Unclassified

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

AD A095 985

REPORT DOCUMENTATION PAGE

READ INSTRUCTIONS BEFORE COMPLETING FORM

1. REPORT NUMBER: Technical Report A-81-1

2. GOVT ACCESSION NO.: AD-A095985

3. RECIPIENT'S CATALOG NUMBER:

4. TITLE (and Subtitle): BIOLOGICAL CONTROL OF AQUATIC PLANTS WITH PATHOGENIC FUNGI,

5. TYPE OF REPORT & PERIOD COVERED: Final report,

6. PERFORMING ORG. REPORT NUMBER:

7. AUTHOR(s): T. E. Freeman, R. Charudattan, K. E. Conway, R. E. Cullen, R. D. Martyn, D. E. McKinney, M. T. Olexa, and D. F. Reese

8. CONTRACT OR GRANT NUMBER(s): Contract No. DACW39-76-C-0097

9. PERFORMING ORGANIZATION NAME AND ADDRESS: Department of Plant Pathology, University of Florida, Gainesville, Fla. 32611

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: January 1981

13. NUMBER OF PAGES: 67

14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office): U. S. Army Engineer Waterways Experiment Station, Environmental Laboratory, P. O. Box 631, Vicksburg, Miss. 39180

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	Fungi
Aquatic plants	Pathogenic fungi
Biological control	Waterhyacinths

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

The endemic fungal plant pathogen Cercospora rodmanii was shown to have a high potential as a biocontrol agent for waterhyacinths (Eichhorniae crassipes). During this study, methods of culturing and dissemination of this fungus for biocontrol purposes were developed and the host-parasite relationship was elucidated. The fungus has been patented based on the results, and it is being developed for biological control purposes. Researchers at the U. S. Army Engineer Waterways Experiment Station will evaluate the fungus in a Large Scale

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20. ABSTRACT (Continued)

Operational Management Test in Louisiana. The endemic pathogen Acremonium zonatum and the exotic rust Uredeo eichhorniae, as well as C. rodmanii, exhibit biocontrol potential for waterhyacinth. An additional exotic pathogen, Fusarium roseum 'Culmorum', shows promise for biological control of the submerged aquatic weed Hydrilla verticillata. Research with the two exotic pathogens has been slowed because of the necessity of conducting studies in quarantine.

PREFACE

This report presents results of a study performed in part under Contract No. DACW39-76-C-0097 with the Department of Plant Pathology, University of Florida, Gainesville, Florida, for the Office, Chief of Engineers, U. S. Army, Washington, D. C. Funds were provided through the Aquatic Plant Control Research Program (APCRP), U. S. Army Engineer Waterways Experiment Station (WES), Vicksburg, Mississippi. Additional support was provided by the U. S. Department of Interior, Office of Water Resources Research and Technology; the Florida Department of Natural Resources; and the University of Florida, Institute of Food and Agricultural Sciences. Drs. T. E. Freeman, R. Charudattan, and K. E. Conway were the principal investigators for the project.

This report was prepared by T. E. Freeman, R. Charudattan, K. E. Conway, R. E. Cullen, R. D. Martyn, D. E. McKinney, M. T. Olexa, and D. F. Reese. Appendix A was written by D. F. Reese. Appendix B was written by R. D. Martyn. Appendix C was written by R. Charudattan. Appendix D was written by D. E. McKinney.

The authors wish to acknowledge the technical assistance provided by B. M. Reber, John Dennis, Susan Broos, Carole Hennan, Elizabeth Shepeck, Meredith Chester, Patty Hill, and a constant flow of student assistants. Special thanks are due to Clark Allen for the scanning electron micrographs of Cercospora rodmanii.

The work was monitored at the WES by Messrs. W. N. Rushing and D. R. Sanders, Sr., of the Aquatic Plant Research Branch (APRB), under the general supervision of Mr. W. G. Shockley, Chief of the Mobility and Environmental Systems Laboratory, and Mr. B. O. Benn, Chief of the Environmental Systems Division, and under the direct supervision of Mr. J. L. Decell, Chief of the APRB. As a result of reorganization at WES, Mr. Decell is now the manager of the APCRP, which is a part of the Environmental Laboratory of which Dr. John Harrison is Chief.

The Commanders and Directors of the WES during this study and the preparation and publication of this report were COL John L. Cannon, CE, and COL Nelson P. Conover, CE. Technical Director was Mr. F. R. Brown.

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BIOLOGICAL CONTROL OF AQUATIC PLANTS WITH PATHOGENIC FUNGI

PART I: INTRODUCTION

1. Plant pathogens have many characteristics that make them ideal candidates as biocontrols for aquatic weeds. They are numerous and diverse; are frequently host specific; are easily disseminated and self-perpetuating; will not completely eliminate a host species; and do not normally affect man or other animals. With these points in mind, a modest program was begun in 1970 at the University of Florida, Institute of Food and Agricultural Sciences (IFAS). Objectives were to evaluate and utilize plant pathogens as biocontrol agents for aquatic weeds. The program was expanded with the aid of a matching grant from the U. S. Department of Interior, Office of Water Resources Research, and subsequent support from the Florida Department of Natural Resources, the U. S. Army Corps of Engineers, and from the annual allotment program of the Florida Office of Water Resources Research.

2. The program progressed rapidly considering the lack of initial background information. A considerable backlog of information about diseases affecting aquatic plants has been developed. The objective of the utilization of plant pathogens in biocontrol programs for at least one noxious aquatic plant is nearing fruition. The point has been reached where large-scale, operational-type field evaluations of the fungus Cercospora rodmanii Conway for control of waterhyacinth are warranted. An additional three or four organisms also should soon reach this point. Efforts have also been made to find and research diseases with biocontrol potential for other aquatic weeds.

Relevance of Research

3. The aquatic weed problem is one of considerable proportion that is growing in magnitude rather than diminishing or even stabilizing. This is occurring despite the expenditure of considerable sums of money and human and fossil energy in the application of conventional methods

of mechanical and chemical controls.

4. The Florida Department of Natural Resources estimates that over 20 million dollars is expended annually in Florida for aquatic weed control. These control efforts are concentrated primarily on the estimated 100,000 hectares of waterhyacinth (Eichhornia crassipes (Mart.) Solms) and 40,000 hectares of hydrilla (Hydrilla verticillata Royal) that occur in the state. Lesser attention is given to the approximately 20,000 hectares of other aquatic weeds, such as Eurasian watermilfoil (Myriophyllum spicatum L.) and alligatorweed (Alternanthera philoxeroides (Mart.) Griseb.) (Burkhalter, personal communication). Despite these efforts, aquatic weed infestations have increased steadily in the years since these plants were introduced. The range of these plants has expanded to include virtually all water bodies of Florida. Within the last 5 years, Eurasian watermilfoil was found in the St. Johns River watershed and hydrilla has infested Rodman Reservoir on the Cross Florida Barge Canal, Okeechobee, Orange, and Lochlossa Lakes.

5. Florida is not unique in having a tremendous aquatic weed problem. Proliferating water weed populations are of concern in the rest of the United States, middle Europe, Africa, Asia, and South and Central America. The problem is worldwide, but is more acute in the warmer latitudes where waterhyacinth, hydrilla, alligatorweed, salvinia (Salvinia spp.), and waterlettuce (Pistia stratiotes L.) are the major offenders. Reasons for the increasing aquatic weed problem are complex but definitely related to man's activities. The increase in human population with its accompanying environmental problems made it apparent that new ways to control aquatic weeds must be found. Conventional methods have not been entirely satisfactory because of cost, overall ineffectiveness, or environmental pollution. The energy problem as it relates to fossil fuel supply has also served to emphasize the need for low-energy methods of control.

6. Biological control methods in recent years have received considerable attention. Various species of herbivorous insects, fish, snails, birds, and mammals either have been or are being investigated for their ability to exert some control pressure on noxious aquatic

plants. Some of them, such as the alligatorweed flea beetle (Agasicles hygrophila Vogt.), have been reasonably effective, especially in an integrated control program. Suprisingly, until this program was initiated, plant pathogens had rarely been considered as biocontrol agents. They have all the prerequisities of a biocontrol agent and thus offer an untapped reservoir of potential usefulness. They may be utilized either alone or in integrated programs with other control agents and methods. Research efforts reported herein have been aimed at bringing to fruition the use of plant pathogens in control programs for aquatic weeds.

Research Approach

7. Two approaches have been used in efforts to utilize plant pathogens to control aquatic weeds:

a. The use of endemic or native plant pathogens as a type of "biological herbicide" through the artificial induction of epiphytotics. It is the most rapid approach from an operational standpoint.

b. The search for and ultimate utilization of exotic plant pathogens. This has been the classical approach used successfully by entomologists in their biological control efforts toward imported weeds. This facet involves the search for pathogens near the center of origin of the noxious species, in an area where climatic conditions are similar to those where the pest is a problem in this country. This is the slower of the two approaches from an operational standpoint.

8. Publications Number 23 and 36 of the Florida Office of Water Resources Research (Freeman, Charudattan, and Zettler 1973 and Freeman et al. 1976a) and Contract Report A-76-2 of the U. S. Army Corps of Engineers (Freeman et al. 1976b) summarize the first 6 years of research work.

9. During the past 3 years, efforts have been directed primarily toward those pathogens with definite biocontrol potential. These are the endemic pathogens of waterhyacinth, Acremonium zonatum (Sawada) Gams and C. rodmanii; and two exotic ones, Uredo eichhorniae Fragoso and

Ciferri on waterhyacinth; and Fusarium roseum 'Culmorum' (Lk. ex Fr.) Synd. & Hans. for hydrilla control. Extensive cultural laboratory studies, greenhouse studies, and, in the case of the endemic pathogens, small-scale field tests were conducted. These latter tests showed A. zonatum and especially C. rodmanii to have considerable potential as biocontrols. Both were tested at locations in Florida and in Lake Concordia in Louisiana. In this latter test, the two pathogens were combined with two insects (Neochetina eichhorniae Warner and Arzama densa Walker) in all possible combinations. This test was conducted in cooperation with the U. S. Army Engineer Waterways Experiment Station (WES) and the U. S. Department of Agriculture with the approval of the Louisiana Department of Agriculture and the Louisiana Wildlife and Fisheries Commission (Addor 1977). Cercospora rodmanii is believed to have caused a decline of waterhyacinths in Rodman Reservoir in 1971. This natural decline saved the Corps of Engineers approximately $35,000 in spray cost in that body of water (Zeiger, personal communication). Laboratory and greenhouse studies with A. zonatum on waterhyacinth have elucidated a general resistance mechanism in this plant that accounts for its disease reaction.

10. Work with the two exotic pathogens is being done in a quarantine facility, which is limited in size. Therefore, progress is slower paced than with the endemic pathogens.

11. This report summarizes the research conducted under Contract DACW39-76-6-0097 between the University of Florida and the WES.

PART II: WATERHYACINTH PATHOGENS

Cercospora rodmanii

Introduction

12. During the winter of 1973-74, waterhyacinth plants in the area of Gainesville, Florida, were affected by a leaf-spotting disease not previously noted. The disorder was found to be incited by a species of Cercospora subsequently identified as C. piaropi Tharp (Freeman and Charudattan 1974). This was only the second reported occurrence of this organism since it was originally described from Texas in 1914 by Tharp (1917). The other occurrence was in India (Thirumalachar and Govindu 1954). The fungus did not appear to be causing appreciable damage to the waterhyacinth plant at the time it was first noted in Florida.

13. In December of 1973, Dr. K. E. Conway isolated a Cercospora species, along with many other fungi, from declining waterhyacinths in Rodman Reservoir (Conway, Freeman, and Charudattan 1974). Preliminary tests showed the fungus to be pathogenic on waterhyacinth and a secondary test showed it to inflict considerable damage on this plant. Affected plants eventually died and sank to the bottom of the test vats. Therefore, this fungus was programmed for more detailed laboratory study and eventual field testing.

14. Microscopic examination revealed the fungus to be a typical Cercospora. However, spore measurements showed the spores to be much longer than those recorded by Freeman and Charudattan (1974) for C. piaropi, the only previously reported Cercospora on waterhyacinth. Spores of C. piaropi rarely exceed 150 µ in reports of its occurrence, whereas those of the Rodman Cercospora frequently exceed 400 µ. In addition, symptoms caused by this fungus differed from those recorded for C. piaropi. Symptoms caused by the former are a general blighting of the foliage, whereas the latter fungus produces more discrete spots on the leaves. However, there was the distinct possibility of the occurrence of two manifestations of the same disease. When leaves exhibiting C. piaropi symptoms that contained fruiting structures

characterized by small spores were brought into the laboratory and placed under moist conditions, long spores frequently developed on the dead tissue. Therefore, it could have been either a long spore variant of C. piaropi or a new species of Cercospora on waterhyacinth (Cercospora spp. are distinguished by the host upon which they occur). Further study revealed that the spore not only differed in size but in basal morphology. These differences, along with the difference in symptoms, prompted its description as a new species of Cercospora designated C. rodmanii by Conway (1976a).

15. Because of the biocontrol potential exhibited by C. rodmanii, preliminary small-scale field evaluations were conducted in Lake Alice at Gainesville, Florida, in Rodman Reservoir near Orange Springs, Florida, and in Lake Concordia, Louisiana. All of these tests indicated that C. rodmanii, either alone or in combination with other biotic agents, was capable of exerting a high degree of biological control of waterhyacinths (Conway 1976a and b and Freeman et al. 1976a). In addition, the fungus was highly host specific (Conway and Freeman 1977 and Freeman et al. 1976a) These studies prompted the further research reported herein.

Symptoms on plant

16. Cercospora rodmanii causes small punctuate spots on the leaves and petioles of the plant. These spots are more numerous at the distal portion of the leaf but can and do occur over the entire leaf surface and the upper portions of the petiole. Because of the distribution of these spots, leaves die from the tip back. Death of tissue gradually spreads towards the base of the leaf until the entire leaf is killed. The progress of these symptoms is shown in the disease severity rating scale depicted in Figure 1.

