BIOLOGICAL CONTROL OF WATER WEEDS WITH PLANT PATHOGENS

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Abstract

Work was conducted on the evaluation of both endemic and exotic plant pathogens as biological control agents for water weeds. Additional studies investigated the potential use of an integrated program of biological control using plant pathogens and insects. Of the endemic pathogens tested, *Rhizoctonia solani*, *Acremonium zonatum* and *Cercospora rodmanii* exhibited potential as biocontrols. The latter fungus was the most promising. In fact, *C. rodmanii* was established as the causal agent of a decline disease which effectively controlled waterhyacinths in Rodman Resevoir in two out of the last five years. It has the additional advantage of being highly host specific for waterhyacinths. The two exotic pathogens showing the most potential were the waterhyacinth rust, *Uredeo eichhorniae* and *Bipolaris stenospila*. Results of the integrated control studies showed that the action of a combination of pathogens and insects would significantly reduce biomass accumulation in waterhyacinth populations.

As a result of these studies, significant advances have been made in realizing the goal of the utilization of plant pathogens in biocontrol programs for water weeds. It is anticipated that the results of this study will help bring this goal to fruition within the next few years.

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Introduction

As pointed out by Zettler and Freeman (Annual Review Phytopathology 10:455-470) and by Freeman, et al (Florida Water Resources Research Center Pub. 23), plant pathogens have many characteristics that make them ideal candidates as biocontrols for aquatic weeds. They are: (1) numerous and diverse; (2) frequently host specific; (3) easily disseminated and self-perpetuating; (4) will not completely eliminate a host species; and (5) do not formally affect man or other animals. With these points in mind, a modest program was begun at the University of Florida in 1970, with the object of the evaluation and subsequent use of 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's Office of Water Research and Technology, subsequent support from the Florida Department of Natural Resources and the U.S. Army Corps of Engineers (Contracts DACW 73-71-C0002 and DACW 73-73-C-0049), and additional support from the annual allotment program of the Florida Water Resources Research Center.

The program has progressed rapidly considering the lack of initial background information. We have developed a considerable backlog of information (see list of project publications) about diseases affecting aquatic plants. The objective of the utilization of plant pathogens in biocontrol programs for noxious aquatic plants has not yet been completely fulfilled. However, we have reached the critical stage when field evaluation of at least two pathogens of waterhyacinth is fully warranted. An additional three or four organisms also should soon reach this point. All of these efforts are aimed at waterhyacinths. We have also attempted to find and research diseases with biocontrol potential for other aquatic weeds. To do these studies, we have requested and received continuing support of this critical area of research by the U.S. Army Corps of Engineers and the Office of Water Research and Technology. Such support is vital in order to enable us to more completely reach our objective and to begin reaping the benefits of an operational biological control program in the shortest possible time.

Relevance of Research

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

The Florida Department of Natural Resources estimates over 15 million dollars are expended annually in Florida for aquatic weed control. These control efforts are concentrated primarily on the estimated 100 thousand hectares of waterhyacinth (*Eichhornia crassipes*) and 40 thousand hectares of hydrilla (*Hydrilla verticillata*) that occur in the state. Lesser attention is given to the approximately 20 thousand hectares of other aquatic weeds, such as Eurasian watermilfoil (*Myriophyllum spicatum*) and alligatorweed (*Alternathera philoxeroides*) (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 also expanded. Within the last 2-3 years, Eurasian watermilfoil was found in the St. Johns River watershed and hydrilla was found to infest Rodman Reservoir on the Cross Florida Barge Canal, Okeechobee, Orange, and Lochlossa Lakes.

Florida is by no means 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. Indeed the problem is world-wide, but is more acute in the warmer latitudes where waterhyacinth, hydrilla, watermilfoil, alligatorweed, salvinia (*Salvinia* spp.), and water lettuce (*Pistia stratioties*), are the major offenders. Reasons for the increasing aquatic weed problem are complex, but are definitely related to man and his activities. With the increase in population and the accompanying environmental problems, it has become apparent that new methods of aquatic weed control must be found. Conventional methods have not been entirely satisfactory either 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.

