

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Miscellaneous Paper A-81-2, U. S. Army Engineer Waterways Experiment Station, CE, Vicksburg, Miss.

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

U. S. customary units of measurement used in this report can be converted to metric (SI) units as follows:

<u>Multiply</u>	<u>By</u>	<u>To Obtain</u>
miles (U. S. statute)	1.609347	kilometres
pounds (force) per square inch	6894.757	pascals

EVALUATION OF THE INFECTIVITY OF A *CERCOSPORA RODMANII*
FORMULATION USING TWO APPLICATION SYSTEMS

PART I: INTRODUCTION

Background

1. *Cercospora rodmanii* Conway, a fungal plant pathogen, was isolated by researchers from the University of Florida in 1973 (Conway 1976). Several years of laboratory and field research revealed that *C. rodmanii* was sufficiently host-specific and had great potential as a biological control agent of waterhyacinth (Conway, Freeman, and Charudattan 1974; Conway and Freeman 1976). Subsequently, the University of Florida patented *C. rodmanii* for this use and granted production rights to Abbott Laboratories, Inc., Chicago, Ill. Abbott Laboratories then developed an experimental wettable powder formulation for testing.

2. The U. S. Army Engineer District, New Orleans, has had a severe problem with waterhyacinth infestations since 1898 (Klorer 1909). In order to combat this problem, the New Orleans District funded a Large-Scale Operations Management Test (LSOMT) with insects and pathogens for the biological control of waterhyacinth in Louisiana (Sanders et al. 1979). The Abbott Laboratories formulation of *C. rodmanii* was one of the organisms to be tested on an operational scale.

Rationale

3. Prior to the initiation of the LSOMT, the pathogenicity and capability of *C. rodmanii* to control waterhyacinth had been demonstrated (Conway and Freeman 1976), and studies conducted at the University of Florida and the WES had demonstrated infectivity of the formulation on waterhyacinth in outdoor experimental tanks (Freeman, Charudattan, and Conway 1979; Theriot, Theriot, and Sanders 1981). However, infectivity of *C. rodmanii* in the Abbott Laboratories formulation had not been

demonstrated on field populations of waterhyacinth, and methods for large-scale application of the formulation had not been developed. While the obvious advantage of using conventional herbicide spray equipment was realized, it was also recognized that high-pressure pump systems could possibly destroy the fungal cells, thus reducing the viability. To evaluate the ability of C. rodmanii to infect field populations and to evaluate methods of applying the formulation on a large scale, an equipment evaluation study was designed and conducted in Louisiana.

Purpose and Objectives

4. The purpose of the equipment evaluation study was to assess the infectivity of the C. rodmanii formulation when applied with conventional high-pressure application equipment and a low-pressure pump system. The objectives of this study were as follows:

- a. To evaluate the infectivity of the C. rodmanii formulation on a natural field population of waterhyacinth in Louisiana.
- b. To determine the effect of two application systems on the viability of the C. rodmanii formulation.

PART II: MATERIALS AND METHODS

Test Organism

5. Cercospora rodmanii is a member of the Class: Fungi Imperfecti, Order: Moniliales. Spore-bearing structures (conidiophores) are produced in clusters that emerge through leaf stomata. The filiform, septate conidia have several cells with a truncate to obconic base, and they occur on the new growing tip of the conidiophore (Figure 1). The initial symptoms of infection are small punctate leaf spots. The spots are more numerous in the apical portion of the leaf due to the greater density of stomata in this area. As a result, dieback begins at the leaf tip and gradually progresses down the petiole until the entire leaf is killed. The optimal temperature for mycelial growth is 25°C with a favorable range of 20° to 30°C (Freeman, Charudattan, and Conway 1979).

Experimental Formulation and Treatment Rate

6. The viable propagules, which Abbott Laboratories characterized as "thick-walled vegetative cells," were contained in a formulation (Figure 2) milled sufficiently to pass through a 24 mesh screen (1.06 mm). The viability of the formulation used in the equipment evaluation study was 1×10^4 Colony Forming Units per gram (CFU/g), much less than that used for previous infectivity studies (1×10^6 CFU/g) (Theriot, Theriot, and Sanders 1981). The low viability was due to problems encountered during the experimental mass production of the formulation. The method of production of the formulation was known only to research staff members of Abbott Laboratories and was of a proprietary nature. The formulation was applied to the test plots at a rate of 5 g/m^2 ($5 \times 10^4 \text{ CFU/m}^2$).