17. Severely diseased plants become chlorotic with spindly petioles. Newly emerged leaves remain small because of the disease stress on the plant. Under severe disease conditions, leaves are killed faster than they can be regenerated and the entire plant eventually succumbs and sinks to the bottom. In the latter stages, a root deterioration is frequently evident.

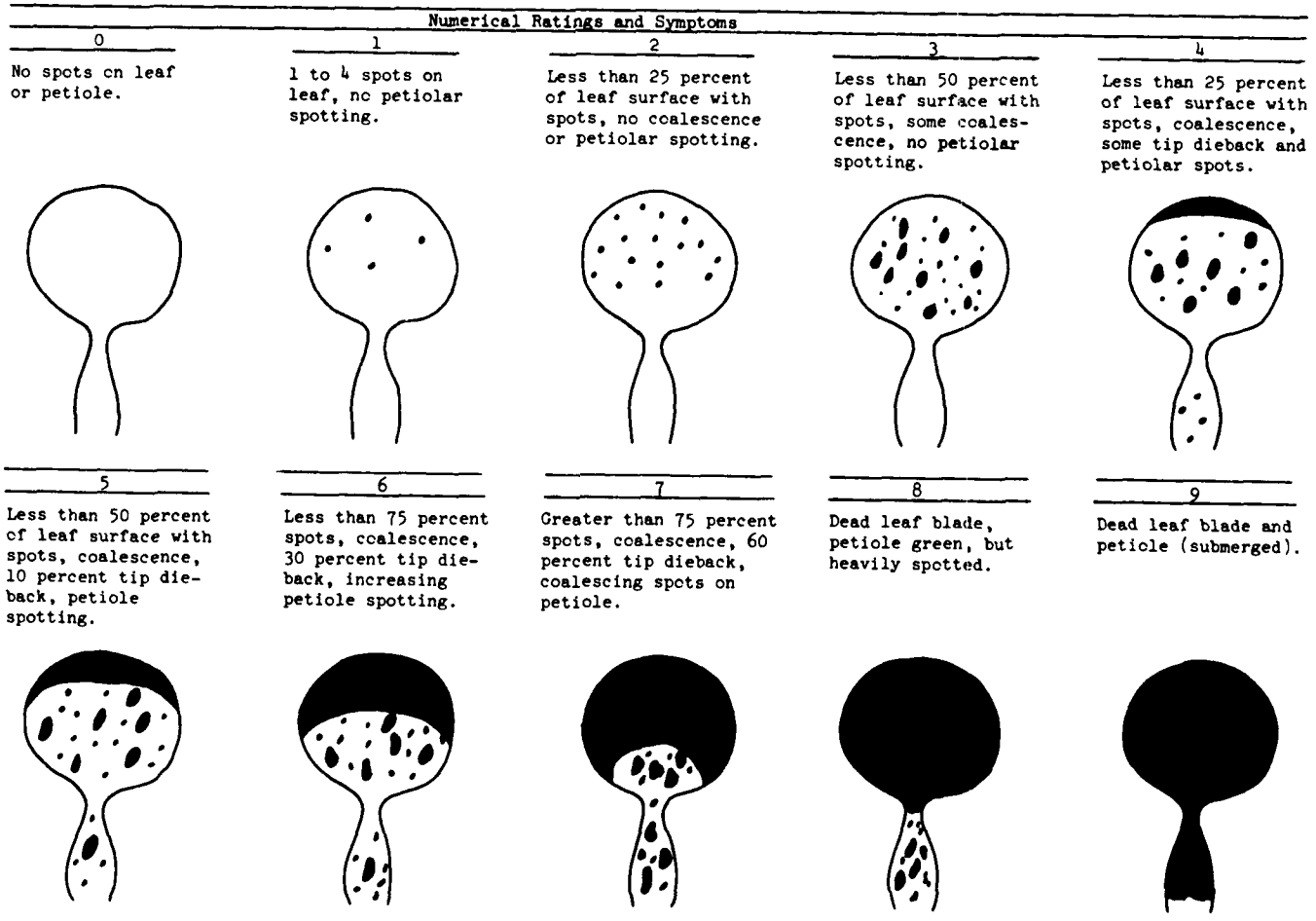


Figure 1. Rating scale system for damage to leaves of waterhyacinth by Cercospora rodmanii

18. Disease stress within a population of waterhyacinths is manifested initially as an overall chlorotic appearance of the plant. Numerous severely spotted to dead leaves will soon become evident in the plants. As the disease progresses the entire plant community has a "browned off" appearance. At this stage the population begins to thin and open water becomes evident in areas where there had been none. As the disease continues, the mat of vegetation begins to break up and small clusters of obviously stressed plants float away from the mat. The plants in these clusters will have only one to three small chlorotic leaves and numerous dead leaves still attached but sunken beneath the water surface. Finally the entire cluster will gradually sink to the bottom. This disease progression in a dense population of waterhyacinths may take several weeks to months for completion.

19. The symptoms described in paragraphs 16-18 and depicted in Figure 1 are typical and easy to diagnose and follow on inoculated plants because of the historical knowledge available. However, the disease may not be easily diagnosed in latter stages without the benefit of either historical knowledge or early observations. In such cases, it is necessary to examine disease tissue for the presence of the causal agent. To accomplish this, a knowledge of the morphology of the pathogen is essential.

Morphology of C. rodmanii

20. Cercospora rodmanii is a typical Cercospora. It has long, frequently flexuous, hyaline spores that may be up to 400 μ in length. They are borne on dark-colored conidiophores arising in fascicles through the stomata. The morphological characteristics of C. rodmanii occurring in and on host tissues are shown in Figures 2 and 3. Photographs in Figure 2 were made using a compound microscope, whereas those in Figure 3 were made with a scanning electron microscope.

21. As noted previously, C. rodmanii can be distinguished from C. piaropi, the other species of Cercospora that occurs on waterhyacinths, by conidiophore size and morphology. The bases of C. rodmanii conidia are truncate and those of C. piaropi are obconic; spores of C. piaropi seldom exceed 150 μ and those of C. rodmanii may be up to 400 μ

a. Mycelial infection through stomatal opening (25 μ) 400×

b. Conidiophores on leaf. (Note amphigenous fruiting on leaf (350 μ thick) 40×)

c. Conidiophores, 200×

d. Conidiophores, 400×

Figure 2. Microscopic (light) details of Cercospora rodmanii morphology and pathological histology (Continued)

e. Conidiophores (5 μ wide) showing conidium attachment (arrow) 400×.

f. Conidium showing truncate base, 400×.

g. Conidia of Cercospora rodmanii, arrow points to 300-μ-long conidium.

h. Top view of fascile of conidiophores on leaf surface, 200×.

i. Subepidermal mycelium, arrow points to base of conidiophores, 400×.

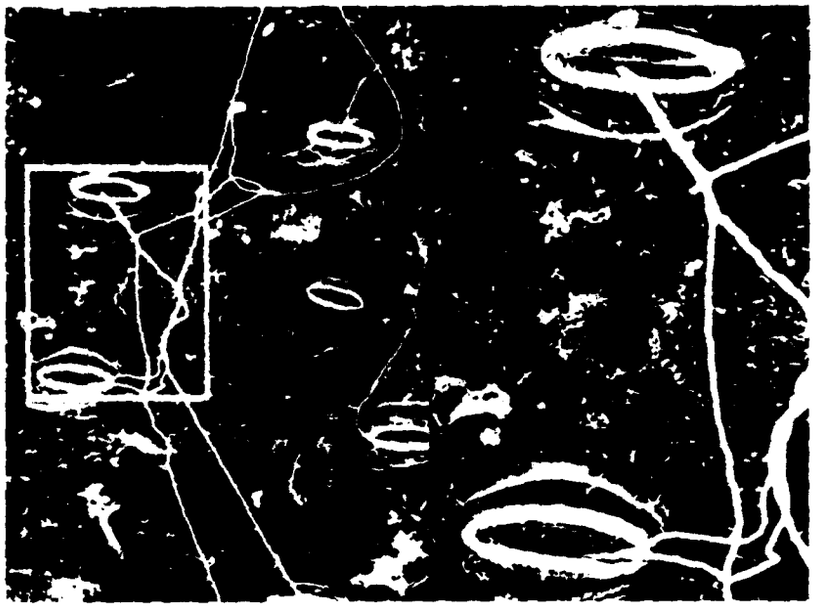
Figure 2. (Concluded)

a. Conidiophores emerging from stomata. (Note spores lying on leaf surface)

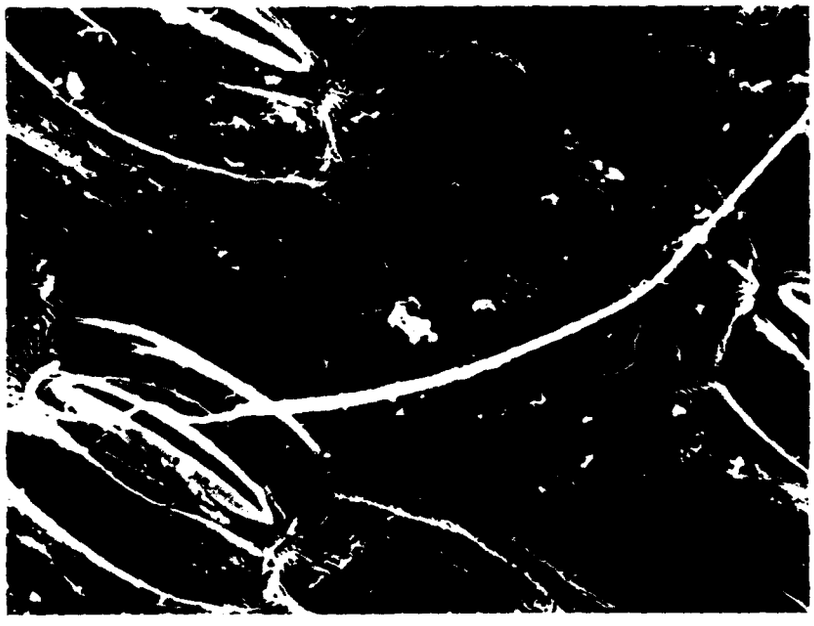
b. Single fascicle of conidiophores. (Note basal end of spore lying on surface at lower left corner)

c. Tip of conidiophores showing spore rear

Figure 3. Scanning electron micrographs of Cercospora rodmanii on waterhyacinth leaf (Continued)



d. Hyphae growing into stomata (infection process)



e. Hyphum from germinating spore growing over leaf surface to stomatal area

Figure 3. (Concluded)

in length. In addition, spores of the latter are frequently flexuous, whereas those of the former are usually straight or only slightly curved. Conway (1976a) used these spore characteristics as a primary basis for the establishment of C rodmanii as a new species. Conway (1976a) described it as follows:

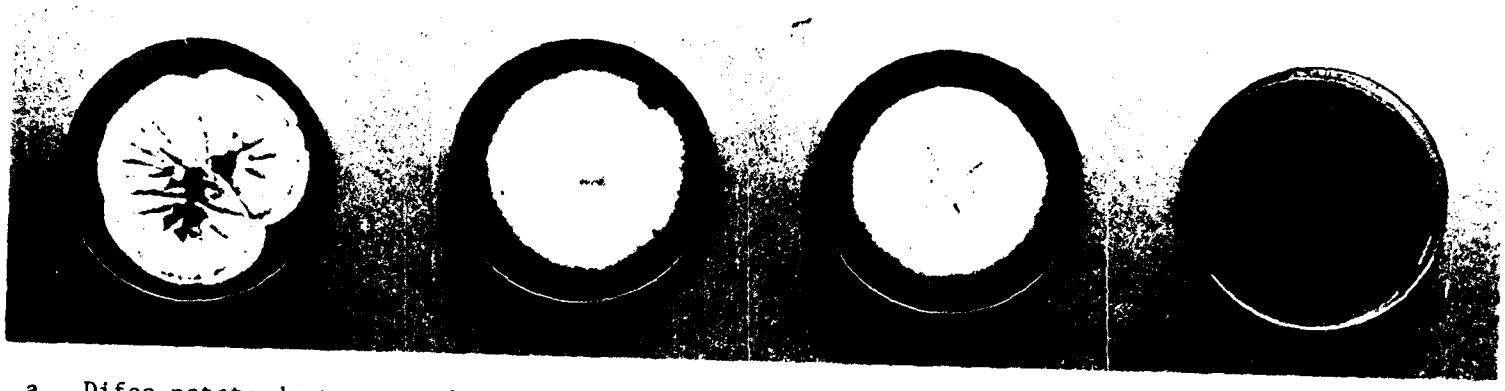
> Leaf spots black, punctate to circular (1-3 mm diam.) leaf and petiole chlorotic, tip of leaf necrotic, conidiophores amphigenous, 3-12 in each fascicle, brown, sympodial, arising from a well developed stroma, emerging through stoma, 84-(145)-284 X 4-(4.5)-5 µm; conidia hyaline, truncate at base, acicular, multi-septate, 66-(172)-374 X 3-(4)-5 µm.

Culturing and
cultural characteristics

22. Cercospora rodmanii has some unique features in regard to its cultural characteristics. This fungus grows well on a variety of both solid and liquid media. However, it varies considerably in growth and cultural characteristics on the various media tested (see Figure 4 and Table 1). It grew best on potato dextrose agar (PDA). The addition of 5 g/ℓ yeast extract (Y) further enhanced growth on all media tested. The growth increase with Y was especially evident on Difco Czapek-Dox agar (C-D).

23. In order to further determine the best growth medium, various types of PDA were tested both with and without Y. Results indicated that freshly prepared PDA (broth from 200 g cooked potatoes, 20 g dextrose, and 17 g agar per litre) was superior to Difco brand of dehydrated PDA, which in turn was superior to BBL brand of dehydrated PDA (Table 2). The same pattern was also evident after the addition of Y to each of the three PDA variations. The difference between fresh PDA and Difco PDA was not significant. Therefore, since the preparation of fresh PDA is time-consuming, Difco PDA plus Y was adopted as the standard medium for growing C. rodmanii on solid media. In liquid culture, Difco potato dextrose broth (PDB) plus Y was adopted for general use.