In recent years, biological control methods have received considerable attention. Various species of herbivorous insects, fish, snails, and even the manatee 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, have been reasonably effective, especially in an integrated control program. Surprisingly, until our program was initiated, plant pathogens had been rarely considered as biocontrol agents. They have all the prerequisites of a biocontrol agent and thus offer an untapped reservoir of potential usefulness, either alone or in an integrated control program with insects and, perhaps, chemicals. Our research efforts are aimed at bringing to fruition this use of plant pathogens in control programs for aquatic weeds.

Research Approach

We are using two approaches in our efforts to utilize plant pathogens to control aquatic weeds. They are:

1) The use of endemic or native plant pathogens as a type of "biological herbicide" through the artificial induction of epiphytotics. We consider this to be the most rapid approach from an operational standpoint.
2) The search for and ultimate utilization of exotic plant pathogens. This has been the classical approach successfully used 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.

Our research efforts since the inception of this project are indicated by the titles on the list of publications. Publication Number 23 of the Florida Water Resources Research Center summarizes the first three years of our research work.

During the past three years our efforts have been directed primarily toward those pathogens with definite biocontrol potential. These are: the endemic pathogens of waterhyacinth; Acremonium zonatum, Cercospora piaropi, C. rodmanii, Rhizoctonia solani, and three exotic ones; Bipolaris stenospila, Uredo eichhorniae, and Rhizoctonia sp. We have carried out extensive cultural studies in the laboratory, greenhouse studies, including cross inoculation onto other plant species, host range studies and, in the case of the endemic pathogens, small scale field tests. These latter tests have shown A. zonatum and C. rodmanii to have considerable potential as biocontrols. We are presently testing both of these at locations in Florida and in Lake Concordia in Louisiana. In this latter test, we have the two pathogens combined with two insects (Neochetina eichhorniae and Arzama densa) in all possible combinations. This test is being conducted in cooperation with the U.S. Army Corps of Engineers, Waterways Experiment Station and the U.S. Department of Agriculture with the approval of the Louisiana Department of Agriculture and the Louisiana Fish and Game Commission. We believe C. rodmanii to have been the cause of a spectacular decline of waterhyacinth in Rodman Reservoir in 1971. This natural decline saved the Army Corps of Engineers approximately $35,000 in spray cost in that body of water (Zeiger, personal communication). Acremonium zonatum and N. eichhorniae combinations are also being tested in South Florida near Ft. Lauderdale.

Work with the exotic pathogens is being done in our quarantine facility, which is limited in size. Therefore, the work is progressing at a slower pace than with the endemic pathogens.

This report summarizes the research conducted under project A-027-Fla. For more detail on specific points, the reader is referred to published articles, reprints of which have been furnished our granting agencies. The project is being continued.

Endemic Pathogens

Rhizoctonia solani: Fungal isolate RhEa from Panama was shown to be highly pathogenic to waterhyacinths (see Freeman & Zettler, Phytopath. 61: 892.) It was identified as a species of Rhizoctonia closely related to R. solani. We, therefore, deemed it advisable to test endemic isolates of this same fungus. Several isolates (see Joyner and Freeman, Phytopath. 63: 681-685.) were found to be very nearly as pathogenic to waterhyacinth as was RhEa. An isolate from bean was selected (H287) for further study as a biocontrol agent. In the meantime, studies were continued under quarantine conditions with RhEa.

After completion of sclerotial survival studies with RhEa, we were convinced that the fungus had potential as a biocontrol agent for aquatic plants. Sclerotia of RhEa survived for over 26 months when submerged in lake water. Survival rate was probably longer, but the supply of sclerotia for testing purposes was exhausted after 26 months. The pathogenicity of cultures derived from submerged sclerotia was equal to that of the stock culture and cultures derived from day sclerotia (see Freeman, Plant Disease Reptr., 57: 601-602). In addition, it was shown that certain R. solani isolates were capable of attacking underwater portions of waterhyacinth and other aquatics (see Joyner M. S. Thesis and Freeman, McGraw-Hill Yearbook.) These later results were further encouragement to proceed with field tests of domestic isolates of R. solani.

On the negative side R. solani possesses some distinct disadvantages. It is a common soil-inhabiting parasite that affects numerous species of plants. Under ideal conditions for disease development it will attack a large number of commercial crop plants (see Table I.) Many consider this to completely preclude the use of this organism for biological control purposes. However, its use as a biological herbicide in the aquatic environment would not necessarily increase its potential as a pathogen of terrestrial plants. Indeed, when terrestrial crop plants were sprayed with inoculum of R. solani, fewer of them were attacked than when these same plants were inoculated under ideal disease conditions in the greenhouse (see Table I.).

