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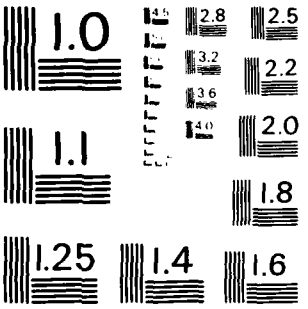

US Army Corps of Engineers

ENVIRONMENTAL LABORATORY

AQUATIC PLANT CONTROL RESEARCH PROGRAM

MISCELLANEOUS PAPER A-83-6

COMPATIBILITY AND INFECTIVITY OF A *CERCOSPORA RODMANII* FORMULATION WITH ENHANCING AGENTS

by

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June 1983
Final Report

Prepared for Office, Chief of Engineers, U. S. Army
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REPORT DOCUMENTATION PAGE	READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER Miscellaneous Paper A-83-6	2. GOVT ACCESSION NO. AD-A134603
3. RECIPIENT'S CATALOG NUMBER	
4. TITLE (and Subtitle) COMPATIBILITY AND INFECTIVITY OF A CERCOSPORA RODMANII FORMULATION WITH ENHANCING AGENTS	5. TYPE OF REPORT & PERIOD COVERED Final report
	6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) Judith C. Pennington Edwin A. Theriot	8. CONTRACT OR GRANT NUMBER(s)
9. PERFORMING ORGANIZATION NAME AND ADDRESS U. S. Army Engineer Waterways Experiment Station Environmental Laboratory P. O. Box 631, Vicksburg, Miss. 39180	10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS Aquatic Plant Control Research Program
11. CONTROLLING OFFICE NAME AND ADDRESS Office, Chief of Engineers, U. S. Army Washington, D. C. 20314	12. REPORT DATE June 1983
	13. NUMBER OF PAGES 23
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

Available from National Technical Information Service, 5285 Port Royal Road, Springfield, Va. 22161.

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

Additives
Aquatic plant control
Biological control
Pathogenic fungi
Waterhacinth

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

A formulation of the fungus Cercospora rodmanii Conway has been produced by Abbott Laboratories, Chicago, Ill., as a biocontrol of waterhyacinth (Eichhornia crassipes (Mart.) Solms.). To ensure the most efficient germination of the formulation, 12 potential enhancing agents were tested for addition during the spray application. The agents were aspartic acid, glucose, glutamic acid, gum xanthan, nutrient agar, Ortho X-77 Spreader, Tween 20, Tween 60, Tween 80, sodium alginate, Super Slupper, and yeast extract.
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20. ABSTRACT (Continued).

Compatibility of test agents and combinations of test agents with two lots of the formulation was determined in the laboratory. All agents tested, except Ortho X-77 Spreader, were compatible with the first lot of formulation and four agents--Super Slupper, yeast extract, gum xanthan, and aspartic acid--resulted in significantly greater colony counts than controls. All agents, including Ortho X-77 Spreader, were compatible with the second lot and one agent, sodium alginate, resulted in significantly greater colony counts than controls. All combinations of test agents were compatible with both lots of the *C. rodmanii* formulation.

The *C. rodmanii* formulation was sprayed with test agents on waterhyacinth pseudolaminae. Damage was monitored each week for 8 weeks by assigning a disease index to each original and new pseudolaminae.

No spots having characteristics suggestive of *C. rodmanii* infection were observed at any time during the study. Microscopic and cultural examinations of pseudolaminae at the end of the study yielded no evidence of infection by the *C. rodmanii* formulation. Analysis of variance of mean disease indices showed no significant differences between treatments and controls. All results indicated that the *C. rodmanii* formulation was not infective. Therefore, the effects of test agents on infectivity could not be determined.

Lack of infectivity could be remedied by isolating a virulent strain of *C. rodmanii* from the field. Agents determined to be compatible in this study could then be reexamined for enhancing infectivity on a virulent *C. rodmanii* formulation.

PREFACE

This report describes laboratory studies to determine the feasibility of using selected humectants and nutrients to enhance the infectivity of a formulation of *Cercospora rodmanii* Conway on waterhyacinth. Funding for this study was provided by the Office, Chief of Engineers, under appropriation number 96X3122, Construction General, to the U. S. Army Engineer Waterways Experiment Station (WES), Vicksburg, Miss., Aquatic Plant Control Research Program (APCRP).