24. On PDA plus Y, cultures are light to dark grey on top and deep red on the bottom. A diffusible red pigment is present in the agar surrounding the culture. An exudate frequently forms on the culture.

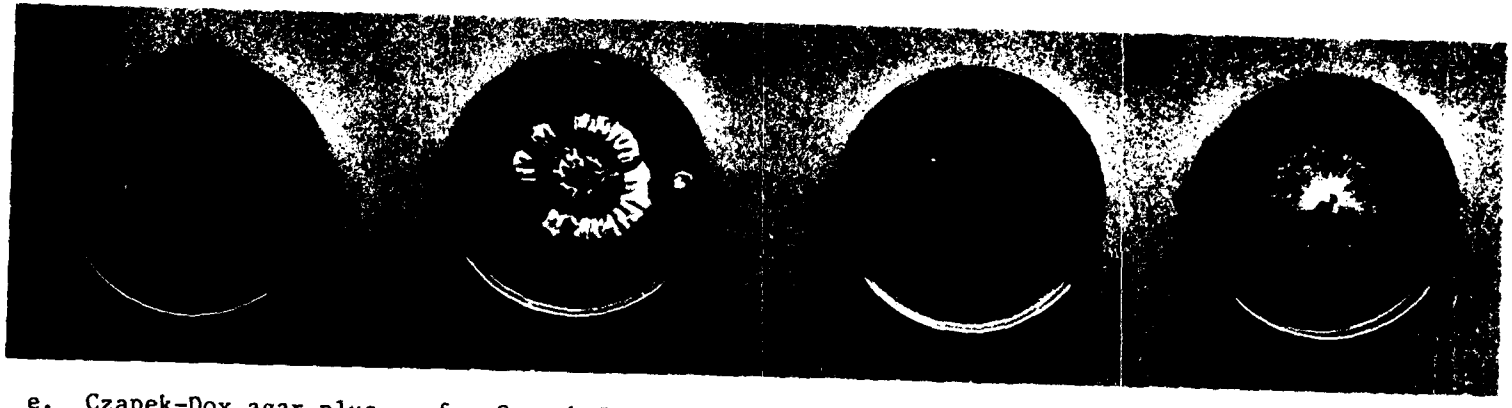


a. Difco potato dextrose agar plus yeast extract

b. Difco potato dextrose agar

c. Freshly prepared potato dextrose agar

d. Nutrient agar



e. Czapek-Dox agar plus yeast extract

f. Czapek-Dox agar

g. Cornmeal agar plus yeast extract

h. Cornmeal agar

Figure 4. Comparative growth characteristics of Cercospora rodmanii on various culture media after 14 days incubation at 25°C

Table 1

Average Diameter of Cercospora rodmanii Cultures Grown at 25°C on Various Media

Medium	Diameter of Culture, mm 7 days	14 days
Fresh Potato Dextrose Agar (PDA)	31.6	56.0
Difco PDA	29.5	55.0
Difco PDA + yeast extract (Y)	39.3	67.0
Difco corn meal agar (CM)	24.0*	44.3*
Difco CM + Y	21.0	24.6
Difco Czapek-Dox agar (C-D)	19.6	30.3
Difco C-D + Y	38.0	66.3
Difco Nutrient Agar	20.3	23.6

* Very little aerial mycelium formed.

Table 2

Average Diameter of Cercospora rodmanii Cultures After 7 Days Growth at 25°C on Variations of PDA

Medium	Diameter of Culture, mm With Yeast Extract	Without Yeast Extract
Fresh PDA	34.6	31.3
Difco PDA	33.3	27.6
BBL PDA	27.8	23.5

In addition, invaginations radiate from the center to the outer edges. Sectors, some of which do not produce pigmentation and are mostly sterile, sometimes form.

25. Conway (1976a) reported that good sporulation occurred on V-8 agar (200 mℓ V-8 juice, 3 g $CaCO_3$, and 15 g agar/ℓ). It will also sporulate on PDA plus Y. Sporulation is augmented by using a mixture of a 12-hr cycle of near ultraviolet light and fluorescent light followed by 12 hr of darkness. Primary conidia in culture frequently produce secondary conidia that are shorter than the primary ones. A stroma and an associated Astromella pycnidial state may also form in older cultures. Conway (1976a and b) described it as follows:

> Astromella pycnidia dark brown, ostiolate, globose, 80-95 X 80-110 µm, substomatal, later erumpent, ostiole 30-40 X 25-30 µm; conidia hyaline, bacilliform 1-1.5 µm.

He considered the presence of this stage as a further criterion for establishing C. rodmanii as a new species.

26. The diffusible pigment that is produced by C. rodmanii in most culture media was identified as the phytotoxin, cercosporin. Identity was based on the 13 criteria listed by Lynch and Geoghyan (1977) for characterizing cercosporin. These authors note that this toxin is a secondary metabolite produced by several species of Cercospora. Its production in culture is enhanced by light. Cercosporin has been implicated as a factor in necrosis of sugar beets by Balis and Payne (1971). These latter authors also studied isolation and chemical characteristics of cercosporin. Preliminary studies conducted under this project on cercosporin from C. rodmanii show that it causes necrosis of waterhyacinths. It may also be involved in pathogenesis, but more study is needed on this point.

27. Yeast extract was found to stimulate growth of C. rodmanii. Since yeast extract is high in vitamin content but may also contain certain amino acids, there was a need to determine if vitamins or amino acids were the stimulating factor for growth. Difco Czapek-Dox broth (C-DB), a chemically defined medium containing essential salts (sodium nitrate, dipotassium phosphate, magnesium sulfate, potassium chloride,

and ferrous sulfate) and sucrose, was used as the base medium. To this medium was added either Y at 5 g/ℓ, vitamin-free casein hydrosylate (CH) at 25 mg/ℓ, or a combination of the two. In addition, CH was substituted as the sole nitrogen source by deleting sodium nitrate from the C-DB. Results are shown in Table 3. These results show that vitamins are more important in growth stimulation than amino acids but that the addition of organic nitrogen may also be a factor in increased growth.

28. The influence of temperature on the growth of C. rodmanii was also determined. The fungus was grown on PDB plus Y (25 mℓ in 250-mℓ flasks) at four different temperatures for periods of 7, 14, and 21 days. Growth was determined by weighing dried mycelium. Results show the optimum growth to occur at near 25°C after 2 weeks incubation (Table 4). However, in reality, the fungus grows quite well over the range of 20 to 30°C, although growth is slightly more restricted at 30°C than at 20°C. Earlier studies (Freeman et al. 1976a and b) had indicated the optimum temperature for growth was near 20°C.

29. In further studies on the influence of temperature on C. rodmanii, it was found that the fungus would not grow at 37°C. However, it did survive at this temperature for at least 2 weeks (limit of test). This is a significant finding because body temperature is 37.1°C. Since C. rodmanii will not grow at this temperature, it would not be expected to be pathogenic on man--a valuable consideration for a potential biocontrol agent.

30. Since there may be a need to relate dry weight of C. rodmanii mycelium to wet weight in future biocontrol studies, this was determined for the optimum growth conditions for C. rodmanii (i.e. in PDB plus Y at 25°C for 2 weeks). The test was conducted in Roux culture bottles containing 100 mℓ of medium. The averages were as follows: wet weight 15.7 g, dry weight 1.3 g, and percent dry weight 8.3.

Infection and pathological histology

31. Infection can originate from either mycelium or spores. In both cases, the hyphae grow into the stomata, ramify in the substomatal cavity, and invade the surrounding tissue. A stroma develops in the

Table 3

Average Diameter of Cercospora rodmanii Cultures Grown at 25°C on Basal and Fortified Culture Media

Medium	Diameter of Cultures, mm	
	7 days	14 days
Czapek-Dox broth (C-DB)	19.3	34.6
C-DB + yeast extract (Y)	32.6	59.6
C-DB + casein hydrosylate (CH)	21.0	24.6
C-DB + Y + CH	33.0	61.6
C-DB + CH substituted for $NaNO_3$	26.0	47.6
C-DB + CH substituted for $NaNO_3$ + Y	34.3	61.0

Table 4

Average Dry Weight of Cercospora rodmanii Mycelium Grown at Various Temperatures on PDB Fortified with Yeast Extract

Temperature, °C	Dry Weight, mg		
	7 days	14 days	21 days
20	114.0	317.3	297.3
25	313.3	327.3	287.0
30	154.0	248.0	251.0
35	29.6	106.3	118.0

stomatal cavity and a fascicle of 3-12 conidiophores arises from it and emerges through the stomata. Primary and secondary conidia are produced on the conidiophores. Figures 2 and 3 show the infection process and the histological aspects of the fungus on and in the tissue.

32. Since infection occurs through the stoma, the number and distribution of stomata on the leaf and petiole influence the infection. Stomata become fewer in number from the leaf tip downward to the base of the petiole (Figure 5). Leaf spot distribution of the infection by C. rodmanii follows the same distribution pattern.

Epidemiology

33. In nature, spores produced on diseased tissue are carried passively and serve to disseminate C. rodmanii between loci. The speed and intensity of the epidemic is directly related to the number of spores produced. This in turn is related to the amount of diseased and dead tissue that is available for sporulation. This condition frequently leads to a cyclic disease pattern analogous to the cyclic nature of pest and predator populations in natural ecosystems.

34. It was previously reported that sporulation by C. rodmanii was curtailed by temperatures below 10°C and reached a maximum in the range of 20-30°C (Freeman et al. 1976a). Extensive spore trapping, using a Roto-Rod system in Lake Alice on the University of Florida campus, showed that sporulation reached a peak during the fall and early winter months (Figure 6). High counts were associated with high disease intensity and low counts with low levels of disease.

35. Since its original discovery, C. rodmanii has been found in areas other than its type location in Rodman Reservoir. Surveys for the presence of the fungus in the state were conducted during the summer of 1978. Twenty-eight locations were checked during the survey and the presence of the fungus was confirmed in 12 out of the 28. It was found to be present at several locations along the St. Johns River ranging from Lake Poinsett near Cocoa, Florida, northward to Lake George. It was also found occurring on waterhyacinths in Lake Rousseau on the Withlacoochee River. However, it was not found on plants in nearby Crystal River where C. piaropi was the predominant pathogen. In north central

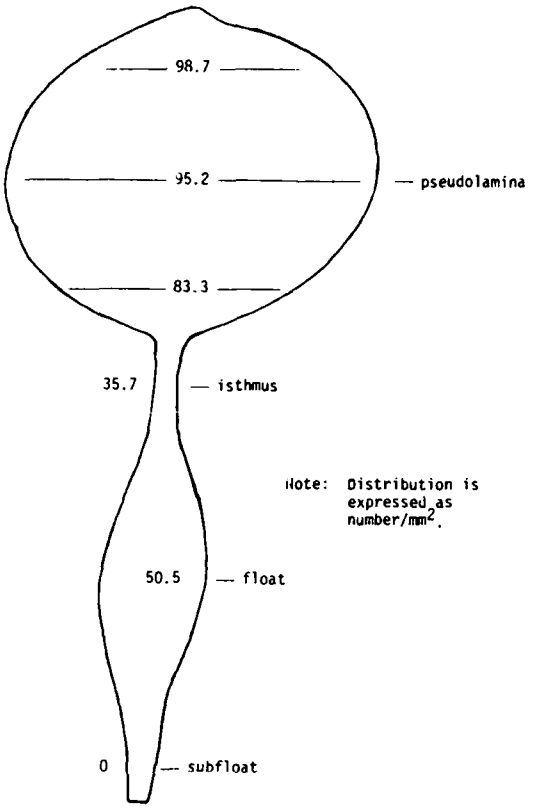


Figure 5. Mean distribution of stomata on the waterhyacinth's petiole adaxial surface

Florida, C. rodmanii was present on plants from Orange Lake. The fungus was not present on plants from either the Santa Fe River or the Suwannee River. Neither was it present in the Aucilla River nor the Wasissa River in west central Florida. It has since been found in an irrigation canal system near Cocoa, Florida, and in Palm Beach and Broward counties. With the exception of the Palm Beach and Broward areas, all the waterways in which the fungus has been found are either connected or in close proximity to connected waterways. Rodman Reservoir is in the connected system. These results confirm earlier reports that the fungus will readily spread from a focus of infection to new areas (Freeman et al. 1976a).