Initial field studies to assess the potential of domestic isolates of R. solani were begun in the fall of 1973. An isolate of Acremonium (Cephalosporium) zonatum described by Rintz (Hyacinth Contr. J., 11: 41-44) was also included in these studies. These fungi were used alone and in combination on a well established stand of waterhyacinths in an isolated area of Lake Alice on the University of Florida Campus. Both fungi were mass grown in liquid culture on a relatively simple chemically defined medium (Difco Czapecks-Dox broth amended with 0.5% yeast extract.) Both fungi were grown in this manner and the mycelial mats collected after 14 days. These mats were ground in a commercial size Waring blender. The resulting mycelial suspensions were sprayed on waterhyacinth plots in Lake Alice on October 10. The two fungi were applied singly and in combination. Spraying was accomplished with a 12 gallon conventional Broyhill sprayer at 100 psi. Plots were approximately 1/20 of an acre.

Infection by R. solani was apparent in less than a week and with A. zonatum in less than two. By the first frost in early December, secondary spread of A. zonatum was apparent and resulting damage was significant. Lesions caused by R. solani persisted, but secondary spread was negligible and damage was less than anticipated. Plots were observed throughout the winter and into the following growing season. No evidence of R. solani infection the following spring was noted, whereas, sporadic A. zonatum infections occurred. These results were disappointing in the case of *R. solani*, but not totally unexpected. The lack of a spore stage was a deterrent in secondary spread on aerial portions of the plant. On the other hand, results obtained with *A. zonatum* were more promising than laboratory and greenhouse studies had indicated. Thus, *R. solani* studies were curtailed in favor of more intense study on *A. zonatum*.

Acremonium zonatum: In addition to the field test noted in the preceding section and host range studies (Table I), we have conducted a wide variety of additional studies with this fungus.

During the course of our preliminary work, it became apparent that the culture medium on which *A. zonatum* was grown affected its pathogenicity to waterhyacinth. Pathogenicity as well as total inoculum production was enhanced by enriching the culture medium. Pathogenicity increased as culturing medium was changed from cornmeal agar to Czapeks agar to Czapeks agar plus yeast extract to potato dextrose agar to potato dextrose agar plus yeast extract.

The fact that pathogenicity of *A. zonatum* changed due to culturing substrate brought forth the idea that other factors may also be used to increase pathogenicity. In at least one other plant pathogen, *Cladesporium fulvum* on tomato, virulence has been increased through irridation of cultures with ultra-violet radiation. Short wave UV radiation in the 253 mμ range was most effective. Since this wavelength is germicidal, dosages were critical. A decision was made to embark upon a study of the effectiveness of UV radiation for inducing mutations for increased virulence to waterhyacinth in populations of *A. zonatum*. Thus far, we have isolated over 400 single spore cultures derived from spores irradiated with UV light (253 mμ) for periods ranging from 30 to 180 seconds. Many of these cultures differ morphologically from the parent cultures.

Table 1 Infection of crop plants by Acremonium zonatum and Rhizoctonia solani after spray inoculation with a mycelial and spore suspension.

Crop	Variety	Infection[1] R. solani	A. zonatum
Cabbage	Charleston Wakefield	-	-
Canteloupe	Hales Best Jumbo	++	-
Carrot	Imperator	-	-
Celery	Giant Pascal	-	-
Collard	Georgia	-	-
Cucumber	Poinsett	+++	-
Eggplant	Florida Market	-	-
Endive	Green curled	-	-
Escarole	Batavian	-	-
Field corn	PAB 751	-	-
Grapefruit	Duncan	-	-
Irish potato	Sebago	-	-
Lettuce	Great Lake	-	-
Lima bean	Henderson	-	-
Mustard	Florida Broadleaf	-	-
Oats	Fulghum	-	-
Okra	Clemson Spineless	-	-
Onion	White Globe	-	-
Orange	Temple	-	-
Peanut	Florunner	-	-
Pole bean	Kentucky Wonder	+	-
Radish	Scarlet Globe	-	-