Experimental Unit

7. A test site near Laplace, La., was selected for this study.



Figure 1. Photomicrograph of C. rodmanii conidia attached to the conidiophore ($\times 200$)

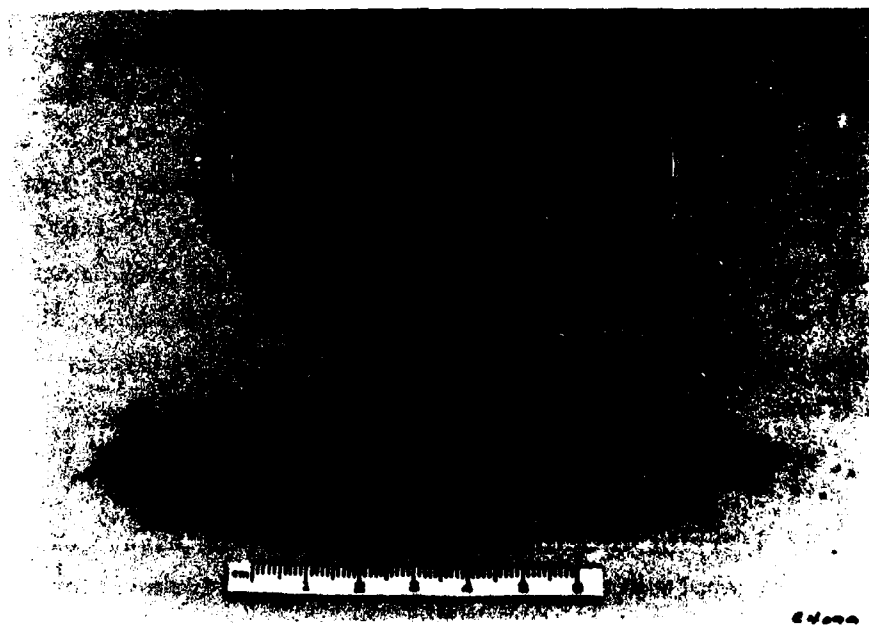


Figure 2. Abbott Laboratories' powder formulation of C. rodmanii

Twelve 28-m² plots were established in a canal paralleling Interstate 10 approximately 2 miles* north of the junction of Interstate 10 and Interstate 55 (Figure 3). Sections of 10-cm-diam polyvinyl chloride (PVC) pipe and nylon cord were tied to 1.2-m metal stakes embedded in the banks of the canal to cordon off the desired area of matted waterhyacinth. Plots were separated by a distance of 100 m.

Application

8. In each block of test plots, one plot was treated with the formulation using a John Beam Roadside R20 Pump (high-pressure piston pump system) (Figure 4) at 150 psi and a second was treated with the formulation using a low-pressure transfer pump (Figure 5) at 25 psi. The other two plots in each block were controls treated with tap water, using each equipment system to treat one plot. A John Beam Deluxe Spray Master adjustable gun was used with both systems to apply all treatments. The gun was adjusted to deliver droplet-sized particles; however, a more direct stream was needed in some plots to reach the plants near the opposite shore. The treatments were applied on 21 September 1979 (Table 1). A surfactant, Ortho x-77, was used in all treatments at a rate of 0.1 ml/m² (2.8 ml per test plot). The formulation was suspended in tap water containing surfactant and applied to each treated test plot at a rate of 0.68 l/m² for a total volume of 19 l/plot. Average output of the equipment was timed and enough material was mixed to apply three replicates of each treatment. Prior to application to the treatment plots, samples of the spray were collected for viability determinations.

Viability Determinations

9. One litre of formulation was obtained from each equipment system prior to application of the spray mix. The formulation was