36. Cercospora rodmanii was also confirmed in samples collected from Louisiana in the experimental sites established by the WES (Addor

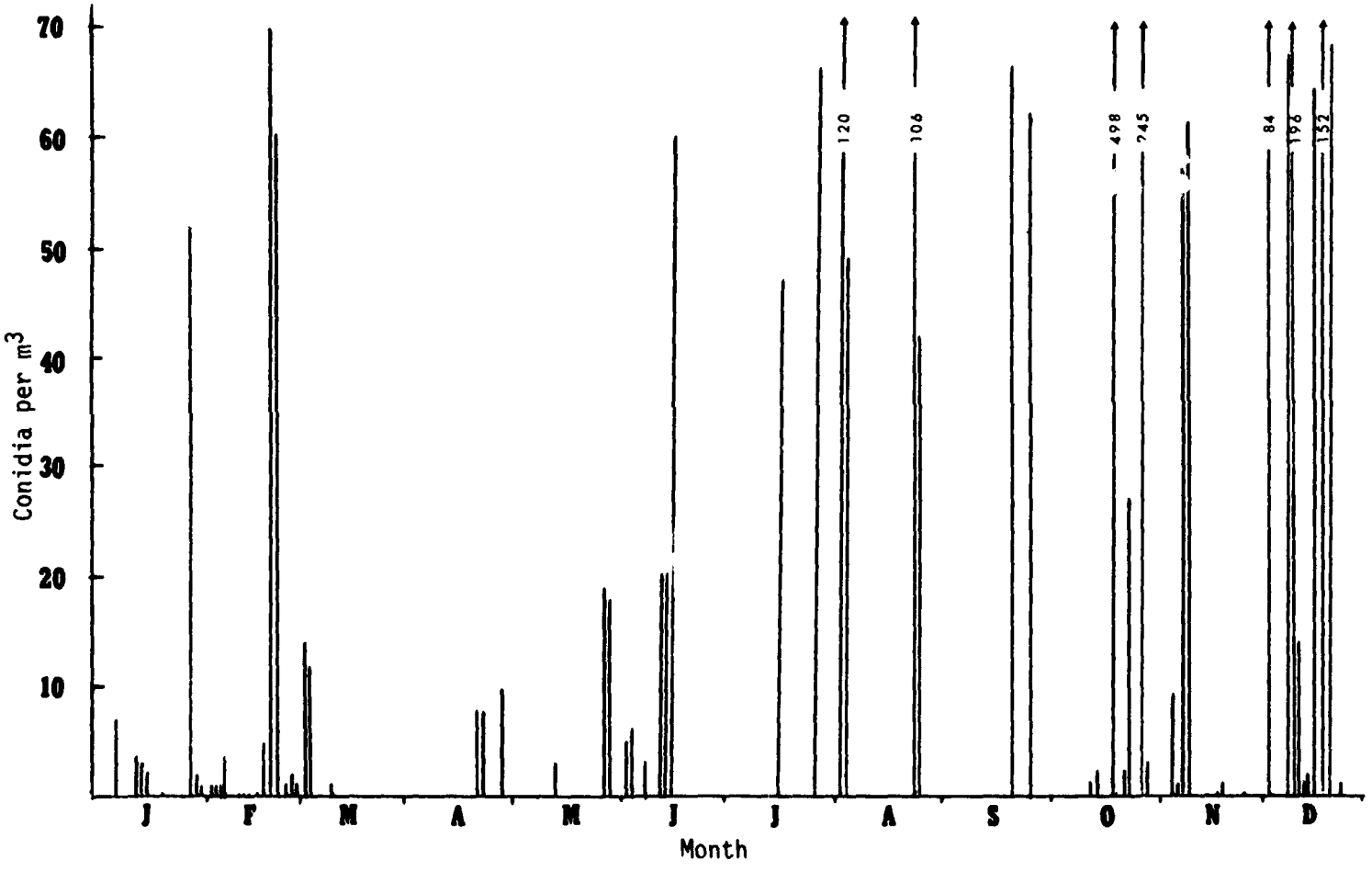


Figure 6. Seasonal variation in conidial production (inoculum potential) of Cercospora rodmanii expressed as conidia per cubic metre of air over Lake Alice, Gainesville, Florida

1977). Cercospora was present on samples collected at Hayes North, Pecan Island I, Pecan Island II, and Manchac I. It was not present in samples from Sorrento II, Hayes West, Centerville C, Bayou Lourse, and Manchac IV.

Biocontrol potential

37. Previous reports of studies conducted in Lake Alice and Rodman Reservoir, Florida, and in Lake Concordia, Louisiana, confirmed that C. rodmanii had high potential, either alone or in combination with insects, as a biocontrol agent for waterhyacinth (Addor 1977, Conway 1976b, and Freeman et al. 1976a). These reports were further confirmed by additional studies conducted in the project reported herein.

38. Notable among these studies was the one on inoculum rate and spread conducted in Fish Prairie. The complete results were published by WES as a separate report (Conway, Cullen, and Freeman 1979). Therefore, they will only be briefly summarized in this report.

39. During the Rodman study, it was determined that under optimal conditions waterhyacinths were capable of producing one new leaf every 5-6 days. This rate will vary with environmental conditions, and during unfavorable periods, this rate may decline to less than one leaf produced over a 3-week period. Therefore, the success of the epidemic will depend upon the rate at which the pathogen can infect and kill these new leaves. To determine if there was an optimal concentration of inoculum necessary to initiate disease, an inoculum rate experiment was begun in a small lake in Fish Prairie southeast of Gainesville, Florida. Waterhyacinths were confined in 9-m^2 frames and treated at three mycelial inoculum concentrations: 48 g/m^2, 96 g/m^2, and 192 g/m^2. Results showed that, regardless of the initial inoculum level, the rate of disease spread became equalized after a period of time due to inoculum buildup on the inoculated plants and cross infectivity between plots. The maximum rate of damage produced by C. rodmanii was assessed at the 192-g/m^2 inoculum level and this rate was not exceeded even with an additional application of inoculum later in the year. The maximum rate of damage caused by C. rodmanii during this experiment corresponded to the death of 1.0-1.3 leaves of the waterhyacinth every 3 weeks. Therefore, when conditions exist that favor disease development and limit leaf production to less

than one leaf per 3 weeks, C. rodmanii can infect and kill leaves faster than the plant can produce new leaves. The plant becomes debilitated and over a period of time will die unless conditions change to favor its regrowth.

40. The use of C. rodmanii as a biological control for waterhyacinth has been patented by the University of Florida. The University is working with Abbott Laboratories (AL), Chicago, Illinois, to produce a commercial product of the fungus. Evaluation of a product has already begun here and at the WES.

41. The first dry product form of C. rodmanii was received from AL in the late fall of 1977. The formulation was plated out to verify viability of the product. It proved to be viable and remained so when last tested 6 months after receipt of the shipment. These results indicated that the production of a viable product form was feasible and should be pursued. Further tests were needed to compare this product with fresh inoculum of C. rodmanii (Isolate WH-9) produced in the laboratory.

42. A second shipment of four formulations of C. rodmanii (Isolate WH-9) was received from AL on 6 April 1978. The four products were dry formulations consisting of coarse and fine vermiculite, perlite, and wheat millings (a by-product of the wheat industry). Viability tests were performed by dilution plating of each product. The highest count was recorded for the coarse vermiculite (3×10^8 colonies/g) and the lowest for the wheat (1×10^6 colonies/g). Microscopic examination, however, showed that the mycelium of C. rodmanii ramified throughout the wheat particles and the low count may have been due to this tight binding. Initial growth in culture also indicated that the wheat product formulation grew almost twice as fast as the other products, indicating that the wheat provided a good food base for the fungus. Waterhyacinth in small pools were inoculated with fresh inoculum of each formulation and compared with untreated control plants. All formulations were capable of infection. Further testing was needed to determine rates of inoculum of the AL product necessary to equal damage obtained with fresh inoculum. The two vermiculite formulations performed quite well in comparison with

the fresh inoculum. Recommendations were made and further tests were planned.

43. On 30 April 1979, 105 g of the C. rodmanii product was received from AL. The dry preparation was 6 days in transit, having been produced on 24 April 1979. A serial dilution ranging from 10^{-1} to 10^{-8} of the product was prepared in sterile, deionized water. Three replications of each dilution were then plated into PDA plus Y. The C. rodmanii product consisted of small mycelial particles less than 1 mm in length. On 2 May, after 2 days incubation at room temperature, only bacteria had grown out on the PDA plus Y plates. Bacteria were present in all 10^{-8} dilution plates. Three days after plating, mycelial growth was noted on one of the 10^{-6} reps. This later proved to be Cercospora. On a later date, Cercospora was found in another 10^{-6} dilution plate. Since good germination was noted from the AL product when it was placed on wet filter paper, it appeared that the high number of bacteria (1×10^{8}/g Al product) may have suppressed the growth of Cercospora in the PDA plus Y plates.

44. Efficacy tests were carried out in five 2.4- by 0.5-m-diam by 0.3-m-deep wading pools that were not divided. The pools were filled with tap water. Pools 7 and 8 were stocked with waterhyacinths collected on 28 March at Cooter Pond in northeast Alachua County. Pool 9-10 was stocked with waterhyacinths collected on 28 March from Payne's Prairie south of Gainesville. The remaining pools were stocked with waterhyacinths collected from the Oklawaha River at Eureka. Pools 1-2 and 11-12 were stocked on 30 March, pool 3-4 was stocked on 13 April, and pool 5-6 was stocked on 27 April. On 1 May, water-soluble fertilizer with minor elements was added to all pools at the rate of 20 mg/ℓ N-P-K. Also, the youngest unblemished leaf of fifteen plants in each pool was tagged with a bright plastic ribbon for future rating.

45. After a 2-day delay due to rain, the test was initiated on 2 May at 5:15 p.m. Inoculum of the AL product was applied with an air driver sprayer at rates of 5 g/m^2 (AL_5) and 10 g/m^2 (AL_{10}). Cercospora rodmanii, which was grown in Roux bottles in the laboratory, was comminuted in a blender. This mycelial suspension was inoculated

onto the waterhyacinths with a sprinkler bottle at a rate equal to 5 g/m^2 dry weight. Check pools were not treated. All treatments and controls were replicated three times. Pool 11-12, because of its isolated position, was assigned control pool status. Treatments for the remaining pools were assigned from a random numbers table.

46. The fifteen tagged leaves in each pool were then rated and the average plant height was recorded at 1, 2, and 4 weeks after inoculation. The leaves were rated on the 0-9 severity scale developed to quantify Cercospora damage on waterhyacinth (see Figure 1). Complete results are presented in Table 5.

47. The initial infection as measured at the 1-week rating time was good. The AL product showed better results than the mycelial inoculum. An unexplained yellowing of waterhyacinths in control pool 11-12 was noted at this time. This resulted in higher damage evaluation for these controls due to the presence of some tip burning. This damage was not due to the pathogen, but in order not to bias the test, the leaves were rated the same as the other treatments. At the second rating, disease progress was good with all treatments being approximately equal. At this time, it was noted that the plants in pool 5-6 were yellowing. Cercospora damage in this pool was noticeably more severe. By this time, the yellowed plants in control pool 11-12 were green and healthy in appearance. Four weeks after the inoculation, disease progress of the AL product had slowed, but that in pools receiving mycelial treatments continued to show good progress. By this time, the tagged leaves were under a canopy of new leaves and in some cases were submerged and could not be rated. The plants in pool 5-6 were even more yellowed and more severely diseased. In fact, this was the only pool in which the average height of plants decreased over the 4 weeks (Table 5).

48. The efficacy of C. rodmanii appears to be closely associated with the physiological state of the waterhyacinth. Under crowded conditions, such as existed during this test, the waterhyacinth produces fewer but larger leaves and few, if any, offshoots. In periods of rapid growth, the pathogen lags behind in development. However, it continues to infect slower growing leaves in the understory of the

Table 5

Waterhyacinth Response to Different Preparations of Cercospora rodmanii

Response	Treatment			
	Abbott 5 g/m^2	Abbott 10 g/m^2	Mycelial 5 g/m^2	Untreated
	Average Damage*			
At inoculation	0	0	0	0
After 1 week	2.7	2.8	2.1	0.7
After 2 weeks	2.8	3.0	2.9	1.5
After 4 weeks	3.4	3.4	5.2	2.2
	Average Plant Height, cm			
At inoculation	14	14	14	13
After 1 week	16	18	17	16
After 2 weeks	27	29	29	25
After 4 weeks	29	27	26	27

* Conway rating scale, 0-9 (see Figure 1).

waterhyacinth canopy and is thus still present in the ecosystem. When conditions occur that stress waterhyacinth, such as cool weather in the fall and various nutrient limitations, Cercospora vigorously attacks the plants. During this experiment, such a condition of stress was noted in control pool 11-12 and treated pool 5-6. Waterhyacinths in the isolated control pool quickly recovered; but in the treated pool C. rodmanii severely infected the plants, and decline symptoms similar to those noted previously in Rodman Reservoir developed. This test has shown that the efficacy of C. rodmanii in infecting waterhyacinth is high under favorable environmental conditions. These conditions should be further elucidated. Also, why the levels of effectiveness of the AL products fell below that of the mycelial inoculum after 4 weeks needs to be determined. The high bacterial content may have played some role in suppressing the fungus.

49. Based on all of the tests conducted thus far, it can be concluded that C. rodmanii shows considerable promise as a biocontrol agent for waterhyacinth. In addition, it has been shown that the fungus can be successfully mass produced commercially.

Other considerations

50. Any organism used as a biocontrol agent must be safe to use in the environment. It has already been shown that C. rodmanii is highly host specific (Conway and Freeman 1977) and that it will not grow at body temperature (see paragraph 29). However, there are other considerations, such as its effect on other aquatic organisms.

51. One of the major concerns in the use of plant pathogens for biological control of aquatic weeds is whether or not they will harm fish. To determine if the pathogen was detrimental to fish, the fish Gambusia affinis was exposed to C. rodmanii in a standard 96-hr bioassay (Conway and Cullen 1978). Ground-up mycelium and spores of C. rodmanii were placed in the fish containers at rates ranging from 0.4 to 6.34 g/ℓ. The lowest rate corresponded to an inoculum level of 48 g/m^2, which was the inoculation rate used in Lake Concordia and the lowest rate used in Fish Prairie. The highest rate is equivalent to a surface area rate of 800 g/m^2, which is four times higher than the highest rate used to

inoculate waterhyacinth with C. rodmanii. None of the fish in any of the treatments was adversely affected. In fact, fish in the tank receiving the highest rate of inoculum ate the fungus, which was subsequently isolated from their feces. Therefore, based on this limited test, C. rodmanii poses no threat to fish; however, other species need to be tested.

52. A biocontrol agent must be easily disseminated to be successful. Studies (Freeman et al. 1976a and b) had shown that an epidemic could be started by simply spraying C. rodmanii onto waterhyacinths. There was a need to determine the influence of nozzle type on the success of sprayed inoculum. Two tests of three nozzle types were conducted during the spring and summer of 1977. The first test was carried out on plants in frames adjacent to the Fish Prairie experiment. The results were largely inconclusive because of contamination by naturally produced C. rodmanii inoculum. However, the results tended to indicate that a mister-type nozzle was superior to either a raindrop or hollow-cone nozzle. The second test was conducted in pools under more controlled conditions. The results again indicated the mister to be the better nozzle for applying inoculum (Appendix A). However, further evaluation is probably warranted because of shortcomings in both tests.