Table 1 continued

Crop	Variety	Infection [1] R. solani	Infection [1] A. zonatum
Rye	Weser	-	-
Slash pine	--	-	-
Snap beans	Harvester	-	-
Soybeans	Bragg	-	-
Southern peas	Cream 40	-	-
Squash	Early Summer Crookneck	-	-
Strawberry	Florida 90	-	-
Sugarcane	CL 41-223	-	-
Sweet corn	Silver Queen	-	-
Sweet pepper	Yolo L	-	-
Sweet potato	?	-	-
Tangerine	Dancy	-	-
Tobacco	Turkish NN	-	-
Tomato	Homestead	-	-
	Manalucie	-	-
	Walter	-	-
	MH 1	-	-
Watermelon	Congo	+	-
	Charleston Grey	+	-
Wheat	Holden	-	-

[1]-no infection; + mild infection; ++ moderate infection; +++ severe

Thus far, approximately 200 cultures have been tested for changes in their pathogenicity to waterhyacinth. Where changes have been noted, in most cases there was a reduction in pathogenicity. However, in three or four cases there appeared to be a slight increase in pathogenicity. The fact that some changes appear to have occurred is encouraging at this stage in these studies.

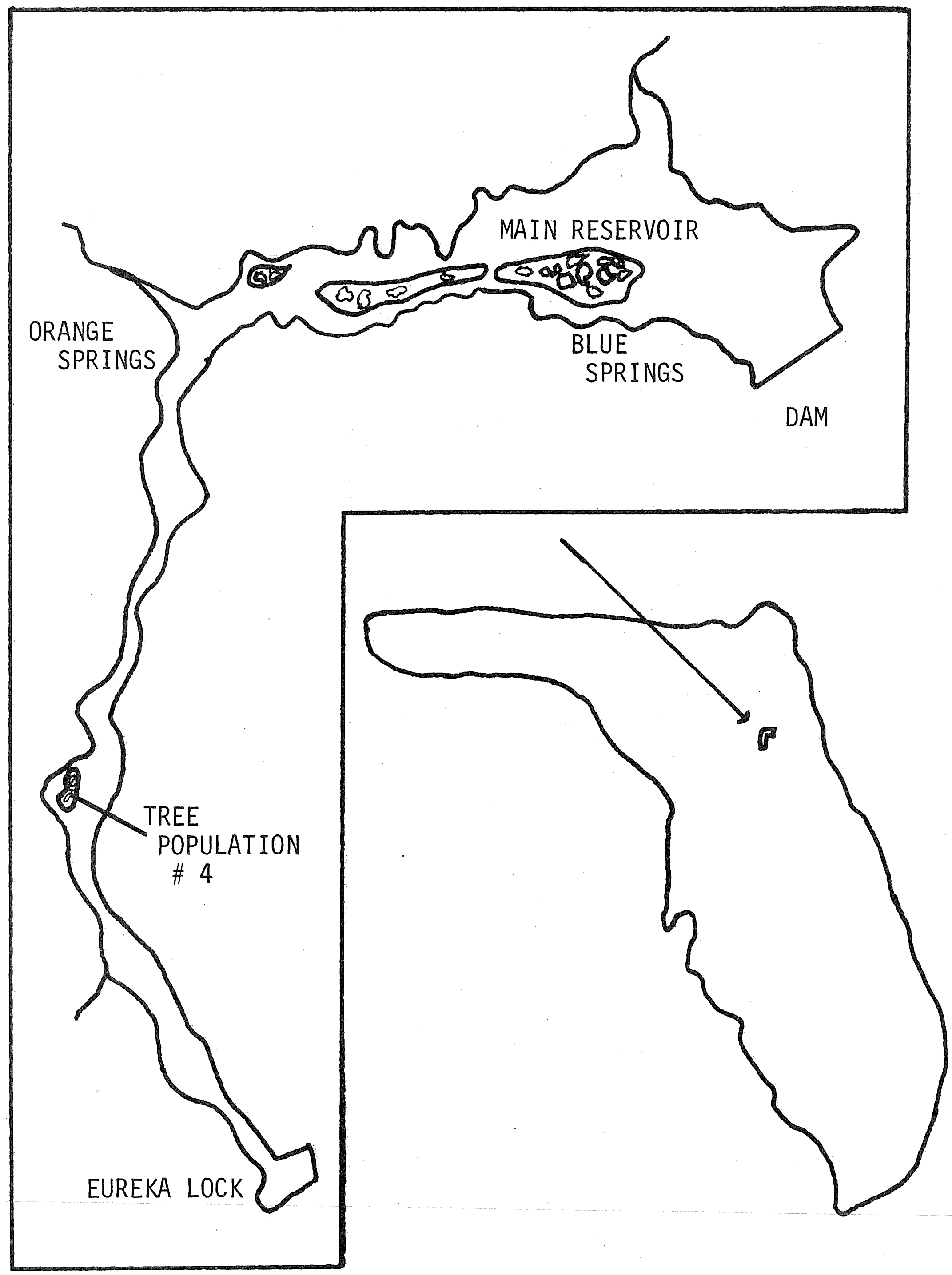
Additional field tests of A. zonatum were conducted during the summer of 1974 both at Gainesville and Ft. Lauderdale. In June of 1975, it was included, along with three other biotic agents in an integrated control test in Lake Concordia, La.