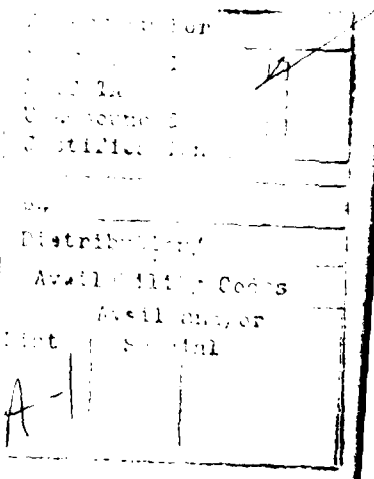
The study was conducted by Mrs. Judith C. Pennington and Mr. Edwin A. Theriot of the Wetland and Terrestrial Habitat Group (WTHG), Environmental Resources Division (ERD), Environmental Laboratory (EL), WES. Abbott Laboratories, Inc., Chicago, Ill., provided the *C. rodmanii* formulation.

The study was conducted under the direct supervision of Dr. Hanley K. Smith, WTHG, and under the general supervision of Dr. Conrad J. Kirby, Jr., Chief, ERD, and Dr. John Harrison, Chief, EL. Manager of the APCRP was Mr. J. Lewis Decell.

Commander and Director of WES during the preparation of this report was COL Tilford C. Creel, CE. Technical Director was Mr. F. R. Brown.

This report should be cited as follows:

Pennington, J. C., and Theriot, E. A. 1983. "Compatibility and Infectivity of a *Cercospora rodmanii* Formulation with Enhancing Agents," Miscellaneous Paper A-83-6, U. S. Army Engineer Waterways Experiment Station, CE, Vicksburg, Miss.



CONTENTS

	Page
PREFACE	1
PART I: INTRODUCTION	3
Background	3
Rationale	3
Purpose and Objectives	4
PART II: MATERIALS AND METHODS	5
The Formulation	5
Test Agents	5
Laboratory Procedures	8
Bioassay Procedures	9
Data Analysis	12
PART III: RESULTS AND DISCUSSION	13
Laboratory Study	13
Bioassay	14
PART IV: CONCLUSIONS AND RECOMMENDATIONS	17
Conclusions	17
Recommendations	17
REFERENCES	18
TABLES 1-5	

COMPATIBILITY AND INFECTIVITY OF A *CERCOSPORA RODMANII* FORMULATION WITH ENHANCING AGENTS

PART I: INTRODUCTION

Background

1. In 1973 *Cercospora rodmanii* Conway was isolated from declining waterhyacinth (*Eichhornia crassipes* (Mart.) Solms.) in Rodman Reservoir, Florida, by Dr. K. E. Conway (Conway, Freeman, and Charudattan 1974). Subsequently, pathogenicity and host-specificity were established at the University of Florida (Conway and Freeman 1977). The University was granted a patent for the use of the fungus as a biocontrol agent of waterhyacinth, and Abbott Laboratories, Chicago, Ill., was given the rights to develop a marketable formulation of the fungus. The *C. rodmanii* formulation subsequently produced is the subject of this study funded by the Aquatic Plant Control Research Program (APCRP) at the U. S. Army Engineer Waterways Experiment Station (WES), Vicksburg, Miss.

Rationale

2. The *C. rodmanii* formulation is a wettable powder consisting of viable mycelial propagules in a white matrix. Applying the formulation with a compatible humectant* to maintain a high level of moisture, with nutrients to sustain the propagules or stimulate their germination and/or with agents capable of stabilizing the spray suspension to ensure more uniform application, will maximize the chances for survival and infection, thereby ensuring the most efficient use of the formulation.

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* The term "humectant" is used in this report to mean any agent capable of maintaining moisture in droplets indefinitely.

Purpose and Objectives

3. The purpose of this study was to enhance the infectivity of the *C. rodmanii* formulation on waterhyacinth. Specific objectives were as follows:

a. Verify compatibility of potential enhancing agents with the *C. rodmanii* formulation.

b. Evaluate the ability of compatible agents to increase the infectivity of the *C. rodmanii* formulation on waterhyacinth.