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

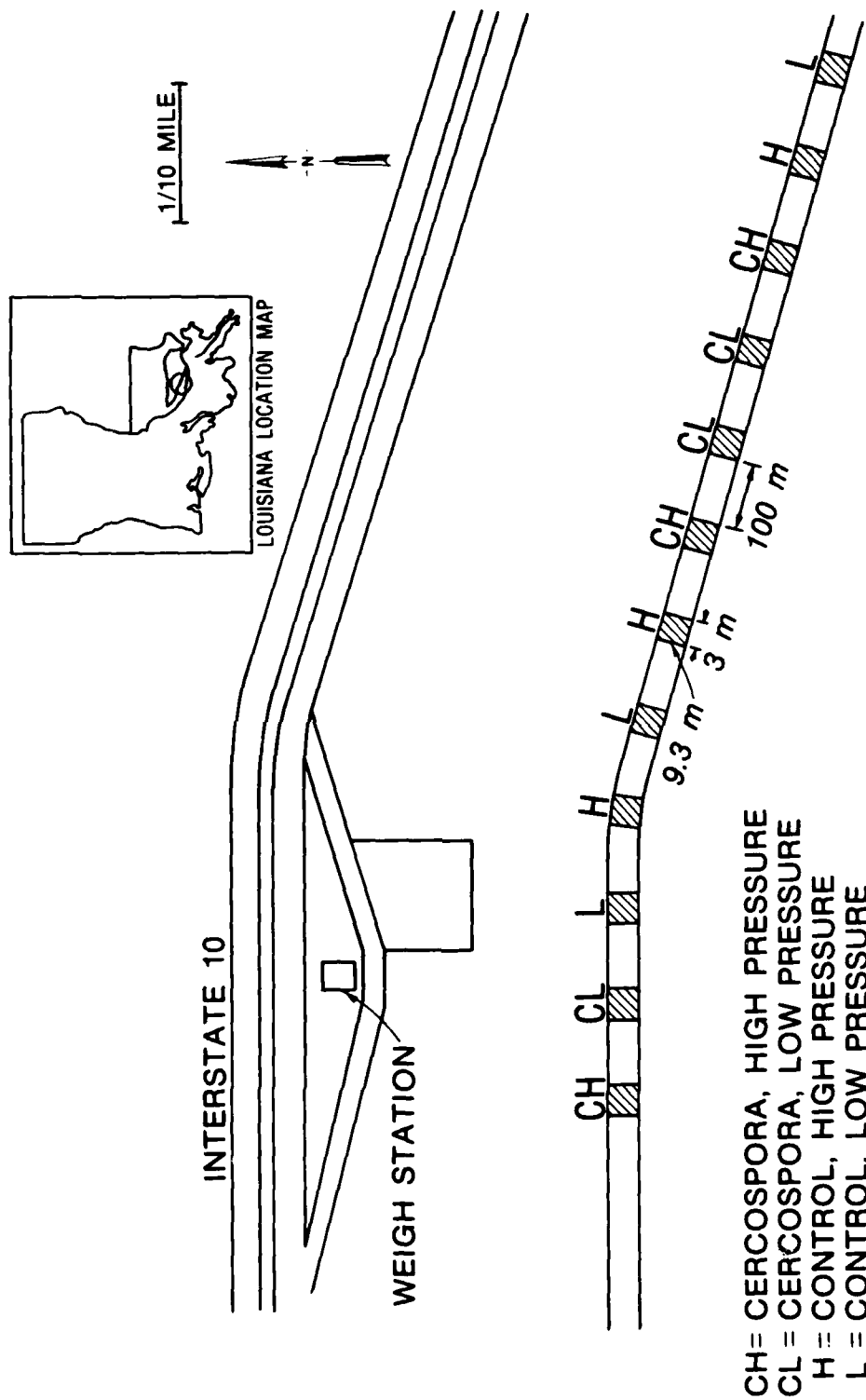


Figure 3. Test plot distribution for the equipment evaluation study



Figure 4. High-pressure piston pump (150 psi) used to apply treatments to test plots

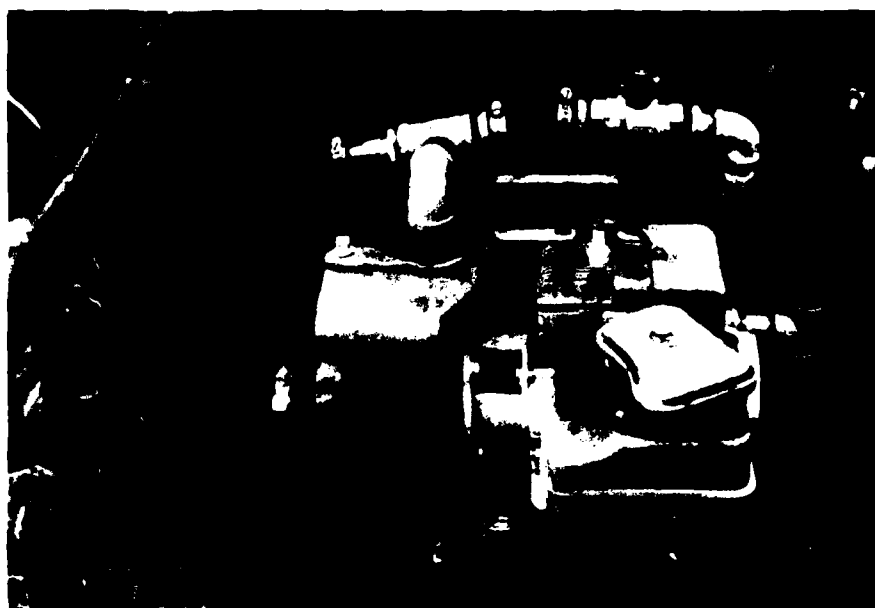


Figure 5. Low-pressure transfer pump (25 psi) used to apply treatments to test plots

suspended in tap water at a 10^{-3} weight by volume dilution rate for application. One millilitre of each sample was transferred to 9 ml of sterile deionized water, which then yielded a 10^{-4} dilution. Three 1-ml samples for each dilution rate were spread on potato dextrose agar (PDA) plates and incubated at room temperature (24° to 28°C) for 5 to 7 days. The number of colonies was then counted and averaged for each dilution rate. This procedure was replicated three times; thus, the average for each rate was derived from nine plate counts.

Data Collection

10. Disease index and pathogen isolation data were collected prior to treatment and at 13, 26, and 48 days after treatment (Table 2). Twelve plants were randomly selected and removed from each plot on each sampling date. Color photographs were taken of the test plots on each sampling date.