Acremonium zonatum

53. The fungus A. zonatum has been the object of previous research to determine its biocontrol potential (Addor 1977, Freeman et al. 1976a, and Rintz 1973). This pathogen causes a zonate leafspot of waterhyacinths that produces considerable damage under certain conditions. The fungus can also invade the vascular tissue of arthropod-damaged waterhyacinths (Charudattan, Perkins, and Littrell 1978). Under such conditions it causes a rotting of the vascular tissues of the plant. Plants appear to vary considerably in regard to this reaction to the fungus. Certain morphotypes are readily infected and severely damaged, while others are not. The work conducted with A. zonatum under this contract was designed to determine the reason for this difference in

disease reaction as well as to further assess its biocontrol potential. The results have been summarized and are presented in Appendix B.

54. Acremonium zonatum does have some biocontrol potential, but plant type and timing of applications of the fungus are critical factors (Martyn 1977 and Martyn and Freeman 1978). More importantly, Martyn's work defined a general disease resistance mechanism in waterhyacinths that may be unique to this plant. This mechanism could account for the fact that this plant is affected by relatively few really serious diseases. These are caused by pathogens, such as A. zonatum, that are able to successfully breach a generalized system of resistance. However, knowing the resistance mechanism will enable future work to concentrate on methods of shunting around it through the use of growth regulators, metabolic inhibitors, and other similar methods to increase the susceptibility of waterhyacinth to plant pathogens.

Uredo eichhorniae

55. A third pathogen with potential for the biocontrol of waterhyacinth is the rust U. eichhorniae. The fungus does not occur in the United States; thus, all studies with it in this country must be conducted in quarantine. In addition, the fungus is an obligate parasite and must be cultured on living material. Also, the perfect stage of the rust, assuming one exists, is not known. All of these factors limit research efforts with this fungus. However, some progress has been made. These results were presented in a request to consider release of U. eichhorniae from quarantine and are presented in Appendix C.

56. The request to release U. eichhorniae was not approved. Therefore, further studies will be necessary before the real biocontrol potential of this fungus can be evaluated. These studies, consisting of efforts to obtain the perfect stage (teliospores) of the fungus, are in progress and will be continued. Teliospores produced on waterhyacinths will make it possible to determine if the rust is autoecious or heteroecious. If the fungus is found to be autoecious, meaning that it is restricted to waterhyacinth for completion of its life cycle, then

the fungus will probably be deemed as host specific and can be released from quarantine. A verdict of heteroecious indicates that the alternate host must be found before the fungus can be considered for biocontrol purposes.

PART III: HYDRILLA PATHOGENS

Fusarium roseum

57. In 1974 a disease of Stratiotes aloides L. (Hydrocharitaceae) was discovered near Wageningen, The Netherlands. Mature plants had symptoms of root and crown rots, and severely diseased plants appeared to sink gradually as a consequence of tissue decay. A few infected plant parts were taken to Gainesville, Florida, where a group of fungi were cultured from them, including predominantly F. roseum 'Culmorum'. In view of the close taxonomic relationship between S. aloides and H. verticillata, the pathogenic potential of these fungi to the latter was of obvious interest. Among the fungi isolated from S. aloides, only F. roseum 'Culmorum' was capable of killing hydrilla (Charudattan and McKinney 1978).

58. The effects of the Dutch isolate of F. roseum 'Culmorum' on hydrilla were determined in three test systems. The first one consisted of incubating 8- to 10-cm-long terminal portions of hydrilla shoots in 3- by 15-cm glass tubes with 40 mℓ sterile water to which were added dense macroconidial suspensions of the fungus. Control tubes received no conidia. Fungal inocula, consisting of filtered macroconidial suspension obtained from PDA cultures, were quantitated with a hemacytometer. Inoculum levels between 2,500 and 250,000 conidia per mℓ (100 thousand and 10 million conidia per tube containing 40 mℓ of water) were set up by mixing suitable concentrations of conidial suspensions. Inoculated and control hydrilla tubes were incubated under diffuse light at 22 ± 2°C for the duration of the test. Damage to hydrilla by the pathogen was usually evident as chlorosis and discoloration of inoculated shoots 10 to 14 days after inoculation. Death and lysis or regrowth of partially damaged hydrilla were observed in 3 weeks. The threshold of inoculum needed to damage hydrilla was found to be 1 million conidia per tube, or 25,000 per mℓ. A dose and effect relationship was seen on inoculated hydrilla; at lower inoculum levels the shoots were only partially damaged or killed, whereas at higher inoculum levels the effects were drastic or lethal.

59. In the second system, 20-ℓ aquarium tanks were layered with river sand, filled with 14 ℓ of water, and planted with 100 terminal hydrilla shoots, each with an active growing bud. After 2 days, the tanks were inoculated with conidial suspensions of the Dutch isolate of F. roseum 'Culmorum' at approximately 80,000 to 90,000 conidia per mℓ of water in tanks. Three weeks after inoculation, hydrilla shoots started to discolor and develop signs of rotting. The shoots broke down completely in about 5 weeks, and some that were still green were defoliated, became uprooted, and floated to the water surface.

60. In the third system, the fungus was grown for 2 weeks on a sterilized mixture of 9 parts sand, 1 part oatmeal, and 3 parts water, and was mixed with the bottom sand in hydrilla tubes at 1:1 and 1:10 proportions (w/w) of inoculum and sand. Controls had sand-oat-water mixture without the fungus, mixed with an equal weight of sand. A hydrilla plant with shoots, roots, and at least one tuber was planted in each tube. The inoculated plants turned pale after a week, and were dead by the end of 14 days.

61. In all these systems, the fungus could be reisolated from inoculated, dead, dying, or green hydrilla shoots after surface sterilization and plating on PDA. Controls did not yield the fungus. The conidia were also observed to germinate on, and penetrate into, hydrilla tissue, which confirmed the pathogenic capability of the fungus.

62. To decide that the effects of the Dutch isolate of F. roseum 'Culmorum' on hydrilla were specifically due to its infectivity and not due merely to massive numbers of fungal spores in water, a comparative inoculation test was set up. In this test, the effects of three unidentified Fusarium spp., isolated from hydrilla in Florida, a F. roseum from Ficus elastica Roxb., a F. roseum 'Graminearum' from waterhyacinth in Florida, and two isolates of F. roseum 'Culmorum' from the State of Washington were compared to effects produced by the Dutch isolate of F. roseum 'Culmorum'. The test tube procedure described in paragraph 58 was used, with inoculum densities between 2,500 and 250,000 conidia per mℓ of treated water.

63. The results confirmed that the Dutch isolate of F. roseum

'Culmorum' was indeed unique in its effects on hydrilla. The three Fusarium spp. from hydrilla and the Ficus isolate of F. roseum did not damage hydrilla even at higher levels of inoculum. The F. roseum 'Graminearum' from E. crassipes and the isolates of F. roseum 'Culmorum' from Washington were capable of damaging hydrilla, inciting similar symptoms as the Dutch isolate. However, the threshold of inoculum needed to cause damage by these domestic isolates was approximately 60,000 conidia per mℓ, or 2.4 times higher than that of the Dutch isolate. Hence, the Dutch isolate was not only pathogenic to hydrilla but was also more virulent than any other Fusarium spp. tested.

64. In another experiment, conidia and mycelial fragments of the Florida isolate of F. roseum 'Graminearum' from E. crassipes were applied to hydrilla shoots maintained in 4-ℓ glass jars containing 2.5 ℓ of water. The fungus for inoculum was grown on PDB for a week. Thirty grams of wet, filtered mycelium and conidia were blended in 125 mℓ of sterile water. The resulting slurry was applied with a 100-mℓ hypodermic syringe fitted with a blunt needle at 10-, 20-, and 40-mℓ portions consisting of 0196 g, 1.92 g, and 3.84 g of conidia and mycelium per litre. The inoculum was suspended over hydrilla in water or injected into the soil. Control plants received equal amounts of sterile water. Inoculum applied as a suspension not only caused considerable turbidity to the water, but also was effective in killing most of the hydrilla by 3 weeks. In jars with soil-injected inoculum, some damage and death of hydrilla shoots was visible, but mostly the plants were healthy, similar to the controls.

65. Since the Dutch isolate of the pathogen is still maintained under quarantine due to its foreign origin, the effects of the local F. roseum 'Graminearum' isolate was tested in an outdoor, large-scale pool test. Plastic swimming pools 3.04 m in diameter and 0.76 m in height were layered with river sand, and each was planted with 45 kg of fresh hydrilla and filled with irrigation water. After 5 weeks, pools were inoculated with mycelial homogenates. One pool was inoculated with a suspension of approximately 0.18 g/ℓ conidia and mycelium and a second pool at 1.0 g/ℓ. Control pools were maintained. There were isolated

patches of dead hydrilla one month following inoculation, but no appreciable control was achieved in the pools. This lack of field efficacy may be due to insufficient levels of inoculum, poor virulence of isolates, or both.

66. Host range of the Dutch isolate of F. roseum 'Culmorum' to some common aquatic plants of Florida has been tested. Rooted aquatic plants in 5- to 200-ℓ glass containers were screened, using an inoculum rate of 125,000 conidia per mℓ. At this level, the isolate was lethal to Ceratophyllum demersum L. (Ceratophyllaceae); Egeria densa Planchon and Vallisneria americana Michx. (both of Hydrocharitaceae); and Najas gudalupensis (Spreng.) Magnus (Najadaceae). It caused severe root rot in waterhyacinth. Alternanthera philoxeroides (Amaranthaceae), Nuphar luteum (L.) Sibth. & Smith (Nymphaeaceae), and Ruppia maritima L. (Ruppiaceae) were not affected by this isolate.

67. Host range tests on terrestrial crops and nontarget plants have been under way. Two types of tests have been undertaken. Sixty-eight crop plants have been screened for their ability to withstand seed infection by the Dutch isolate of F. roseum 'Culmorum'. Fusarium roseum 'Culmorum' isolates can be seed pathogens that may colonize and rot preemergent seeds. Testing was therefore aimed at determining this potential. Conceivably, this isolate could pose problems if water treated with the fungus is used for crop irrigation, assuming that the fungus would survive in water for long periods of time and at large enough inoculum levels (which is unlikely). The test was conducted by growing plants from fungicide-free seeds in soil infested with 38,000 conidia/g soil. Seeds were allowed to germinate and seedlings were observed for 3 weeks or more. The percentage of reduction in seed germination over controls and symptoms or root or collar rots on seedlings were assessed. Any partially germinated seeds and seedlings with symptoms of rots were assayed for F. roseum 'Culmorum'. The results are summarized in Table 6.

68. Of the 70 cultivars belonging to 43 species in 14 families tested, 35 were not affected by the fungus, while 35 others exhibited a reduction in germination. These reductions, expressed as percent reduction from the controls, ranged from 4 to 100, the latter in the case

Table 6

Plants Screened for Susceptibility to the Dutch Fusarium roseum 'Culmorum' in Preemergence Inoculation Test: Effect of the Fungus on Seed Germination

Family, Genus, and Species	Common Name, Variety	Percent Reduction[a]	'Culmorum' Reisolated[b]
Alliaceae:			
Allium cepa L.	Onion, 'Texas Grand'	0	NT
Apocynaceae:			
Vinca rosea L.*	Periwinkle	50	-
Chenopodiaceae:			
Spinacia oleracea L.*	Spinach, 'Bloomsdale'	0	NT
Beta vulgaris L.*	Beet, 'Detroit Dark Red'	11	-
B. vulgaris	Beet, 'Early Wonder'	0	NT
Compositae:			
Cichorium endivia L.	Endive, 'Broad Leaf Batavian'	0	NT
Heliathus annuus L.*	Sunflower	0	NT
Lactuca sativa L.*	Lettuce, 'Bibb'	0	NT
	'Great Lakes'	0	NT
Cruciferae:			
Brassica juncea (L.) Coss	Mustard, 'Florida Broad Leaf'	0	NT
B. oleracea L.	Broccoli, 'Italian Green Sprout'	0	NT
B. oleracea	Cabbage, 'Charleston Wakefield'	14	-
B. oleracea	Cabbage, 'Greenback' (var. capitata)	0	NT
*B. oleracea**	Cauliflower, 'Snowball A.' (var. botrytis)	9	-
*B. oleracea**	Collards, 'Georgia' (var. acephala)	8	-

(Continued)

[a] Percent reduction in germination of fungus-infested seeds in relation to control seeds.

[b] *F. roseum* 'Culmorum' not isolated (-) or isolated (+); NT = not tested.

* These cultivars were also screened in postemergence test.

(Sheet 1 of 4)

Table 6 (Continued)

Family, Genus, and Species	Common Name, Variety	Percent Reduction	'Culmorum' Reisolated
Cruciferae (Cont'd)			
B. oleracea*	Kohlrabi, 'Early White Vienna' (var. gongyloides)	0	NT
B. rapa L.*	Turnip, 'Purple Top'	0	NT
Raphanus sativus L.*	Radish, 'Scarlet Globe Short Top'	0	NT
Curcurbitaceae:			
Citrullus vulgaris Schrad.*	Watermelon, 'Charleston Grey'	0	NT
C. vulgaris	Watermelon, 'Congo'	10	-
Cucumis melo L.	Cantaloupe, 'Hale's Jumbo'	39	-
C. melo L.*	Cantaloupe, 'Smith's Perfect'	16	-
C. sativus Duchesne*	Cucumber, 'Poinsett'	0	NT
Curcurbita maxima*	Squash, 'Early Prolific Straightneck'	34	-
Euphorbiaceae:			
Ricinus communis L.	Castor Bean	100	-
Gramineae:			
Lolium multiflorum Lam.	Annual Ryegrass, 'Gulf'	27[c]	-
Paspalum notatum Fluegge*	Argentine, Bahiagrass	53	-
Secale cereale L.*	Rye, 'Weser'	15	-
Setaria italica	Millet	0	NT
Sorghum vulgare pers.*	Sorghum	0	NT
Triticum vulgare Vill.*	Wheat, 'Coker'	47[c]	-
T. vulgare	Wheat, 'Hadden'	100	-
T. vulgare*	Wheat, 'McNair'	0	NT
T. vulgare	Wheat, 'Nebeaka Bozu'	5[c]	-

(Continued)

* These cultivars were also screened in postemergence test.

[c] Some seedlings of these varieties showed symptoms of collar rot, brown hypocotyl lesions, and/or rots on mummied cotyledons (cotyledons that did not emerge out of seed coats following germination).