PART II: MATERIALS AND METHODS

The Formulation

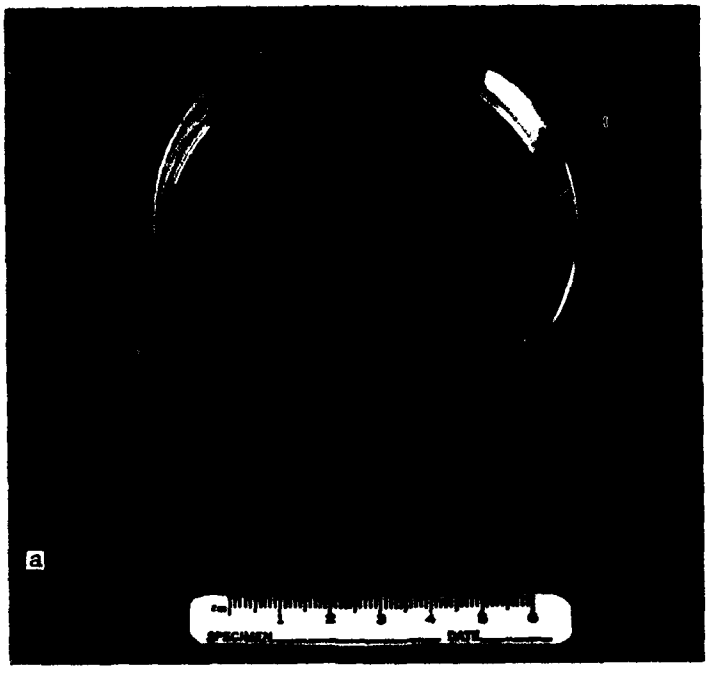
4. The exact nature and method of production of the *C. rodmanii* formulation are proprietary information. Two different formulations have been tested in the WES laboratory. The first was a brown wettable powder containing mycelial fragments (Figure 1a). This formulation consisted of particles varying greatly in size and weight, which made it difficult to suspend in water. Because of its high moisture content, the brown formulation required refrigeration to prevent growth of contaminants. To solve these problems and to extend shelf life, a second formulation was produced.

5. The second formulation, the subject of this study, consisted of fluffy white mycelial fragments (Figure 1b). This formulation was much more uniform in particle size, and the smaller, lighter weight propagules were more easily suspended. Uniform distribution of viable propagules throughout the matrix was confirmed by laboratory tests that showed reduced variability among colony counts in tests of viability. Moisture content and bacterial and fungal contaminants were greatly reduced and laboratory tests indicated that shelf life was extended.

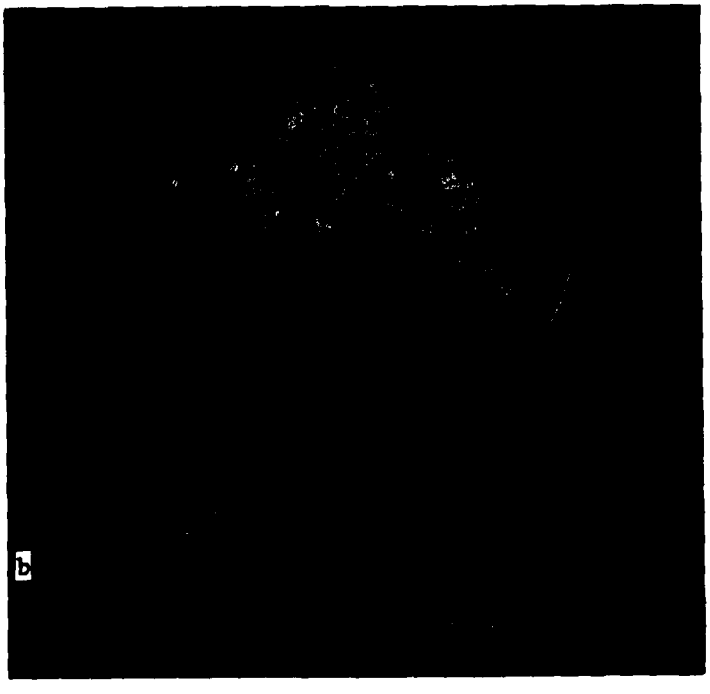
Test Agents

6. This study was limited to a few selected nutrients, humectants, and suspending agents. Concentrations of test agents were determined by considering nutrient levels potentially stimulatory to the *C. rodmanii* formulation, ability of agents to maintain high moisture levels, or physical constraints of spray application. The following is a list of test agents accompanied by a brief description of their relevant characteristics:

a. Yeast extract. Yeast extract (YE) is a commonly used stimulant of growth in nutrient preparations for microorganisms. Freeman et al. (1981) found that YE significantly enhanced growth of *C. rodmanii* on potato dextrose



a. Brown wettable powder tested in a previous study



b. Fluffy white powder tested in this study

Figure 1. Two formulations of *C. rodmanii* made by Abbott Laboratories

agar (PDA). This stimulatory effect is very likely due to the presence of B-complex vitamins. The 0.5-percent solution is suggested in the Difco Manual (Difco Laboratories, Inc. 1953).