11. Disease index. The disease index was determined for each leaf and the values were averaged for each test plot. Damage per leaf caused by disease agents was based on a modification of a rating scale developed by Dr. Conway and associates at the University of Florida (Conway and Freeman 1976). Damage was rated on a scale of 1 to 10, in which 1 represented no apparent infection of the leaf and 10 indicated a dead submerged leaf blade and petiole. Values between 2 and 9 corresponded to increasing coverage of the leaf blade by the disease symptoms (Table 2). However, the values for disease damage per leaf reflected not only symptoms produced by *C. rodmanii*, but also disease symptoms produced by other opportunistic microorganisms.

12. Pathogen isolation. Plant tissues were selected from the plant samples to verify the presence of *C. rodmanii*. Sections of plant tissue (16 mm^2) were excised from regions bordering the disease symptoms. The tissues were surface treated with 0.5 percent sodium hypochlorite for 2 min and rinsed twice in containers of sterile, deionized water. The tissues were placed on PDA plates containing vancomycin and streptomycin, antibiotics used to prevent bacterial growth, and the

plates were incubated at room temperature (24° to 28°C) for 5 to 7 days. The disease agent was identified by colony morphology, type of fruiting structures, and characteristics of spore and hyphal morphology.

Data Analysis

13. The statistical model used to analyze the data was a one-way analysis of variance within sampling periods. The Statistical Analysis System (SAS), version 76.6D (Barr et al. 1976), was used to perform the statistical analyses. Duncan's Multiple Range Test was used to test for significant differences between treatment means (Steel and Torrie 1960).

PART III: RESULTS AND DISCUSSION

Experimental Formulation

14. Cercospora rodmanii was isolated at the 10^{-4} dilution rate from samples taken from both equipment types, but true counts could not be made due to severe contamination of the plates by microorganisms in the tap water used for application.

Pretreatment Plant Conditions

15. Plants in the test plots were large and vigorous. The average height was 87.6 cm and the mean Average Disease Index (ADI) value of all test plots was 2.63. Feeding scars produced by Neochetina spp. (waterhyacinth weevil) were present on the leaves, but the level of feeding activity was slight. No other insects that could significantly impact waterhyacinth were observed.

Disease Development

Thirteen days posttreatment

16. Thirteen days after treatment, the ADI values of the test plots treated with the C. rodmanii formulation were significantly higher ($p < 0.05$) than the controls (Table 3), and C. rodmanii was verified from tissue samples collected from plants in treated plots 13 days after application. These data indicate that the C. rodmanii formulation had infected the test plants. There were no significant differences in ADI values between the two equipment types (Table 4). The ADI values were greater for plots treated with the formulation with the low-pressure equipment than for plots treated with the formulation using the high-pressure system (Figure 6). Although not statistically significant, there may be a difference in the effect of the two equipment types on the viability of the formulation. Additionally, no mechanical damage

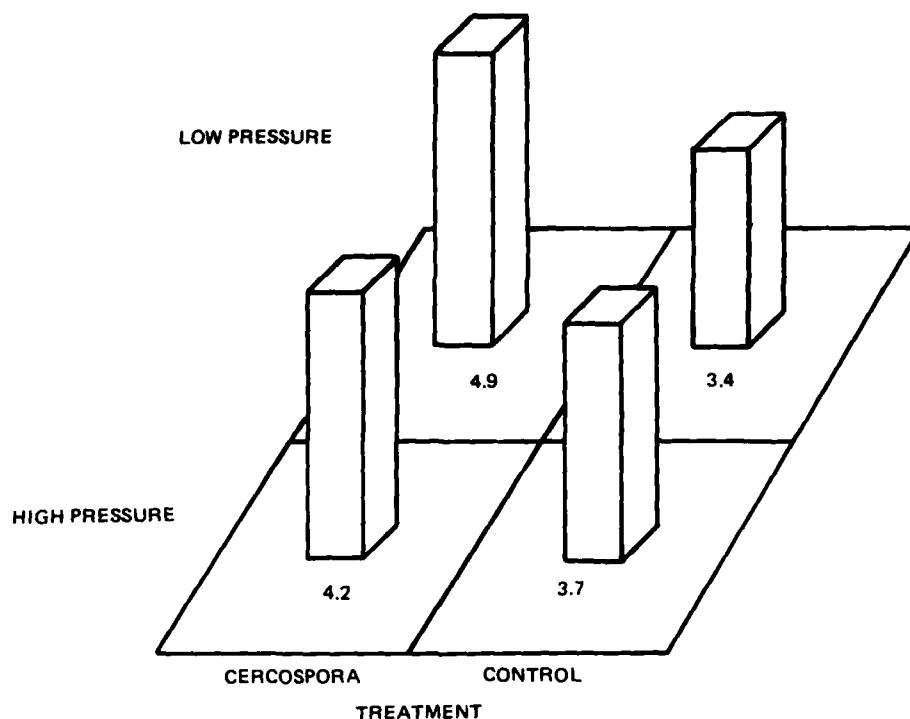


Figure 6. Comparison of Average Disease Indices of waterhyacinth 13 days after application

to test plants was observed with either spray system. Thus, there was no demonstrated difference in the stress produced by the two equipment types on the test plots.