(Sheet 2 of 4)

Table 6 (Continued)

Family, Genus, and Species	Common Name, Variety	Percent Reduction	'Culmorum' Reisolated
Gramineae (Cont'd)			
Zea mays L.	Corn, 'Funks 5945'	4[cd]	+
Z. mays*	Corn, Bantam Golden Cross	18	-
Z. mays*	Corn, 'Silver Queen' (var. rugosa)	0	NT
Leguminosae:			
Archis hypogea L.	Peanut, 'Florerunner'	0	NT
Glycine max L. Merr.*	Soybean, 'Hood'	41	-
G. max*	Soybean, 'Forrest'	0	NT
G. max*	Soybean, 'Pickett'	28	-
Pisum sativum L.	Pea, 'Calif. Blackeye #5'	74	-
P. sativum*	Pea, 'Acre Cream'	83[c]	-
P. sativum*	Pea, Eng. 'Alaska'	0	NT
P. sativum	Pea, Eng. 'Little Marvel'	11	-
Phaseolus limensis MacF.*	Butter Bean, 'Henderson'	0	NT
P. limensis*	Lima Bean, 'Fordhook'	53[c]	-
P. limensis*	Lima Bean, 'Thorogreen'	100[d]	+
Phaseolus vulgaris L.*	Pole Bean, 'Blue Lake'	8	-
P. vulgaris*	Bush Snap Bean, 'Harvester'	9	-
P. vulgaris*	Bush Snap Bean, 'Tendergreen'	4	-
P. vulgaris*	Pole Bean, 'Dade'	0	NT
Trifolium hybridum L.	Clover, 'LA. S-1'	0	NT
Vigna sinensis (Torner) Savin.*	Cowpea, 'Knuckle Purple Hull'	61[cd]	+
Malvaceae:			
Gossypium barbadense L.*	Sea Island Cotton	6	-
Hibiscus esculentus L.*	Okra, 'Clemson Spineless'	14[c]	-

(Continued)

[c] Some seedlings of these varieties showed symptoms of collar rot, brown hypocotyl lesions, and/or rots on mummied cotyledons (cotyledons that did not emerge out of seed coats following germination).

[d] Ungerminated seeds or symptomatic hypocotyls yielded F. culmorum on reisolation in these varieties.

* These cultivars were also screened in postemergence test.

(Sheet 3 of 4)

Table 6 (Concluded)

Family, Genus, and Species	Common Name, Variety	Percent Reduction	'Culmorum' Reisolated
Polygonaceae:			
Rheum rhaponticum L.	Rhubarb	0	NT
Solanaceae:			
Capsicum annuum L.	Pepper, 'Yolo Wonder'	13[c]	-
Lycopersicon esculentum Mill.*	Tomato, 'Homestead'	0	NT
L. esculentum*	Tomato, 'Walter'	0	NT
Nicotiana tabacum L.	Tobacco, 'NN'	0	NT
N. tabacum	Tobacco, 'GCI'	0	NT
Solanum melongena L.	Eggplant, 'Fla. Strain M.'	0	NT
Solanum tuberosum L.	Potato, 'White Kennebec'	26[cd]	+
S. tuberosum*	Potato, 'Red LaSoda'	6[cd]	+
Tetragoniaceae:			
Tetragonia expansa L.	New Zealand Spinach	0	NT
Umbelliferae:			
Apium graveolens L.*	Celery, 'Pascal'	0	NT
Coriander sativum L.	Coriander	0	NT
Daucus carota L. var. sativa DC*	Carrot, 'Imperator'	0	NT
Heracleum sp.	Parsnip, 'Hollow Crown'	50	NT

* These cultivars were also screened in postemergence test.

[c] Some seedlings of these varieties showed symptoms of collar rot, brown hypocotyl lesions, and/or rots on mummied cotyledons (cotyledons that did not emerge out of seed coats following germination).

[d] Ungerminated seeds or symptomatic hypocotyls yielded F. culmorum on reisolation in these varieties.

(Sheet 4 of 4)

of castor bean, 'Hadden' wheat, and 'Thorogreen' lima bean. However, of the 35 varieties with reduced seed germination, only 13 had symptoms of collar rot, brown lesions at the hypocotyls, or cotylendonary rot associated with the inability of the cotyledons to shed seed coats after germination.

69. The Dutch isolate of F. roseum 'Culmorum' was reisolated from only 5 of the 13 cultivars that had symptoms. It appears that at very high inoculum levels, such as used in this test (35,000 spores/g soil), some reduction in germination may result in some crops, together with diseaselike symptoms. However, these results do not negate the biocontrol potential of this pathogen for the following reasons: (a) it is unlikely that crops will be irrigated with the pathogen-treated water when inoculum levels are high; (b) the fungus may not survive or multiply to the inoculum levels used in this test; and (c) the fungus is not likely to survive in the warm soils of Florida. Furthermore, these results are to be evaluated in relation to postemergence and field host-range test.

70. Seedlings of 44 cultivars, including 42 which are identified in Table 6 with asterisks, were tested for foliar, stem, and root infections by the Dutch isolate of F. roseum 'Culmorum'. Seedlings were established in sterilized soil in pots and sprayed with conidial inoculum of approximately 1 million spores/mℓ of water. The fungus was sprayed on leaves and stems until the excess ran off into the soil around the stem. Plants were then incubated in the greenhouse for 3 weeks or more and observed for infections on leaves, stems, and roots. In all cases, the fungus failed to infect the plants. Additional hosts are being tested at this time.

71. The temperature optimum for in vitro growth of the Dutch isolate of F. roseum 'Culmorum' has been determined. On potato dextrose agar, the fungus grew between 15° and 30°C, but the rate of growth was better between 16° and 27°C, and the peak growth was attained earliest at 21°C. The fungus thus appears to be adapted for relative cooler temperature, and is likely to survive in Florida waters, but not in the warm soils of the southeastern United States.

72. The Dutch isolate F. roseum 'Culmorum' was capable of respiring (using O_2) at a rate 2 to 3.5 times higher than an isolate of the same fungus from a culture collection. Whether or not this preliminary finding relates to the ability of the fungus to parasitize and kill hydrilla has not yet been determined.

Other Pathogens

73. Phycomycetes are fungi frequently referred to as water molds. As the name implies, they are adapted to survival in an aquatic environment. Several species are parasitic on higher plants, which they attack under conditions of high moisture. Many of these pathogens have a wide host range and will attack several plant species. Most of the latter pathogens are soil inhabiting. The susceptibility of hydrilla to 25 such pathogens belonging to three genera was tested. Out of these, three would consistently attack hydrilla under conditions of this test (sprigs of hydrilla in test tubes of distilled water). Results are shown in Table 7. However, larger scale tests in 3.8-ℓ jugs and 18.9-ℓ aquaria yielded inconsistent pathogenicity results. Similar inconsistent results were also obtained with unidentified species of Pythium and Phytophthora isolated from declining hydrilla in Orange Lake, Florida. Despite this inconsistency, the search for pathogens of hydrilla in the Phycomycetes should be continued because of the adaption of these fungi to an aquatic mode of existence.

74. Bacteria have often been associated with declining populations of hydrilla. In fact, the so-called milky water disease is associated with high bacterial counts in the cloudy water. When hydrilla began to decline in Orange Lake, Florida, bacteria were incriminated as the causal agent of the decline by some observers. Therefore, a study was set up to determine if pathogenic bacteria could be isolated from declining plants. The results are presented in Appendix D. However, the presence of pathogenic bacteria on declining hydrilla in Orange Lake could not be verified.

Table 7

Reaction of Hydrilla Inoculated with Various Phycomycetes

Phycomycete		Hydrilla Reaction*
Aphanomyces cochliodes Drechs.	Beta vulgaris L.	-
A. euteiches Drechs.	Pisum sp.	-
Phytophthora cinnamoni Rands.	Persea americana Mill.	-
P. citrophthora (R. E. Sm. and E. N. Sm.) Leonian	Citrus sp.	-
P. cryptogea Pethybridge and Lafferty	Aster sp.	-
P. dreschleri Tucker	Citrus sp.	-
P. erythroseptica Pethybridge	Solanum tuberosum L.	++
P. palmivora ButL.	Fiscus sp.	-
P. parasitica Dast.	Lycopersicon esculentum Mill.	+++
P. parasitica	Nicotiana tabacum	-(?)
P. stellata ButL.	unknown	-
Pythium acanthicum Drechs.	unknown	-
P. aphanidermatum (Eds.) Fitz.	Chrysanthemum sp.	-
P. carolinianum Matthews	unknown	-
P. debaryanum Hesse	unknown	-
P. graminicolum Subramaniam	unknown	-
P. helicoides Drechs.	unknown	-
P. irregulare Buisman	unknown	-
P. irregulare	Caladium sp.	-
P. myriotylum Drechs.	unknown	-
P. paroecandrum Drechs.	Zea mays L.	-
P. polytylum Drechs.	unknown	+

* No reaction (-), slightly damaged (+), moderately damaged (++), severely damaged (+++).

PART IV: EFFECTS OF PATHOGENS ON OTHER WEEDS

75. As a part of this program, other weed-pathogen combinations were investigated on a limited basis. Of particular interest were pathogens of alligatorweed and Eurasian watermilfoil.

76. Thus far, no naturally occurring pathogens have been found to affect Eurasian watermilfoil. However, the fungus Rhizoctonia solani Kuehn will attack this plant under laboratory conditions. As with hydrilla, fungi belonging to the Phycomycetes need to be investigated as potential pathogens of milfoil.

77. The fungus Alternaria alternantherae Holcomb and Antonopoulos affects alligatorweed in Louisiana and Florida. A small-scale field test of this fungus was conducted in 1977 and repeated in 1978. It shows some promise as a biocontrol and its use should be considered if present methods of biocontrol using the flea beetle begin to falter. Cultures of the fungus will be maintained for possible future use.

78. Another fungus, Helminthosporium sigmoideum Cav., caused severe damage on giant cutgrass in Lake Seminole, Florida, in the summer of 1978. It would appear to have some potential and will also be maintained for future use if needed.

PART V: CONCLUSIONS

79. During the past 3 years, considerable additional progress has been made in reaching the goal of utilizing plant pathogens in biological control programs for aquatic weeds.

80. The pathogen C. rodmanii has been shown to be effective against waterhyacinth in tests in both Louisiana and Florida. Methods of culturing and disseminating this fungus for biocontrol purposes have been developed, and considerable basic information concerning the host parasite relationship has been elucidated. This fungus shows such promise that the University of Florida has patented it for use in biocontrol programs. In addition, AL has entered into an agreement with the University of Florida to develop the fungus into a marketable product form for possible worldwide distribution. Abbott Laboratories is also cooperating with WES researchers in a Large Scale Operational Management Test in Lousiana.

81. Two exotic pathogens also show biocontrol potential. The rust fungus, U. eichhorniae, from Argentina appears to have potential in programs for the biocontrol of waterhyacinth. The fungus Fusarium roseum 'Culmorum' from Holland shows promise for hydrilla control. Research with these fungi has been slowed because of the necessity of conducting research studies in quarantine. Therefore, additional research is needed.

82. Investigations have also been conducted with other pathogens and potential pathogens on various hosts. As a result, the program has advanced faster than anticipated and is nearing its goal in some areas. Based on this work, it can be concluded that the original proposition is correct--plant pathogens are viable candidates as biocontrol agents for aquatic weeds.

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APPENDIX A:

REEVALUATION OF NOZZLE SYSTEMS FOR APPLICATION OF CERCOSPORA RODMANII*

Introduction

1. A field test of nozzle types was carried out in test pools at the University of Florida, Gainesville, Florida.

2. The objective of this experiment was to retest certain nozzle types to determine if nozzles have any effect on the size of inoculum particles and on the pathogenicity of Cercospora rodmanii Conway, a biological control organism currently being experimented with for waterhyacinth control.

Materials and Methods

Nozzles

3. The following nozzle types were selected for testing: Delavan RD-10, #45 core (raindrop); Delevan WR-25 (mister); and Delevan hollow cone, #10 disc (hollow cone).

4. Each nozzle was used in conjunction with a Spray Systems gun jet #12GH, adapted with a Delevan gun #3160. A portable spray power rotary pump with a modified even flow tank was used to deliver the spray. Spray pressure was 20 psi (138 kPa). Each nozzle was calibrated to determine the amount of flow per second. The raindrop nozzle delivered 300 ml/ sec, the mister 200 ml/sec, and the hollow cone 200 ml/sec.

Inoculum

5. Isolate WH 9 of C. rodmanii was grown on potato-dextrose broth plus 0.5 percent yeast extract (PDBY) for approximately two and one half weeks. It was then chopped in a blender, diluted with water, and sprayed onto plants at a concentration of 48 g/m^2 of wet weight mycelium.

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* Reese, Deborah F., research assignment, 1977, University of Florida, Gainesville, Fla.

6. Plants in each test pool received the same amount of inoculum. This was regulated by varying the length of time of spraying. The pools, made from metal and lined with plastic, were 2.6 m^2 and were filled with floating waterhyacinths of approximately the same number and age. An important technique utilized in this experiment was the color coding of the oldest and youngest living leaves of 15 plants from each pool.

7. The pools were numbered 1, 2, and 3. The inoculum was applied to waterhyacinths in pool 1 using the raindrop nozzle. Waterhyacinths in pool 2 were inoculated using the mister nozzle. Waterhyacinths in pool 3 were inoculated using the hollow cone nozzle. Each pool was rated for damage at 2 and 7 weeks after inoculation.