b. Amino acids. All of the amino acids occurring in plants have been found in leachates from leaves (Blakeman 1973) and some of these have been found to stimulate germination of pathogenic fungi (Blakeman 1975). Glutamic and aspartic acids were selected and tested at 0.09 and 0.06 percent, respectively.

c. Glucose. Glucose is known to play an important role in the germination of pathogenic fungi (Dunn et al. 1971). Many incidences of stimulation of germ tube production and elongation by glucose and other simple sugars have been documented in the literature (Wildman and Parkinson 1981; Brodie and Blakeman 1976; Blakeman 1973). A 1.0-percent concentration was selected for testing.

d. Nutrient agar. Nutrient agar was considered a promising candidate for enhancement of the *C. rodmanii* formulation because the agar is a widely used growth medium for microorganisms in the laboratory and its viscosity could be easily manipulated to a suitable consistency for the suspension and application of the formulation. A concentration of 0.05 percent produced adequate viscosity.

e. Tween. Tween is a viscous biological surfactant having wide use as a wetting and dispersing agent. This agent was considered potentially enhancing to the formulation because of its ability to reduce the surface tension among formulation particles and between infection droplets and the pseudolaminae of waterhyacinth. Tween 20, Tween 60, and Tween 80 differ from each other in their basic fatty acid esters, and their viscosities increase as their numbers increase. These agents may differ in the degree of surfactant action that they exert on the formulation. The test concentration for each was 0.01 percent.

f. Sodium alginate. Sodium alginate is the salt of a gelatinous polysaccharide extracted from kelp (Merck and Company 1976). Sodium alginate has been used as a carrier for herbicides in swift streams because its extreme viscosity and low solubility prevent rapid dissipation of the herbicide.* A concentration of 0.05 percent was selected for ease of spray application.

g. Gum xanthan. Gum xanthan is a polysaccharide produced by the bacterium *Xanthomonas campestris*. When mixed with

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* Personal Communication, Allen Woolridge, April 1981, Chevron, Orlando, Fla.

water in low concentrations, the gum forms a highly viscous solution that can be used as a "sticker" for the application of insect eggs in biological control of noxious terrestrial plants (Baer and Quimby 1983). Once the aqueous phase has evaporated, a strong, heat-resistant, pseudoplastic film develops that cements the eggs to the plants (Merck and Company 1976). The 0.05-percent concentration was selected as most efficient for suspension of the formulation.

h. Ortho X-77 Spreader. This agent is a spray additive commonly used with herbicides for control of emergent aquatic plants. At 0.031 percent (V/V), Ortho X-77 Spreader was the most compatible spray additive tested with the brown *C. rodmanii* formulation in a previous study (Pennington and Theriot 1982). Results of that study suggested that Ortho X-77 Spreader enhanced the formulation. The test concentration was 0.031 percent.

i. Super Slupper. Super Slupper is a starch polymer capable of absorbing 300 to over 1500 times its weight in water (Weaver et al. 1977). It has many and varied uses, one of which is to coat seeds prior to planting. When coated seeds are wetted, the polymer expands, forming thick, moist coverings that ensure protection and early germination of the seeds. The gelatinous matrix would afford similar advantages to propagules of the *C. rodmanii* formulation. The 0.075-percent concentration selected is sufficiently dilute to facilitate spray application.

Laboratory Procedures

7. A 0.100-g sample of the *C. rodmanii* formulation containing 6.1×10^5 colony forming units (CFU) per gram was added to 100 ml of sterile distilled water with stirring. Petri dishes of potato dextrose agar with lactic acid (PDA-LA) added to inhibit contaminating bacteria were inoculated with 0.9 ml of the suspended formulation. Test dishes received 0.1 ml of each test agent (Table 1) at ten times the required concentration to bring the final concentration in the dishes to the desired level. A weighed amount of test agent was used if a tenfold concentration was too viscous to pipette. Control dishes received 0.1 ml of sterile distilled water. Three controls were used--one was inoculated at the beginning, one near the middle, and one at the end of the pipetting procedure--to account for variability in counts between

treatments. The entire procedure was replicated five times. All dishes were incubated at 25° to 27°C (Freeman et al. 1981) for 5 days, after which colonies of *C. rodmanii* were counted on each plate using a Quebec Colony Counter.