17. While collecting data on the test plots 13 days after application, it was observed that severe petiole geniculation had occurred in the plants treated with the C. rodmanii formulation (Figure 7). Since this symptom had not been noted in previous applications with the formulation, water samples collected from the equipment on the day of application were analyzed for chemical residues from the spray equipment. The equipment, which was steam cleaned by personnel of the Louisiana Department of Wildlife and Fisheries prior to the application, had been used previously in the application of 2,4-D and chemicals containing an arsenate compound. Analyses of the samples revealed that a maximum of 0.11 mg/l of 2,4-D residue and 0.07 mg/l of



Figure 7. Test plot treated with C. rodmanii. Thirteen days after application, petiole geniculation was common to all plots treated with the formulation

arsenate residues were present. These low concentrations of residues could possibly have caused the geniculation of the petioles, except that controls were sprayed with the same equipment prior to application of the formulation and no geniculation was observed in control plots. It appeared that the stress of infection by C. rodmanii in combination with the residual amounts of herbicides produced the geniculation.

Twenty-six days posttreatment

18. Twenty-six days after treatment, there were no significant differences between the control plots (high and low pressure) and the plots treated with the formulation (high and low pressures) (Table 3).

Again, a difference was observed between the plots treated with the formulation with the low-pressure equipment as compared to the plots treated with the formulation with the high-pressure system (Figure 8). However, the difference was not statistically significant.

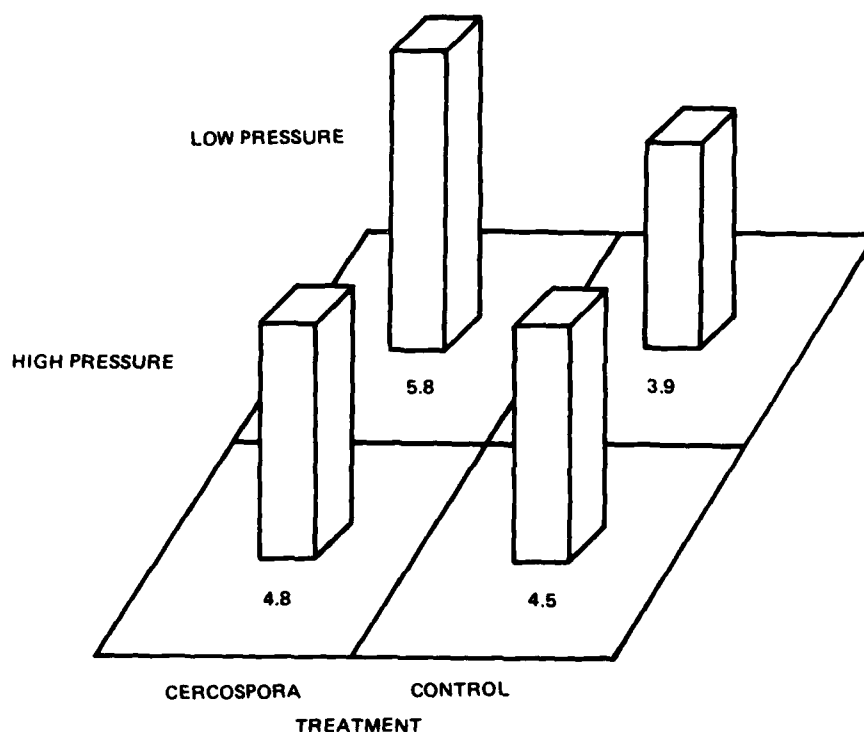


Figure 8. Comparison of Average Disease Indices of waterhyacinth 26 days after application

Forty-eight days posttreatment

19. Forty-eight days after treatment, mean ADI values for test plots treated with the formulation were higher than mean ADI values for control plots (Figure 9, Table 3). Although the ADI values were higher in the plots treated with the formulation using the low-pressure equipment than those treated with the high-pressure equipment, there was no statistically significant difference (Table 4).