Results with A. zonatum have been erratic. Infection appears to be related to the growth habit of the waterhyacinth. Large robust plants seem to be more readily infected than smaller ones. Where infection does take place, subsequent spread by the pathogen is slow and does not keep pace with the prolific growth of the host. Acremonium zonatum is most effective when used in an integrated system with insects and other fungi.

Cercospora spp: During the winter of 1973-74, waterhyacinth plants in the area of Gainesville were found 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 (see Freeman and Charudattan, Plant Disease Reptr. 58:277-278). This was only the second report of the occurrence of this organism since it was originally reported from Texas in 1914. The other occurrence was in India. The fungus did not appear to be causing appreciable damage to the waterhyacinth plant at the time it was first noted.

In December of 1973, Dr. Conway isolated, along with many other fungi (see Conway et al., Fla OWRR Publ. No. 30), a Cercospora species from declining waterhyacinth in Rodman Reservoir. Preliminary tests showed the

RODMAN RESERVOIR



fungus to be pathogenic on waterhyacinth and a secondary test showed it to inflict considerable damage on this plant. In fact, 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.

Microscopic examination revealed the fungus to be a typical Cercospora. However, spore measurements showed the spores to be much longer than those recorded for C. piaropi (see Freeman and Charudattan, Plant Disease Reptr. 58:277=278) which is 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 appear to differ from those recorded for C. piaropi. Symptoms caused by the former are a general blasting of the foliage whereas the latter produces more discreet spots on the leaves. However, there was the distinct possibility that we were dealing with two manifestations of the same fungus and disease. When leaves exhibiting C. piaropi symptoms with fruiting characterized by small spores are brought into the laboratory and placed under moist conditions, long spores frequently develop on the dead tissue. Therefore, we were not sure if we had a long spore variant of C. piaropi or a new waterhyacinth species of Cercospora (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 symptomology prompted its description as a new species of Cercospora designated C. rodmanii (see Conway, Canad. J. Bot. in press.)

Optimum temperature for growth of C. rodmanii is near 20°C, however, excellent growth occurs over the range of 20° to 30°C. Pathogenicity appears correlated with growth but this has not been definitely determined. The fungus grows rapidly for a Cercospora reaching a maximum growth on potato-dextrose-agar plus yeast extract in 10-14 days at 20^{o}C. It sporulates sparsely on waterhyacinth extract agar but profusely on the dead leaf tissue both in the laboratory, greenhouse and field.

Temperature studies tended to indicate the fungus would more likely attack the plant during mild weather. Therefore, field studies were begun in the Fall on established stands of waterhyacinth in an isolated area of Lake Alice on the University of Florida Campus. An initial application was made September 4, 1974. Infection took place within two weeks but subsequent spread was slow and a second application was made October 3, 1974. Inoculum consisted of the mycelial growth from 60 Roux bottles culture two weeks in age (approx. 1000 g of wet weight.).

By mid-November extensive damage had occurred in the immediate area sprayed and spread was apparent around the entire perimeter of the lake. By the first frost on December 2, considerable damage was apparent in areas of secondary spread and spore trappings of the fungus were made at considerable distance from the original inoculation site indicating extensive secondary spread. After this first frost and subsequent ones on December 9 and 17, it was apparent that diseased plants were more severely damaged than more healthy ones. It should be noted that all of these frosts were relatively light and healthy plants were not severely damaged. Above average temperatures occurred in the latter part of January, and February, 1975, and some of the waterhyacinths began sending out offshoots. Evidently the apical meristem of the plants had not been killed and the plants were able to resume growth. However, it was apparent that a severe stress had been placed on the waterhyacinths when the plants in the pool area which were normally 2-3 ft. tall were less than 6 in. high. In comparison to these plants, waterhyacinths in the main lake where the disease was less severe were their normal 3-4 ft. Thus, *C. rodmanii* fulfills the purpose of a biological control organism for waterhyacinths by increasing the stress on the plant and not necessarily eliminating the entire population.