8. The same procedure was used for testing 12 combinations of agents. Three humectants (Super Slupper, gum xanthan, and sodium alginate), three nutrients (yeast extract, aspartic acid, and glutamic acid), and one combination of nutrients (aspartic acid plus glutamic acid) were selected on the basis of results of individual agent tests. Each humectant was paired in turn with each nutrient for testing. All combinations of agents and the concentrations used are presented in Table 2.

9. Immediately prior to initiation of the bioassay, a new lot of *C. rodmanii* formulation was received from Abbott Laboratories. The new lot possessed greater viability than the lot already tested. The test of individual agents was repeated to eliminate any possible variations inherent in a different lot (Table 1). In addition, modifications in the concentrations of test agents were made because it was discovered that the spray apparatus would easily dispense a more viscous solution. The greatest possible viscosity was desirable to maintain maximum uniformity and stability in the suspension of the formulation. The test with combinations of agents was also repeated using the second lot of formulation and the higher concentrations of test agents (Table 2). Selection of agents for this combinations test was based on results of both individual agent tests.

Bioassay Procedures

10. Eighty-four healthy waterhyacinth plants having bulbous petioles were selected from greenhouse stock. Two plants were placed in each of 42, 8.5-ℓ plastic pails. Older, damaged pseudolaminae, daughter plants, and flowers were removed leaving a minimum of four healthy leaves per plant. The newest unfurling pseudolamina was tagged with a loose plastic ring in order to distinguish untreated original from treated pseudolaminae.

11. A nutrient solution for waterhyacinth was made by mixing 9 g of 23-19-17 fertilizer in 750 ℓ of tap water. This provided sufficient quantity to fill each pail with 6 ℓ and to supplement pails whenever necessary throughout the study.

12. The tiny aquatic macrophytes duckweed (*Lemna* spp.) and watermeal (*Wolffia columbiana*) were added to each pail. Experience with waterhyacinth in small containers has shown that these floating plants greatly limit algal growth and formation of slime on the water surface. Equal numbers of these macrophytes were added to controls and treatments.

13. The waterhyacinth plants in pails were placed in a small building adapted for control of temperature, humidity, and light. Temperature was adjusted to 27°C using the thermostat system present in the building. Relative humidity was maintained over a range of 60 to 90 percent with two small room humidifiers programmed to mist for 10 min at 1-hr intervals. Both temperature and relative humidity were continuously monitored. Twelve hours of light alternating with twelve hours of darkness were maintained and all external light sources to the building were sealed with opaque material. Sixteen fluorescent lights were supplemented by six 122-cm plant growth lights, which produced an average intensity of 21.5 $\mu E/m^2/sec$.

14. Plants were acclimated for 1 week before the study was initiated.

15. Five test agents for bioassay (asparic acid, gum xanthan, Super Slupper, sodium alginate, and yeast extract) were selected from results of the four laboratory tests. Each agent was diluted to its test concentration with 500 ml of sterile distilled water containing 4×10^6 CFU of the *C. rodmanii* formulation. The suspension was poured into a 7.6-ℓ garden mist sprayer for inoculation of plants. Treatments were assigned by dividing the pails into two groups. Within each group, treatments were randomized by using a table of random numbers. For each treatment, six pails of waterhyacinth were placed in a 1-m-square area outside the building and misted until all inoculum had been dispensed. Six treated controls were sprayed with the *C. rodmanii* formulation in

water, and six untreated controls were sprayed with water only. The pails were then replaced in numbered positions inside the building.

16. To determine damage to the plants, a modification of the disease index developed by Conway for rating pseudolamina damage on waterhyacinth was used (Freeman et al. 1981). The index is based on the number of spots on the pseudolaminae, coalesced spots on the pseudolaminae and petioles, and percentage of pseudolamella tip dieback. For this study, Conway's scale was condensed from 0 through 9 to 0 through 5 (Figure 2). This index does not reflect damage caused only by the *C. rodmanii* formulation, but is a broad evaluation of pseudolaminae condition that includes damage by many factors.