Summary

20. Test plots treated with the formulation exhibited higher mean

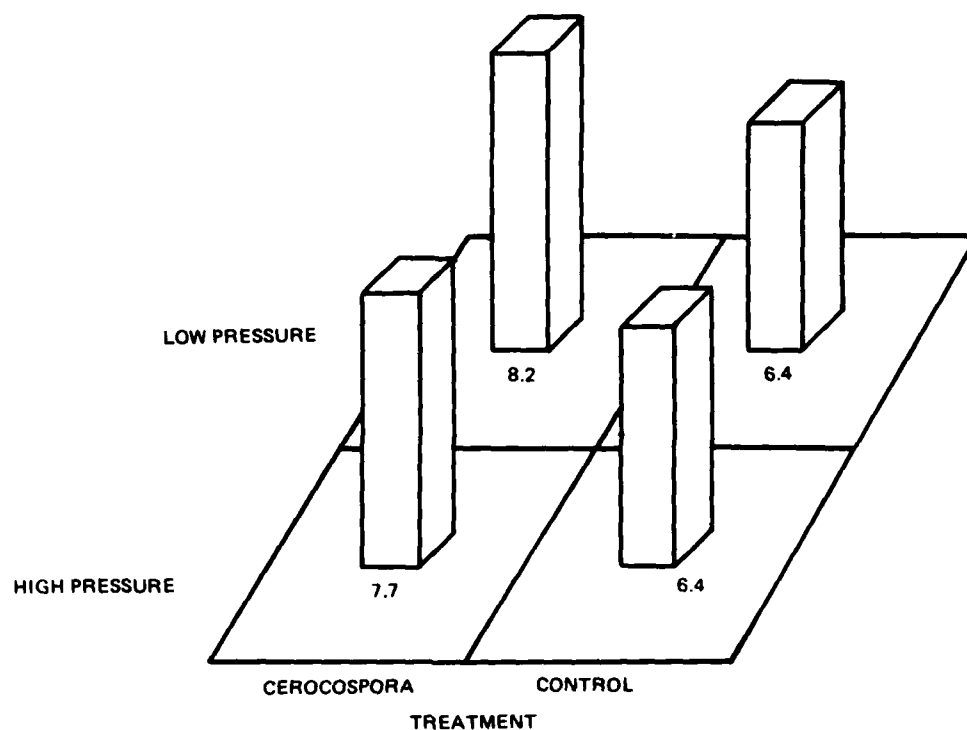


Figure 9. Comparison of Average Disease Indices of waterhyacinth 48 days after application

ADI values than the controls throughout the study period (Figure 10) and *C. rodmanii* was reisolated from plants in plots treated with the formulation; thus, it can be concluded that *C. rodmanii* in the formulation was infectious on waterhyacinth. Mean ADI values for test plots treated with the formulation using the low-pressure equipment were consistently higher than mean ADI values for test plots treated with the formulation using the high-pressure equipment. However, the differences were not statistically significant. Therefore, there is no significant difference in infectivity that can be achieved with a high-pressure piston pump system at 150 psi and a low-pressure impeller pump system of 25 psi. However, the consistently higher mean ADI values that occurred in plots treated with the low-pressure system suggest that the use of a low-pressure system is preferred to ensure the maximum initial infection possible.