We wanted to know what effect infection would have on the plants over a period of time. Four more sprays were applied on the pool area; two in March, and one each in May and July.

In April we were able to determine that most of the small waterhyacinth in the pool were infected again with *C. rodmanii*. However, with the approach of summer the waterhyacinth began their rapid growth phase, and by June, the new leaves were outgrowing the disease. The infection of *C. rodmanii* during the summer was confined to the older, lower leaves. This condition prevailed throughout the summer until September when the waterhyacinth growth was slowed by cooler night temperatures. Increased infection was now apparent in the pool area. The waterhyacinths in the pool area showed a general browning by mid-September. This browning has continued into October until definite disease symptoms could be seen on the plants throughout the pool. Damage to waterhyacinth was approximately one month ahead of last year and we were looking for increased damage this fall and winter. We will not be able to totally evaluate the extent of damage until regrowth occurs in the early Spring of 1976.

The disease that occurred in Lake Alice as a result of our inoculation with *C. rodmanii* is strikingly similar to the Rodman disorder noted in 1971 and to a lesser extent in 1972-1973. Dave Bowman, Reservoir Manager, observed the disease in Lake Alice and believes what he saw to be the same disease he had observed in Rodman. In addition, the root rotting phase so notable in Rodman has begun to appear in Lake Alice. We now believe

C. rodmanii to be the causal agent of the Rodman disorder.

Encouraged by the success in Lake Alice, test plots were set up in Rodman Reservoir. A site was chosen behind tree population No. 4 to exclude outside interference with the tests. The purpose of this experiment was to reestablish the disease in the reservoir. Although the disease was very prevalent in 1971, its severity on the waterhyacinth population has lessened each year.

Five sprays were to be applied from the shoreline, one every two weeks. The multiple sprays were felt necessary to begin infection and to increase the inoculum to a high enough level to create an epiphytotic. Spray operations were begun on February 28, 1975. The next day, the U.S. Army Corps of Engineers began to raise the water level of the reservoir from its winter draw-down depth of 4.67 m to a level of 5.49 m. The water level had risen and the waterhyacinth were growing and floating free of the shoreline by the second spray date. By the third spray application, the spray plots were moving with the rising water and, therefore, one of our objectives of increasing the inoculum in one area could not be achieved.

In mid-April, damage on the plants due to C. rodmanii was present along a gradient from the opening on to the reservoir from tree population No. 4 to our spray site on shore. By this time there was a definite reduction in plant growth in our plots when compared to the untreated waterhyacinths that surrounded these areas. In addition to the disease in the tree population No. 4 area there was also a heavy natural infection of C. rodmanii in the main part of the reservoir in the Orange Springs and Blue Springs areas.

During May, the disease in the main reservoir continued to stress and brown the waterhyacinths. On May 30, another spray plot was established in the Cypress tree stand in tree population No. 4.

In June, the waterhyacinth in the Orange and Blue Springs areas were completely browned. The symptoms on the plants were typical for _C. rodmanii_ damage.

By July, the waterhyacinths in the Cypress stand were beginning to brown at the tips of the leaves, and in mid-July the waterhyacinths in our original spray plots were showing the typical _C. rodmanii_ symptoms. Water lettuce was invading soon after the severely infected waterhyacinths died and sank to the bottom. By late July, the waterhyacinths in the Cypress stand were also dying out and open water was beginning to show.

On August 7, aerial inspection of the original spray site showed continued browning and drop out of the waterhyacinths. In the Cypress tree area there was 10-20 acres of open water. By mid-August, the estimate of open water in the Cypress tree area exceeded 20 acres. Large mats of waterhyacinth showed typical symptoms which included many dead plants with floating, spindly petioles and leaves. It was also noted that completely dead plants continued to float until broken apart by wind and water action.

By mid-October the area of open water was estimated to be 35-40 acres, with an additional 20 acres invaded by water lettuce. The area now is picturesque.