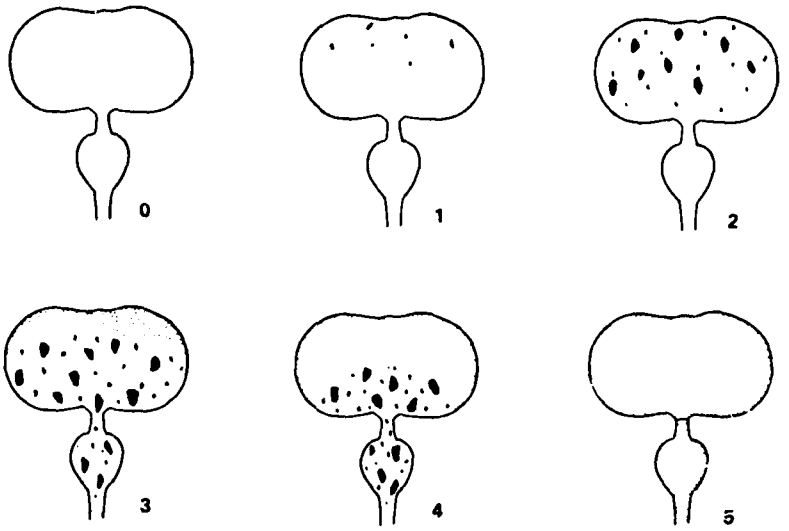

Figure 2. Disease index. 0 = no apparent damage or disease; 1 = presence of noncoalescent spots; 2 = presence of coalescent spots; 3 = less than 50 percent pseudolamina tip dieback; 4 = more than 50 percent but less than total pseudolamina tip dieback; 5 = dead pseudolamina

17. The disease index was assigned to each original and new pseudolaminae on the plants prior to treatment and then at weekly intervals after treatment. Data were collected for an 8-week period after which any spots suggestive of *C. rodmanii* infection were examined microscopically and cultured.

Data Analysis

18. For the laboratory study, colony counts for each test agent and for controls were subjected to an analysis of variance (ANOVA). Duncan's New Multiple Range Test was used to separate mean values.

19. For the bioassay, a three-level, nested ANOVA was used to compare original pseudolamina damage and new pseudolamina damage to controls. The three levels of evaluation were groups, test agents, and time (i.e. sampling dates). Duncan's New Multiple Range Test was used when significant differences occurred.

PART III: RESULTS AND DISCUSSION

Laboratory Study

Individual agents

20. Four agents tested with the first lot resulted in mean colony counts significantly greater than controls (Table 3). These four agents were 0.075 percent Super Slupper, 0.5 percent yeast extract, 0.05 percent gum xanthan, and 0.06 percent aspartic acid. It is possible that the nutrients, yeast extract and aspartic acid, stimulated the germination of small and/or weak propagules of the formulation accounting for an increase in colony counts over controls. The enhancement effects of Super Slupper and gum xanthan could be due to (a) increased moisture availability to propagules and/or (b) surfactant action of the test agents on clumps of viable propagules, which, when separated, produced more colonies than controls. One test agent, Ortho X-77 Spreader, exhibited a mean colony count significantly lower than controls. This result was unexpected because previous studies of compatibility of the brown *C. rodmanii* formulation with this agent had shown significant enhancement (Pennington and Theriot 1982). This suggested that the newer formulation differed in important physical, or possible chemical, characteristics from the earlier brown formulation. It also indicated that Ortho X-77 Spreader was incompatible with this lot of the new formulation. Remaining agents tested exhibited no significant differences from controls.

21. No trends were observed in colony counts of controls inoculated at the beginning, near the middle, and at the end of the pipetting procedure. Therefore, it was assumed that the Petri dishes received uniform amounts of formulation.

22. Results of the second laboratory test of individual agents with the new lot of the *C. rodmanii* formulation and with the modifications in concentrations of test agents discussed earlier (paragraph 9), indicated that one test agent, sodium alginate, produced significantly higher colony counts than the controls (Table 3). This result

represented a contrast to results with the first lot in which sodium alginate was not significantly different from controls. No other agents produced colony counts significantly different from controls. This indicated that all of the agents, including Ortho X-77 Spreader which was inhibitory to the first lot, were compatible with the new lot of formulation.

23. Examination of differences between results obtained with the two lots of formulation suggested that raising the concentrations of Super Slupper and gum xanthan may have decreased their effectiveness in enhancing the formulation, and that raising the concentration of sodium alginate may have increased its effectiveness. Other differences in results involving agents whose concentrations were not changed (e.g. aspartic acid, yeast extract, and Ortho X-77 Spreader) suggested that variation between lots of *C. rodmanii* formulation affected their performance.

Combinations of agents

24. Results from ANOVA and Duncan's New Multiple Range Test of treatments and controls with the first lot of formulation (Table 4) showed that none of the combinations of agents produced colony counts significantly different from controls. Results with the second lot of formulation also showed no significant differences from controls (Table 4). Therefore, the *C. rodmanii* formulation was compatible with all combinations of agents tested.