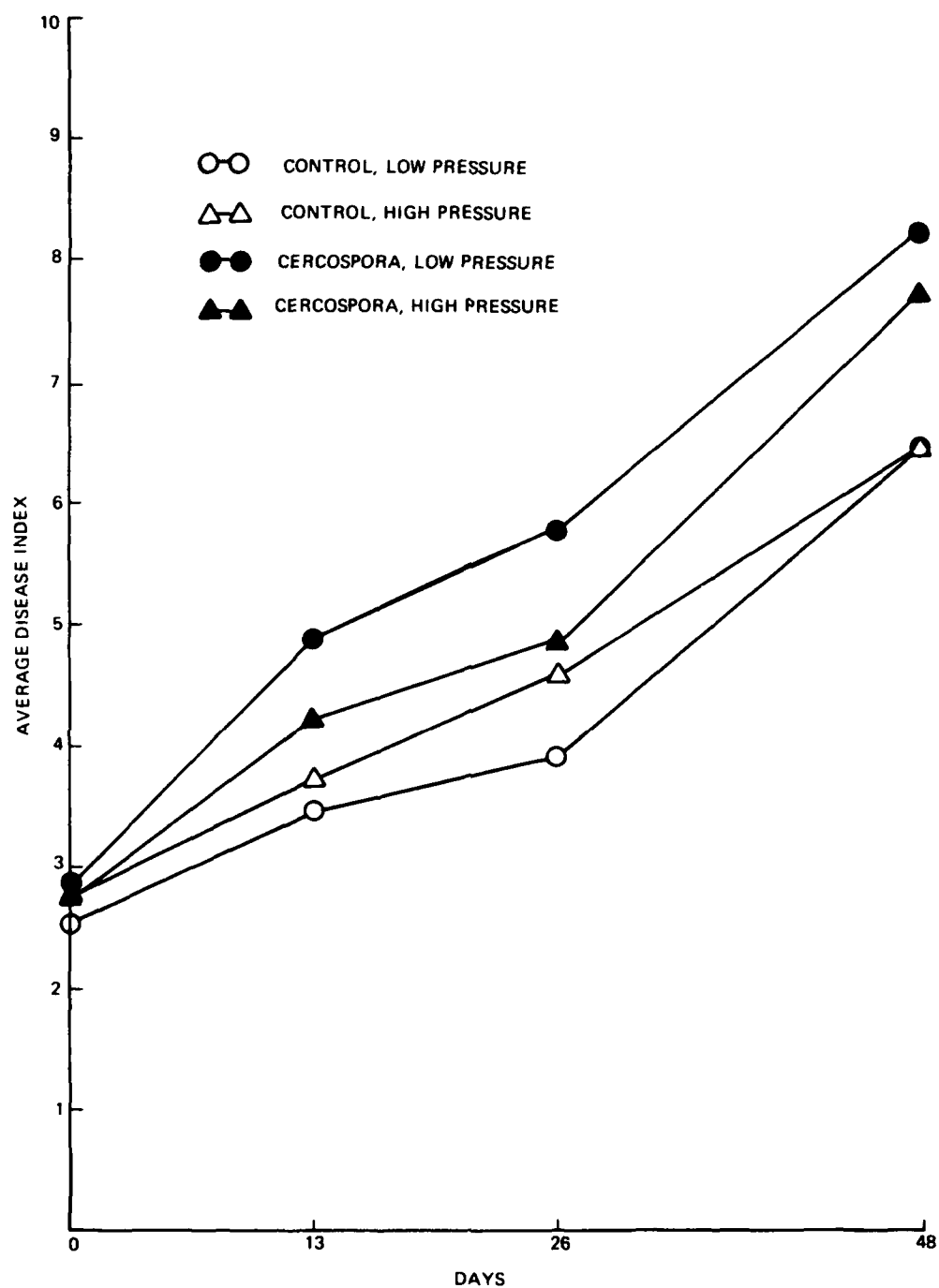


Figure 10. Mean Average Disease Index values among treatments for the equipment evaluation study

Subsequent Observations

21. Observations of the test plots were continued until mid-May 1980. In December 1980, a dense, frost-killed winter mat was present in all the test plots and in the areas between plots. By late March 1980, the winter mat had dropped out and no regrowth of seedling plants had occurred in any of the treated plots or between them (Figure 11). The old winter mat was present in the canal within 100 m of the east end of the test area, and regrowth of the waterhyacinth had occurred in that area. However, as late as mid-May, no waterhyacinth plants were present in the test area.



Figure 11. Test plot condition in March 1980. The entire test area was devoid of waterhyacinth

PART IV: CONCLUSIONS

22. The following conclusions were drawn from the study:
- a. Cercospora rodmanii contained in the formulation can infect field populations of waterhyacinth in Louisiana.
 - b. Cercospora rodmanii contained in the formulation can be applied with either a low-pressure impeller pump at 25 psi or a high-pressure piston pump system of 150 psi with no significant difference in infectivity.

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Table 1
Timetable for the Equipment Evaluation Study

Event	Date
Establishment of test plots	17-18 September 1979
Pretreatment data collection	18-19 September 1979
Application of treatments	21 September 1979
Posttreatment data collection	
13 days	4 October 1979
26 days	17 October 1979
48 days	8 November 1979
Poststudy observations	
74 days	4 December 1979
179 days	18 March 1980
230 days	9 May 1980