Results

8. Immediately after they were sprayed, 10 leaves per plot were removed. Using a binocular microscope, each leaf was examined for average size of inoculum particles and average number of particles per unit area (3.14 cm^2). Dimensions of the 10 particles per leaf were recorded, and the area of an ellipse formula was used to calculate the area of each particle. The unit area was chosen at random on each leaf. The following tabulation is a breakdown of data on particle size and number of particles/unit area:

Nozzle	Average Area mm^2	Range mm^2	Particles/ Unit Area
Raindrop	0.85	0.90-1.5	7.9
Mister	0.86	0.47-1.7	9.0
Hollow cone	0.76	0.46-1.3	8.1

9. The leaves of 10 waterhyacinth plants from each of the three pools were rated using the Conway system of rating disease damage from 0 to 9. Zero indicates no infection and nine indicates death of the leaf. The following tabulation is a breakdown of the damage caused by inoculum sprayed from each of the three nozzle types:

	27 June	5 August
Raindrop: (Pool 1)		
Total Damage	220	507
Average damage/plant	22.0	50.7
Average damage/leaf	3.4	8.2
Mister: (Pool 2)	294	526
Total Damage		
Average damage/plant	29.4	52.6
Average damage/leaf	4.1	8.5
Hollow cone: (Pool 3)	233	472
Total Damage		
Average damage/plant	23.3	47.2
Average damage/leaf	3.6	8.1

10. Following inoculation, plants in pool 2 had the greatest amount of total damage, average damage/plant, and average damage/leaf. Plants in pool 3 had an intermediate amount of total damage, average damage/plant, and average damage/leaf. Plants in pool 1 had the least total damage, average damage/plant, and average damage/leaf. The average damage/leaf was studied because each plant did not have the same number of leaves. On 27 June, the difference in total damage to waterhyacinth in the pools was apparent from visual inspection alone.

11. On August 5, the plants in the pools were rated again to see if nozzle type had had any effect on the total damage of the plants over a period of time. Again, the plants in pool 2 had the greatest amount of total damage, average damage/plant, and average damage/leaf. This was the same result obtained in the earlier experiment. Plants in pool 1 had intermediate damage in all three categories, and plants in pool 3 had the least damage in all three categories. It was difficult to visually distinguish damage on plants in the pools that had been sprayed with inoculum using different nozzles.

Discussion

12. Infection of waterhyacinth by C. rodmanii is through penetration of hyphae through stomatal openings. Hyphae are prone to drying, necessitating the delivery of larger inoculum particles. Delivery

of larger inoculum particles ensures less drying before hyphae invade the stomata of the waterhyacinth leaf. It was felt that, with greater initial infection, greater amounts of conidia would eventually be produced. This increase in conidial production would increase the infection rate and ultimately increase the progress of an epidemic.

13. Damage values obtained in a previous tes' included the counting of leaves that may have been actually sprayed with inoculum. This gave possible misleading results. By color coding the oldest and youngest leaves of the plants at spray time, analysis for damage could be limited to only those leaves that had actually been inoculated.

14. This test did not include a control or adequate replicates. However, results from this test replicated the results obtained in the spring test, 1977.

Conclusions and Recommendations

15. This experiment, a retest to check previous results, used a different method that recorded and rated only those leaves actually sprayed. This experiment confirmed that the mister nozzle sprayed the largest inoculum particle; inoculum sprayed from it caused the greatest initial damage and the greatest long-term damage. Therefore, it is recommended that the mister nozzle (Delevan WR-25) be used in application of C. rodmanii as a biological control organism.

APPENDIX B:

DISEASE RESISTANCE MECHANISMS IN WATERHYACINTHS AND THEIR SIGNIFICANCE IN BIOCONTROL PROGRAMS WITH PHYTOPATHOGENS*

1. The waterhyacinth is a free-floating vascular hydrophyte that has colonized much of Florida's inland water. In 1970, a program was initiated at the University of Florida to study biological control of this noxious plant with phytopathogens. One of the pathogens currently being studied is the fungus Acremonium zonatum that causes severe spotting on both leaves and petioles of the waterhyacinth under conditions of high humidity.

2. During field trials with this fungus, it was observed that small, young plants displayed fewer symptoms after infection than did larger plants in the same plots. It also appeared that large plants infected with A. zonatum exhibited a faster rate of leaf regeneration than did smaller plants. The present study was initiated to determine if small plants were in fact more resistant to A. zonatum than large plants; if meristematic activity in the plants was altered after infection; and, if so, to what extent host phenolic compounds and their oxidizing enzymes, namely polyphenoloxidase (PPO), were responsible.

3. Waterhyacinths displayed various degrees of resistance to A. zonatum depending on their morphotypic state of development. Results of this study indicated that these differences in resistance were due to the variations in phenol chemistry among plants in different sizes and to subsequent changes induced by infection (Table B1).

4. Small plants are more resistant to fungal attack than are medium or large plants, based upon the number of lesions per leaf after infection. It appears that the presence of high concentrations of phenolic compounds does not itself impart resistance to the pathogen. Rather it is the oxidation of these compounds by enzymes, such as polyphenoloxidase (PPO), which is responsible for the resistance. This view is supported by qualitative and quantitative data on the phenols

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* Martyn (1977).

in plant morphotypes and is coincident with the observed differences in resistance.

5. Small plants, by virtue of having fewer phenol cells/mm^2 leaf area, have less total phenol content/leaf than larger plants. If phenol content alone was responsible for disease resistance, then small plants would be more susceptible than large plants; but they were not. In this case PPO activity is apparently the mediating factor. The rate of enzyme activity in small plants is threefold that in large plants; presumably therefore, oxidation of polyphenols to quinones is much greater in small plants. Thus, small plants are initially more resistant to pathogenic attack than are larger plants.

6. After the disease has progressed for several weeks, the differences in resistance among the morphotypes is no longer evident. Each plant size exhibits a percent-total-diseased leaf area which is statistically the same (approximately 40 percent). It is believed that this equalization of disease severity results from a gradual loss in resistance by small plants while at the same time there is a gradual increase in resistance by large plants. Again, quantitative data of the phenol metabolism can be correlated with this change.

7. The total phenol content decreases significantly after infection in small- and medium-sized plants. This is coincident with a reduction in PPO activity. The coupling of these two phenomena may account for the decrease in resistance in small plants. Large plants, on the other hand, retain their total phenol content and at the same time exhibit a threefold increase in PPO activity. Therefore, an increase in polyphenol oxidation would be expected to occur and could account for the increase in resistance in large plants.

8. In essence, then, the point being made is: if infected small plants retain the phenol content and PPO activity of healthy plants, then disease severity would probably be limited to much less than 40 percent. Similarly, if infected large plants retain the PPO activity of healthy plants, disease would progress to much higher percentage, perhaps 60 to 70 percent.

9. However, because each morphotype responds to infection

differently (in most cases in contrast to each other), disease severity balances among the plant size at approximately 40 percent of the leaf-surface area.

10. If disease severity is viewed, not from a percentage of leaf-area infected, but as a reduction in plant growth, then data on leaf regeneration rates among the morphotypes become of prime importance. It has been observed that infected large plants regenerate two to three times as many new leaves as do infected small plants. This too is correlated with the plant's phenol chemistry.

11. It has been shown that A. zonatum is capable of synthesizing indolacetic acid (IAA) in vitro and that this is one explanation for the increased growth observed in large plants. More important, however, is the fact that phenols are known inhibitors of IAA oxidase, the enzyme responsible for controlling IAA level in the plant. It has already been pointed out that the different waterhyacinth morphotypes vary in phenol content, both prior to and after infection. The higher phenol content in large plants could account for the increased growth in large plants by inhibition of the IAA oxidase system.

12. Perhaps the most significant data supporting a positive role for phenols in disease resistance come from the localization studies of PPO in healthy and diseased plants. Enzyme activity is localized in the thylakoids of chloroplasts in only three cell types in healthy plants. After infection there is a "turn on" in PPO activity in all cells which contain chloroplasts. This turn on in PPO activity is highly suggestive of a vital role for enzymatic oxidation of polyphenols during disease.

13. Before disease can ensue, the pathogen must come into contact with and penetrate its host. In this regard, A. zonatum can enter the waterhyacinth by either of two ways: through open stomata or by directly penetrating the unbroken cuticle of the leaf. Intracellular colonization is enhanced by the diffuse secretion of cellulolytic enzymes and perhaps by the localized secretion of pectolytic enzymes.

14. Growth of A. zonatum was either unaffected or stimulated by seven different phenolic acids in concentrations up to 1000 ppm in minimal

media. When yeast extract was added to the media as a growth supplement, one phenolic acid, p-coumaric, was found to be inhibitory. In addition, fungal growth was enhanced on media containing healthy leaf extracts.

15. Several cytological changes were observed in the cells from infected waterhyacinth leaves. First, chloroplasts in cells of healthy leaves had an abundance of starch granules that disappeared after infection. Second, there were only a few plastoglobuli in chloroplasts in healthy cells, but after infection, they increased both in size and in number. Third, there was a noticeable increase in microbodies in the cytosol of infected cells. It was believed that each of these cytological changes was the result of a shift in host metabolism induced by infection.

16. It is concluded that waterhyacinths have at least two distinct biochemical defense mechanisms that are related to phenol metabolism and plant size. The first is the presence of high concentrations of polyphenols in specialized phenol cells that, under the proper conditions, can serve as toxicants to potential pathogens. The second proposed defense mechanism of waterhyacinths is an acceleration of its growth rate brought about by the inhibition of IAA oxidase by the phenolic compounds.

17. Of the above mechanisms, the one that is operational is dependent upon the plant's morphotypic stage of development. It is believed that, initially, small plants defend against pathogenic attack by virtue of their high PPO activity whereas large plants respond by increased leaf production. Medium-sized plants appear to have a combination of both mechanisms.

18. In consideration of A. zonatum as a potential biocontrol agent for waterhyacinths, it is concluded that best control would be achieved with small, young plants rather than with the larger, more mature plants. In this regard, control procedures should be initiated early in the spring when new plants start to grow and colonize the body of water.

Table B1

Differences and Similarities Among Healthy and A. zonatum-Infected Waterhyacinth Morphotypes

Assessment Criteria	Morphotype		
	Small	Medium	Large
Mean number of lesions per leaf	3.7	12.8	18.3
Percent total disease	41.3	37.0	39.5
Mean number of phenol cells/mm^2	33.6	41.8	48.7
PPO rate (healthy)	1.53	0.80	0.47
PPO rate (diseased)	0.90	0.70	1.36
PPO localization (healthy)	3 cell types*	3 cell types*	3 cell types*
PPO localization (diseased)	All cells**	All cells**	All cells**
Type of phenolic acids (healthy)	6	6	9
Type of phenolic acids (diseased)	7	7	9
Total phenols (healthy)	92 g/g tissue	106 g/g tissue	104 g/g tissue
Total phenols (diseased)	80 g/g tissue	96 g/g tissue	105 g/g tissue
Fungal growth (healthy)	Stimulative†	Stimulative†	Stimulative†
Fungal growth (diseased)	Stimulative††	Stimulative††	Stimulative††
Leaf regeneration (healthy)	27.3%	28.5%	46.1%
Leaf regeneration (diseased)	21.6%	33.9%	93.3%

* Vascular parenchyma, bundle sheath, and phenol cells.
** All cells which contain chloroplast.
† P = 0.05: growth increased over control.
†† P = 0.05: growth increased over healthy counterpart.

APPENDIX C:

A REQUEST TO CONSIDER RELEASE OF UREDO EICHHORNIAE FROM QUARANTINE FOR ESTABLISHMENT OF A CULTURE IN LAKE ALICE*

Pathogen

1. Uredo eichhorniae Fragoso and Ciferri is the only known obligate pathogen of waterhyacinth. It was described in 1927 from the Dominican Republic (Cifferi and Fragoso 1927).** Presently, it appears to be restricted to portions of Argentina, Uruguay, and Southeastern Brazil (Charudattan et al. 1976). Being a member of the highly specific Uredinales, U. eichhorniae may be a desirable biological control agent for waterhyacinth. A 2-year research on U. eichhorniae has been aimed at discovering (a) the telial stage of the pathogen either from nature or by artificial induction, (b) its relation to rusts in Pontederiaceae, and (c) its host-range in Pontederiaceae.

Current Knowledge

2. Telia have not been found thus far in nature or under artificial treatments of uredia-bearing leaves of waterhyacinth in laboratory. In the absence of knowledge concerning its teliospore, the life cycle of U. eichhorniae remains imperfectly understood. However, efforts in this study have led to the discovery of the teliospore in the rust on Eichhornia azurea (Swartz) Kunth. and the confirmation of this rust as Uromyces pontederiae Gerard. The fact that another species of Eichhornia (E. azurea) is susceptible to U. pontederiae strongly suggests that the waterhyacinth rust is also a Uromyces, possibly U. pontederiae (Charudattan et al. 1976).

3. The rusts in Pontederiaceae are difficult to separate on the basis of uredospore morphology (Charudattan and Conway 1975, Charudattan

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* Charudattan, R., request for quarantine release, 1976.
** References refer to items presented in alphabetical order in the list of references following the main text on page 46.

et al. 1976). However, on three host genera, Pontederia, Heteranthera, and Eichhornia (E. Azurea), the rusts have been recognized as belonging to the genus Uromyces. On E. crassipes and Reussia spp., rusts are known only in uredial stages. In Florida, species of Pontederia and Heteranthera and E. crassipes occur. Other members of Pontederiaceae which have rusts, namely E. azurea and Reussia spp., do not occur in North America and hence their rusts have not been reported in the United States. Uromyces pontederiae has been found in the United States only on Pontederia cordata L. and P. lanceolata Nutt. in New York, Florida, Pennsylvania, Virginia, North Carolina, Georgia, Missouri, and Texas (Anon. 1969). It occurs on Pontederia spp. and E. azurea in Argentina, Uruguay, and Brazil (Anon. 1969 and Allen 1971). Uromyces heterantherea and U. eichhorniae have not been reported in the United States. Apparently, the U. pontederiae from Pontederia spp. in the United States is pathologically unrelated to U. eichhorniae even though taxonomically the latter may fall into U. pontederiae (Charudattan and Conway 1975 and Charudattan et al. 1976).

4. Several attempts in Florida to cross-infect waterhyacinth with isolates of U. pontederia from Florida, Argentina, Uruguay, and Brazil have failed. Similar failure resulted in Argentina from cross-inoculation of waterhyacinth with a local isolate of U. pontederiae. However, waterhyacinth from Florida has been shown to be susceptible to U. eichhorniae from Argentina (Charudattan et al. 1976).