Bioassay

25. Any of the individual agents at any of the concentrations tested, with the possible exception of Ortho X-77 Spreader, could be used in the bioassay since all were compatible with the *C. rodmanii* formulation. However, space and time comstraints required that the number of test agents be limited. Therefore, only agents from both individual agents tests that exhibited significantly greater colony counts than the controls were selected (Table 5).

26. Previous outdoor studies indicated that the time from

unfurling until senescence for a waterhyacinth pseudolamina averaged 6 weeks (Theriot, Theriot, and Sanders 1981). Under the conditions of this study, the senescence rate was somewhat reduced, but, after 8 weeks, many of the original pseudolaminae had dropped out and most of those remaining were entering senescence. This natural process was reflected in disease indices when tip dieback began. Therefore, the high mean disease index for original pseudolaminae on untreated controls on the last sampling date of the study (4.3) was attributable to natural senescence rather than disease damage. The mean index for new pseudolaminae on untreated controls on the last sampling date (2.4) indicated that they were subject to normal stresses associated with their confinement. For example, the recirculating air system necessary for the maintenance of experimental conditions favored contaminating fungi and bacteria; therefore, indices were slightly higher than they might have been under natural field conditions. The use of untreated controls allowed compensation for these factors.

27. There was some concern that humidity, light, and temperature lacked uniformity throughout the study area, so results for the two groups of plants, one group on each end of the building, were compared using ANOVA. No significant difference was found.

28. Analysis of variance showed no significant differences between treated controls sprayed with *C. rodmanii* formulation and untreated controls sprayed with water, and no significant differences between treatments and controls. Table 5 shows that test mean disease indices through time were extremely consistent with control indices. These results indicated that the formulation did not produce significant levels of infection and, therefore, effects of various test agents on infectivity could not be determined.

29. Test data taken each sampling date were subjected to an ANOVA, but no significant differences between treatments and controls were found at any time during the study.

30. No spots suggestive of *C. rodmanii* infection developed. At the fifth week two pseudolaminae exhibited spots, but these were not typical of *C. rodmanii* infection and microscopic examination failed to

reveal spores. During the sixth and seventh weeks, additional spots were found on 13 plants and 10 plants, respectively, but still none were typical of *C. rodmanii* and no spores could be isolated. When the data sampling period was concluded, all spots found were examined. At that time there were spots on 12 original and 3 new pseudolaminae. No spores of *C. rodmanii* were seen upon microscopic examination and cultures yielded typical environmental contaminants, except for one PDA-LA dish that yielded a single mycelium having properties suggestive of *C. rodmanii*.

31. A bioassay for determining the enhancement of infectivity by combinations of agents could not be conducted since the *C. rodmanii* formulation did not infect waterhyacinth.

PART IV: CONCLUSIONS AND RECOMMENDATIONS

Conclusions

32. The conclusions of this study are as follows:

a. All of the individual agents at the tested concentrations except Ortho X-77 Spreader were demonstrated to be compatible with the *C. rodmanii* formulation with respect to propagule viability. Due to inconsistencies in the results obtained when Ortho X-77 Spreader was tested with the two different lots of fungal formulation, no determination of its compatibility with this formulation of *C. rodmanii* was possible.

b. All combinations of agents tested were compatible with the formulations at test concentrations.

c. Effects of compatible test agents on infectivity of the *C. rodmanii* formulation could not be determined because the formulation, although viable in laboratory cultures, did not infect waterhyacinth.

Recommendations

33. The Abbott Laboratories formulation of *C. rodmanii* has been greatly improved since first produced, at least with regard to its physical suitability for spray application and its increased shelf life as determined by laboratory cultures. Lack of virulence on waterhyacinth should not be a difficult problem to remedy. We recommend that a virulent strain of *C. rodmanii* be obtained from infected waterhyacinth in the field, processed by current methods, and tested for virulence on waterhyacinth. No other modification in procedure should be necessary. Once a virulent formulation is produced, test agents determined in this study to be compatible with the formulation can be examined for enhancing infectivity.

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Table 1

Concentrations of Test Agents (in percent)*

Test Agent	First Lot	Second Lot
Aspartic acid	0.06	0.06
Glucose	1.0	1.0
Glutamic acid	0.09	0.09
Gum xanthan	0.05	0.1
Nutrient agar	0.05	0.5
Ortho X-77 Spreader	0.031**	0.031**
Tween 20	0.01**	0.1**
Tween 60	0.01**	--
Tween 80	--	0.1**
Sodium alginate	0.05	0.5
Super Slupper	0.075	0.1
Yeast extract	0.5	0.5

* All concentrations based on weight to volume percentage except where indicated.