Table 2

Disease Index Used to Determine Damage to Waterhyacinth Caused by Pathogens

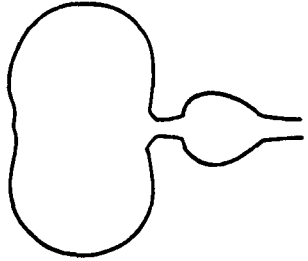
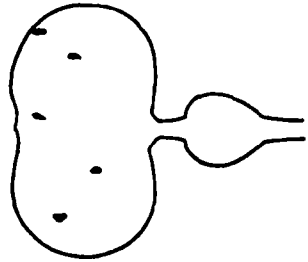
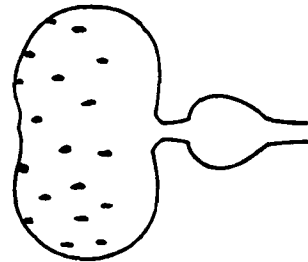
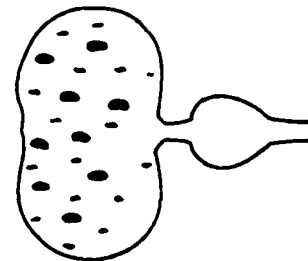
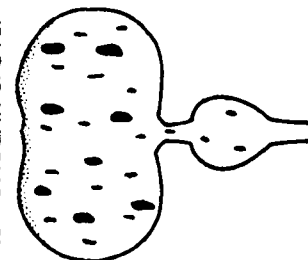
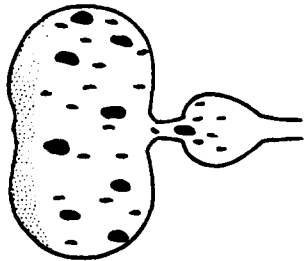
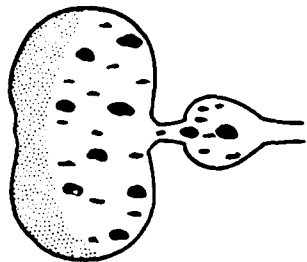
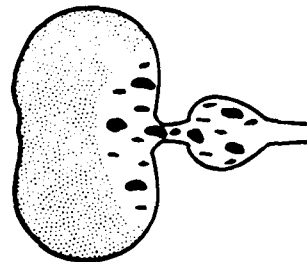
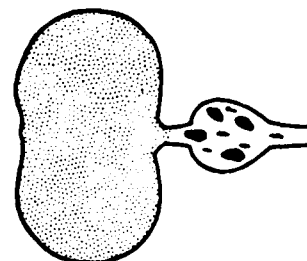
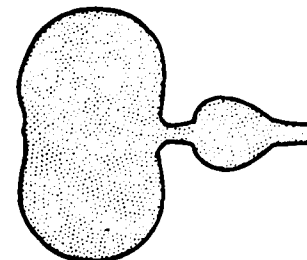
NUMERICAL RATING SYMPTOMS	1	2	3	4	5
	NO SPOTS ON LEAF OR PETIOLE.	1-4 SPOTS ON LEAF, NO PETIOLAR SPOTTING.	LESS THAN 25% OF LEAF SURFACE WITH SPOTS, NO COALESCENCE OR PETIOLAR SPOTTING.	LESS THAN 50% OF LEAF SURFACE WITH SPOTS, SOME COALESCENCE, NO PETIOLAR SPOTTING.	LESS THAN 25% OF LEAF SURFACE WITH SPOTS, COALESCENCE, SOME TIP-DIEBACK AND PETIOLAR SPOTS.
					
	6	7	8	9	10
	LESS THAN 50% OF LEAF SURFACE WITH SPOTS, COA- LESCENCE, 10% TIP-DIEBACK, PETIOLE SPOTTING.	LESS THAN 75% SPOTS, COALESCENCE, (30%) TIP-DIEBACK, IN- CREASING PETIOLAR SPOTTING.	GREATER THAN 75% SPOTS, COALESCENCE, (60%) TIP-DIEBACK, COALESCING SPOTS ON PETIOLE.	DEAD LEAF BLADE, PETIOLE GREEN, BUT HEAVILY SPOTTED.	DEAD LEAF BLADE AND PETIOLE (SUBMERGED).
					

Table 3
Comparison of Average Disease Indices on Waterhyacinth in Control
Plots with Plots Treated with *Cercospora rodmannii*

Treatment	Time, days			
	0	13	26	48
0 (controls)	2.6 a*	3.6 a	4.2 a	6.4 a
5×10^4 CFU/m ²	2.8 a	4.5 b	5.3 a	8.0 b

* Data within a column followed by the same letter are not significantly different ($p < 0.05$) using Duncan's Multiple Range Test.

Table 4
Average Disease Indices for Waterhyacinth After Treatment
Using Two Equipment Types

Treatment	Time, days			
	0	13	26	48
Low-pressure equipment	2.7 a*	4.2 a	4.8 a	7.3 a
High-pressure equipment	2.7 a	4.0 a	4.7 a	7.1 a

* Data within a column followed by the same letter are not significantly different ($p < 0.05$) using Duncan's Multiple Range Test.

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.

Theriot, Edwin A.

Evaluation of the infectivity of a *Cercospora Rodmanii* formulation using two application systems : final report / by Edwin A. Theriot, Russell F. Theriot, Dana R. Sanders, Sr. (Environmental Laboratory, U.S. Army Engineer Waterways Experiment Station). -- Vicksburg, Miss. : The Station ; Springfield, Va. ; available from NTIS, [1981].

23, [3] p. : ill ; 27 cm. -- (Miscellaneous paper / U.S. Army Engineer Waterways Experiment Station ; A-81-2).

Cover title.

"June 1981."

"Prepared for U.S. Army Engineer District, New Orleans and Office, Chief of Engineers, U.S. Army."

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1. Aquatic weeds. 2. Biological control of weeds.
3. Fungi, Pathogenic. 4. Insects. 5. Water-hyacinth.

Theriot, Edwin A.

Evaluation of the infectivity of a *Cercospora* : ... 1981.
(Card 2)

I. Theriot, Russell F. II. Sanders, Dana R., Sr.
III. United States. Army. Corps of Engineers. New Orleans District. IV. United States. Army. Corps of Engineers. Office of the Chief of Engineers. V. U.S. Army Engineer Waterways Experiment Station. Environmental Laboratory. VI. Title VII. Series: Miscellaneous paper (U.S. Army Engineer Waterways Experiment Station) ; A-81-2.
TA7.W34m no.A-81-2

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