5. In an attempt to learn about the incidence of U. eichhorniae on waterhyacinth, annual surveys for this rust in Argentina were undertaken. The rust occurred throughout 1975-76 (June to May) in uredial stage, strongly suggesting cyclic uredospore infections. The highest incidence of rust occurred in the fall with notable incidence also in the spring. However, the rust is very localized in distribution in Argentina, Uruguay, and Brazil, and, in comparison with incidence of U. pontederiae in Florida and in South America, U. eichhorniae was not considered epiphytotic in Argentina (Charudattan and Conway 1975).

6. Thus, results obtained so far point to U. eichhorniae as being pathologically distinct from Uromyces pontederiae, very localized in

distribution in South America, and potentially host specific to waterhyacinth.

7. So far stages 0 and I (spermatium and aecium) are unknown in U. pontederiae, U. heterantherae, and U. eichhorniae. It is possible that no alternate hosts of economic importance are infected by stages 0 and I in nature. It is assumed that the Uromyces spp. and U. eichhorniae are probably heteroecious rusts. However, they may not be; they may be autoecious and microcyclic.

Reason for Seeking This Quarantine Clearance

8. To evaluate U. eichhorniae as a biological control agent, information is needed on several basic aspects on its pathology. The inadequate spore supplies and infected waterhyacinth plants, along with limited instrumentation and space in the quarantine greenhouses, have hampered speedy experimentation. Several futile attempts at maintaining adequate U. eichhorniae cultures on waterhyacinth have suggested that conditions in the quarantine greenhouse at Gainesville are inadequate for research on this rust. An evaluation of this rust as a biological control agent must include its ability to stress waterhyacinth under field conditions. Currently, this cannot be done due to the quarantine restriction on U. eichhorniae.

9. Many of these problems can be solved by working with the rust outside the quarantine facility. Outside the quarantine facility, several instrumental analyses on spores of U. eichhorniae can be done and attempts can be made to establish a rust nursery on the University of Florida campus.

Safeguards

10. Considering the restricted occurrence of U. eichhorniae in Argentina, Uruguay, and Brazil, the rust may not spread far and wide in Florida after release on the University of Florida campus. To date, no economically important alternate hosts are known for rusts in

Pontederiaceae. Uredo eichhorniae may not be an exception. Being a rust pathogen, U. eichhorniae will have a very narrow host range (assuming the presence of alternate host(s) in Florida) and will be safe to release on the University of Florida campus.

Proposed Handling of Uredo eichhorniae During and After Release

11. Uredospores will be collected under quarantine, free of any contaminants, and transferred to waterhyacinths at designated sites in Lake Alice, University of Florida. Infection and spread will be monitored. A maximum of 12 releases in 1977-78 will be attempted (starting from the date of approval of this request). Further releases may be negotiated with the regulatory agencies in 1977 or in later years, as needed.

12. Studies on (a) greenhouse inoculation, (b) spore physiology, and (c) cross-inoculations on species of Pontederia, Heteranthera, and E. crassipes will be attempted in addition to monitoring the rust at introduced sites.

13. Controlled studies on eradication of U. eichhorniae with fungicides and other methods will be run. Should the need for eradication of this pathogen from release sites arise, necessary steps will be taken to accomplish this.

APPENDIX D:

SURVEY OF HYDRILLA UNDERGOING ANNUAL DECLINE FOR PATHOGENIC BACTERIA*

Introduction

1. Hydrilla verticillata (L. f.) Royle is a submersed vascular aquatic macrophyte. It is a monocot and belongs to the Hydrocharitaceae family. The plant is thought to have been introduced into Florida around 1960 (Haller 1976).** Since its introduction, the plant has spread to many major freshwater lakes and streams and is threatening all those not currently infested. It grows in dense mats that may quickly destroy the public usefulness of any infested water body.

2. Florida may have been the first site of introduction, but the plant has now become a problem in many southeastern and southwestern states. In these areas and in northern Florida its growth is similar to an annual plant.

3. In early spring, as the water temperature increases and days become longer, new shoots arise from stem fragments, tubers, and turions that have survived the winter in the hydrosoil. Hydrilla soon outgrows native aquatic plants since it utilizes light more efficiently (Van, Haller, and Bowes 1976). It grows towards the surface of the water from depths as great as 12 to 15 m. The hydrilla may then form dense, entangled mats that can reduce light penetration to less than 5 percent at a 30-cm depth (Haller 1976). Throughout the summer, hydrilla stores starch in its stems, stolons, and underground rhizomes. By late summer, the mat is at maximum density. An increase in epiphytic growth is commonly observed at this time (Berg 1977). As the summer passes into fall, hydrilla begins to undergo annual decline. At this time of the year, the weather is the warmest, and the growing season and light intensity are near maximum. Plants undergoing annual decline exhibit

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* Daryl F. McKinney, special research project, University of Florida, Gainesville, Fla.

** References refer to items presented in alphabetical order in the list of references following the main text on page 46.

chlorotic leaves and stems that may become transparent. Leaf abscission is common and stems fragment easily. These symptoms are always associated with the surface growth of the hydrilla.

4. There are several possible explanations for annual decline. It is possible that hydrilla loses an excessive amount of polysynthate as dissolved organic matter (DOM) through its leaves; epiphytes then use the DOM. This would correlate with the observed growth increase of epiphytes (Allen 1977 and Snad-Jensen 1977). These epiphytes (bacteria and algae) may then interfere with CO_2 diffusion or photosynthesis by the hydrilla (Van, Haller, and Bowes 1976). When photosynthetic processes subside, the plant would begin to die. The epiphytes may produce toxin metabolites that damage hydrilla tissue. Another theory is that plant pathogens are involved in annual decline (Berg 1977).

5. This research project was initiated to study the possibility that annual decline of hydrilla is caused by a plant pathogenic bacterium. The criteria of the study are that (a) the pathogen will be isolated from diseased hydrilla, (b) it will be grown in pure culture, (c) it will be inoculated on healthy hydrilla where it would have to cause symptoms associated with annual decline, and (d) the bacterium will have to be reisolated from the test plants.

Materials and Methods

Isolation

6. Bacterial isolations were made from samples of hydrilla expressing symptoms of annual decline. The samples were collected on 14 October 1977 near the middle of Orange Lake. Isolations were made at the site of collection and also in the lab.

7. At the site, isolations were made by cutting necrotic hydrilla stem and leaf tissue into approximately 0.5-cm pieces and surface sterilizing 20 of these pieces. Sterilization was accompanied by rinsing the hydrilla pieces with a 10 percent Clorox solution in a petri plate for 1 min followed by two rinses of sterile deionized water. The surface-sterilized pieces were then plated on hydrilla infusion agar

(10 g crushed hydrilla and 15 g Bacto agar in 1 ℓ of deionized water), nutrient agar, and potato dextrose agar. Plates were then incubated at 25°C for 3 days.

8. Lab isolations were made from hydrilla sprigs maintained in sterile deionized water. The sprigs were shaken vigorously in three sterile water rinses to remove most of the epiphytic bacteria. Sprigs were then crushed with a glass rod in small tubes containing 2 ml of sterile saline solution. Loopfuls of the resulting suspensions were streaked on hydrilla infusion agar, NA and PDA. Plates were incubated at 25°C in the dark for 3 days.

9. Seven apparently different bacteria were than selected from the site and lab isolations based on colony morphology and color. Stock cultures of these seven bacteria were maintained on NA slant tubes under paraffin oil in the refrigerator.

Inoculum preparation

10. Two-litre flasks containing 500 ml of nutrient broth were inoculated from stock cultures and the culture was shaken at approximately 60 strokes per minute for 24 hr. Isolates 6 and 7 grew extremely slow at these conditions. They were examined microscopically and found to be myceloid, apparently actinomycetes. They were not tested for pathogenicity due to their slow growth and the fact that few actinomycetes have been found to be plant pathogens.

11. The remaining five bacterial isolates were prepared for inoculum by first sedimenting them from the nutrient broth by centrifugation (10,000 G for 10 min). The supernatant was decanted and the pellet was resuspended in 50 ml of sterile saline (0.85 percent) solution. Each isolate was then adjusted at 0.25 transmittance with a colorimeter. This equalled approximately a 10^8 cells/ml concentration.

Inoculation

12. Healthy hydrilla was collected from Rodman Reservoir on 18 October 1977. Sprigs of hydrilla were washed thoroughly with running tapwater before being rinsed twice with sterile deionized water.

13. Two inoculum systems were used. The first consisted of incubating an 80- to 100-mm-long growing hydrilla shoot in a 30- by 150-mm

glass tube in a bacterial suspension. The suspension was prepared by adding 4 ml of 10^8 cells/ml bacterial concentration to 36 ml of sterile water. This resulted in a 10^7 cells/ml concentration around each hydrilla sprig. Three replications were made of each treatment. A 4-ml saline solution was added to each of three control tubes. All the tubes were mixed with a Vortex mixer.

14. The second inoculation method was similar to the first except that tubes were vacuum infiltrated 2 min at 25 mm Hg vacuum after inoculation.

15. All tubes were incubated at approximately 22°C on the lab windowsill for 3 weeks after inoculation.

16. Disease assessment after 3 weeks was made by visually comparing inoculated tubes with the control tubes. Hydrilla sprigs were rated as either healthy (H), chlorotic (C), or necrotic (N) (see Table D1).

17. Besides visually assessing the inoculated hydrilla, reisolations were made from all inoculated and control hydrilla sprigs. A central piece of each stem containing one node and three leaves was surface sterilized and rinsed twice with sterile water before being crushed with a glass rod in a 2-ml saline solution.

Results

18. The seven colony types are listed in Table D1. The majority (4/7) of the colonies were gray or white. This agreed with Berg's (1977) finding of three white colony types commonly isolated from hydrilla undergoing annual decline. Colony types 2 and 3 may have been the same bacterium, with 3 being a rough mutant. All bacterial types grew similarly on NA as they did on hydrilla infusion agar. This indicated a lack of specific growth requirements.

19. Results from inoculated hydrilla sprigs showed that bacteria tested were not pathogenic to hydrilla (Table D2). The chlorosis observed was probably due to nutrient deficiency and not a pathogenic response.

20. Reisolations (Table D3) from the inoculated and control hydrilla resulted in a random pattern of bacteria reisolated. Many of the reisolations contained bacteria that had not been inoculated on that particular hydrilla sprig. These bacteria were compared to stock tubes and related visually on the basis of the similarities in gross morphology.

Discussion

21. Bacterial diseases are associated with enormous concentrations (10^9 cells/ml) of bacteria in diseased tissue. In concentrations of this magnitude, it is seldom that they are not present in reisolations. There was no preponderance of any one bacterial type found in any of the reisolations and none of the colony types tested produced symptoms of annual decline in test inoculations.

22. Berg (1977) previously isolated three white bacterial types from hydrilla expressing annual decline and inoculated unknown concentrations of these on healthy hydrilla. He found symptoms similar to annual decline only to occur when he mixed the three different bacteria and again inoculated unknown concentrations on hydrilla. He reasoned that annual decline was due in part to toxins (or toxin) produced by the three bacteria. It is more probable that he used excessive numbers of bacteria in his inoculations and that normally nontoxin metabolites produced by the bacteria became concentrated to such an extent that they were toxic. His tests with the toxins found them to be nonspecific on other aquatic plants; this supports the idea that they are superconcentrated metabolites and not toxins per se.

23. It is probable that the bacteria found in this survey were epiphytic and not associated directly with the symptoms of annual decline of hydrilla. This view is supported by the results of reisolations (Table D3) of inoculated and noninoculated hydrilla, which indicate the presence of these bacteria on healthy hydrilla.

Table D1

Description of Seven Isolated Bacteria

Grown on NA Plates

Isolate	
1	White, gummy, some slime production
2	Gray, some slime, smooth colony
3	Gray, some slime, rough colony
4	Yellow, some slime (nonfluorescent on KMB)
5	Yellow, copious slime, myceloid
6	White, very slow growth, myceloid
7	Pink, some slime, gummy, slow growth, myceloid

Table D2

Visual Comparison of Hydrilla Sprigs

Three Weeks After Inoculation

Inoculation Method	Tube	Isolate Inoculated* 1	2	3	4	5	Control (Saline Only)
Method 1	1	H	H	H	H	H	H
	2	H	H	H	H	H	H
	3	H	H	C	H	C	H
Method 2	1	H	C	H	C	H	H
	2	H	H	H	H	H	C
	3	H	H	H	H	H	H

* H = Healthy, C = Chlorotic.

Table D3

Reisolation (2-3 Days on NA)

Inoculation	Tube	Treatment					
		Control	1	2	3	4	5
Method 1	1	(5)	(4)(5)	---	(3)	(4)(2)	---
	2	(2)(3)(4)	(1)	(2)(7)	---	---	(5)
	3	---	---	(2)(5)	---	---	(5)(7)
Method 2	1	---	(4)(5)	(2)	(3)	(4)	(2)(5)
	2	(7)(6)	---	(2)(5)	(4)	(4)(7)	---
	3	---	(1)	---	---	(7)	---

NOTE: ( ) contain the bacterial type reisolated, compared with stock cultures.

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.

Freeman, T E
Biological control of aquatic plants with pathogenic fungi / by T. E. Freeman ... [et al.], Department of Plant Pathology, University of Florida, Gainesville, Fla. Vicksburg, Miss. : U. S. Waterways Experiment Station ; Springfield, Va. : available from National Technical Information Service, 1981.
47, [20] p. : ill. ; 27 cm. (Technical report - U. S. Army Engineer Waterways Experiment Station ; A-81-1)
Prepared for Office, Chief of Engineers, U. S. Army, Washington, D. C., under Contract No. DACW39-76-C-0097.
References: p. 46-47.

1. Aquatic plant control. 2. Aquatic plants. 3. Biological control. 4. Fungi. 5. Pathogenic fungi. 6. Water hyacinths. I. Florida. University, Gainesville. Dept. of Plant Pathology. II. United States. Army. Corps of Engineers. III. Series: United States. Waterways Experiment Station. Vicksburg, Miss. TA7.W34 no.A-81-1