Results with _C. rodmanii_ have been very encouraging and it is programmed for more intense study. The host range tests (Table 2) were especially encouraging as they indicate the fungus to be highly host specific.

The field symptoms of _C. rodmanii_ suggested that toxin may be involved. Symptoms were typical of those incited by toxic byproducts in other disease syndromes. However, studies conducted by a graduate student, Mr. Ray Martyn, did not verify the presence of a toxic product in culture filtrates of the fungus.

Table 2 PLANTS INCLUDED IN GREENHOUSE AND FIELD HOST RANGE TEST FOR SUSCEPTIBILITY TO CERCOSPORA RODMANII

	Plant	Resistance
1.	avocado	-
2.	beans -- "Harvester"	-
3.	beans -- lima "Fordhook" bush	-
4.	beans -- runner "Kentucky wonder" -- pole type	-
5.	beans -- speckled butter "Jackson wonder"	-
6.	beans -- white baby lima "Henderson" bush	-
7.	beets -- "Detroit Dark Red"	-
8.	cabbage -- "Charleston Wakefield"	-
9.	canteloupe -- "Hale's Best"	-
10.	carrots -- "Imperator"	-
11.	celery -- "Giant pascal"	-
12.	collard -- "Georgia"	
13.	*Colocasia* sp.	-
14.	corn -- field corn "PAG 751"	-
15.	corn -- Funks 5945[b]	-
16.	corn -- Funks 4762[b]	-
17.	corn -- "Florida Sweet"	-
18.	corn -- "Golden Hybrid NK 199"a	-
19.	corn -- "Silver Queen"	-
20.	cotton -- "Sea Island"	-
21.	cotton -- "DPL 16[b]"	-
22.	cotton -- "Stoneville 213[b]"	-
23.	cowpeas -- "Zipper cream"	-
24.	cucumber -- pickling F1 hybrid[a]	-

Table 2 continued

	Plant	Resistance
25.	eggplant -- "Florida market"	-
26.	*Hydrocotyle umbellata*[b]	-
27.	grapefruit	-
28.	lemon	-
29.	lettuce -- "Great Lakes" bibb -- sporulated on senescent leaves	
30.	red mangrove	-
31.	mustard -- "Florida Broadleaf"	-
32.	oats -- "Fulghum"	-
33.	okra -- "Clemson Spineless"	-
34.	onion -- purple	-
35.	onion -- red	-
36.	onion -- white	-
37.	orange -- sour	-
38.	orange -- sweet	-
39.	peanuts -- "Florunner"	-
40.	peas -- "Cream acre"	-
41.	peppers -- banana	-
42.	peppers -- hot "Hungarian"	-
43.	pine tree -- slash	-
44.	*Pontedaria cordata*	-
45.	potatoes -- Irish "Red La Soda"	-
46.	pyracantha	-
47.	radishes -- "Scarlet Red Globe"	-
48.	rice -- "Saturn[b]"	-
49.	rye -- "Weser"	-
50.	squash -- "Early Summer crookneck"	-
51.	strawberry -- "Florida 90"	-

Table 2 continued

	Plant	Resistance
52.	soybeans -- "Bragg"	-
53.	soybean -- Davis[b]	-
54.	sugarcane	-
55.	sweet potatoes -- "Georgia Red"	-
56.	tangerine	-
57.	tobacco -- turkish	-
58.	tomatoes -- "Homestead"	-
59.	tomatoes -- "MH-1"	-
60.	tomatoes -- "Manalucie" [b]	-
61.	tomatoes -- "Walter"	-
62.	waterhyacinth -- inoculum check	-
63.	watermelon -- "Charleston Grey"	-
64.	watermelon -- "Crimson Sweet"[a]	-
65.	wheat -- "Holden"	-

- denotes not susceptible

a. field only

b. greenhouse only

We have carried out limited epidemiological studies. Extensive spore trappings were conducted in Lake Alice during the fall of 1975, when temperature variations ranged from below freezing at night to day temperatures in the eighties. Results show that sporulation by *C. rodmanii* was curtailed by temperatures below 50°F. Maximum occurred when day temperatures were in the 70-85 degree range. On Dec. 8, 1975, spore trappings reached a high of 193 spores per cubic meter of air volume. Laboratory studies failed to reveal a forceable discharge mechanism for spores of *C. rodmanii*. Therefore, both spore release and subsequent dispersal is probably passive in nature.