** Concentration based on volume to volume percentage.

Table 2

Combinations of Test Agents*

Humectants	Nutrients
	First Lot
0.075% Super Slupper	0.5% Yeast extract
0.05% Gum xanthan	0.06% Aspartic acid
0.05% Sodium alginate	0.09% Glutamic acid
	0.06% Aspartic acid + 0.09% glutamic acid
	Second Lot
0.1% Super Slupper	0.5% Yeast extract
0.1% Gum xanthan	0.06% Aspartic acid
0.5% Sodium alginate	0.9% Glutamic acid
0.031% Ortho X-77 Spreader	1.0% Glucose

* Each humectant on the left was tested with each nutrient on the right.

Table 3

Mean Colony Counts for Individual Agent Tests

Test Agent	Mean Colonies/Dish*
First Lot	
Super Slupper	410.0^{a}
Yeast extract	350.2^{ab}
Gum xanthan	322.0^{b}
Aspartic acid	316.0^{b}
Glutamic acid	278.0^{bc}
Sodium alginate	272.2^{bc}
Control	229.7^{c}
Tween 20	228.0^{c}
Glucose	222.4^{c}
Nutrient agar	198.2^{cd}
Tween 60	189.8^{cd}
Ortho X-77 Spreader	128.2^{d}
Second Lot	
Sodium alginate	57.4^{a}
Gum xanthan	43.6^{ab}
Glucose	42.4^{ab}
Aspartic acid	40.0^{ab}
Ortho X-77 Spreader	39.4^{ab}
Super Slupper	39.2^{ab}
Yeast extract	36.2^{b}
Glutamic acid	33.6^{b}
Control	30.6^{b}
Tween 20	24.2^{b}
Nutrient agar	23.2^{b}
Tween 80	22.2^{b}

* Means followed by the same superscript(s) within lots are not significantly different from each other ($P < 0.05$), based on Duncan's New Multiple Range Test.

Table 4

Mean Colony Counts for Combinations of Agents Tests

Test Agent	Mean Colonies/Dish*
First Lot	
Sodium alginate/glutamic acid	19.2[a]
Gum xanthan/aspartic and glutamic acids	19.0[a]
Gum xanthan/glutamic acid	18.6[a]
Gum xanthan/yeast extract	18.4[a]
Sodium alginate/yeast extract	16.4[a]
Super Slupper/yeast extract	16.2[a]
Gum xanthan/aspartic acid	15.4[a]
Sodium alginate/aspartic acid	15.2[a]
Super Slupper/aspartic acid	14.2[a]
Super Slupper/aspartic and glutamic acids	13.4[a]
Control	13.3[a]
Sodium alginate/aspartic and glutamic acids	13.0[a]
Super Slupper/glutamic acid	11.2[a]
Second Lot	
Sodium alginate/yeast extract	190.5[a]
Super Slupper/glutamic acid	186.8[a]
Gum xanthan/glucose	180.6[ab]
Gum xanthan/aspartic acid	174.8[abc]
Sodium alginate/glutamic acid	168.0[abc]
Sodium alginate/aspartic acid	162.0[abcd]
Control	161.9[abcd]
Gum xanthan/yeast extract	160.6[abcd]
Gum xanthan/glutamic acid	153.4[abcd]
Ortho X-77 Spreader/glutamic acid	151.4[abcd]
Ortho X-77 Spreader/aspartic acid	141.0[abcd]
Sodium alginate/glucose	139.8[abcd]
Super Slupper/glucose	139.0[bcd]
Ortho X-77 Spreader/glucose	137.2[bcd]
Super Slupper/yeast extract	134.0[cd]
Ortho X-77 Spreader/yeast extract	126.8[d]
Super Slupper/aspartic acid	114.6[d]

* Means followed by the same superscript(s) within lots are not significantly different from each other ($P < 0.05$), based on Duncan's New Multiple Range Test.

Table 5

Mean Disease Indices on Waterhyacinth in Bioassay Through Time

Test Agent	Concentration, %	Mean Disease Index	
		Original Pseudolaminae	New Pseudolaminae
Aspartic acid	0.06	3.3	2.1
Gum xanthan	0.05	3.3	1.9
Super Slupper	0.075	3.3	2.0
Sodium alginate	0.5	3.3	1.7
Yeast extract	0.5	3.3	2.1
Treated control	--	3.2	1.9
Untreated control	--	3.3	1.5