Fungi associated with waterhyacinth: In November 1973, an active research project was initiated when Dr. Conway joined the project to catalogue the mycoflora found on waterhyacinths. His specific assignment was to study the mycoflora associated with declining waterhyacinth in Rodman Reservoir. The mycoflora can be divided into two main catagories; those occurring on living tissue and those occurring on declining tissue (see Table 3).

The fungi that occur on living tissue are studied for their possible role in the control of the prolific hyacinths. Fungi identified and cultured in this group include: the Ascomycetes; *Mycosphaerella* sp., *Didymella* sp., *Leptosphaerulina* sp., and *Asteromella* sp., *Ascochyta* sp., *Cercospora* sp., *Phoma* sp., *Myrothecium striatisperum*, and *Cephalosporiopsis* sp. belonging to the Fungi Imperfecti.

Fungi associated with dead and declining tissue include; (Fungi Imperfecti) *Curvularia lunata* var. *aeria*, *Alternaria* sp., *Dendryphion* sp., *Nigrospora sphaerica*, *Nigrospora oryzae*, *Thysanophora longispora*, *Periconca* sp., *Scolecobasidium constrictum*, *Penecillium* spp., *Tilletiopsis* sp., *Fusarium* sp., *Pestalotia* sp., *Botryodiploidea* sp., *Acremonium* (*Cephalosporium*)

Table 3 FUNGI FOUND ASSOCIATED WITH WATERHYACINTH DURING WINTER AND SPRING MONTHS

Fungi	Pathogenicity
Subdivision	
Ascomycotina	
Class - Pyrenomycetes	
1. Melanospora sp.	none
Class - Loculoascomycetes	
2. *Leptosphaerulina* sp.	none
3a. *Didymella exigua*	none
b. *Ascochyta* (imperfect stage)	
4. *Mycosphaerella* sp.	slight
Deuteuromycotina	
Class - Coelomycetes	
5. *Phoma* spp.	varies-moderate to none
6. *Botryodiploidea* sp.	slight
Class - Hyphomycetes	
7. Unknown synnematal fungus	none
8. *Mycoleptodiscus terrestris*	slight to none
9. *Myrothecium cintum*	none
10. *Epicoccum purpurascens*	none
11. *Alternaria* spp.	none
12a. *Aspergillus flavus*	none
b. *Aspergillus niger*	
13. *Acremonium* (Cephalosporium) *zonatum*	good-excellent
14. *Bipolaris* spp.	good-under investigation
15. *Cercospora* sp.	good-excellent

Table 3 continued

	Fungus	Pathogenicity
16.	Cladosporium spp.	none
17a.	Curvularia brachyspora	none
b.	Curvularia penniseti	slight
18.	Dendryphiella infuscans	not tested
19.	Exserohilum prolatum	under investigation
20.	Memnoniella subsimplex	not tested
21.	Perionia echinochloae	slight
22.	Pithomyces chartarum	none
23a.	Nigrospora oryzae	none
b.	Nigrospora sphaerica	none
24.	Thysanophora longispora	none
25.	Scolecobasidium humicola	none
26.	Stemphylium vericarium	under investigation
27.	Ustilaginoidea sp. (?)	not tested

zonatum, Memnoniella sp., Epicoccum sp., Cladosporium spp., Pithomyces chartatum, Aspergillus spp., an unknown synnematal fungus, Rhizoctonia sp. (Ceratobasidium, Pyrenochaeta sp. and (Phycomycetes) Mucor sp., Circinella sp., and (Ascomycete) Melanospora sp.

The role of saprophytic fungi is often neglected in disease research, however, several important contributions to our knowledge of waterhyacinth control and utilization can be realized through their investigation. Periconia is a saprophytic fungus that produces a toxin which causes disease symptoms on certain plants. When the fungus is inoculated onto the hyacinth a leaf spot is produced with the fungus continuing to grow on the leaf. The presence of Aspergilli and Penicillia may indicate the production of aflatoxins on waterhyacinth. This obviously is of importance to researchers attempting to use this plant as a food source. The presence of Pithomyces chartarum is of even greater importance to nutrition researchers. It is the only fungus known to have caused a natural widespread outbreak of poisoning. The toxin, sporidesmin, causes liver damage, loss of weight, icterus and photosensitivity to grazing animals. An additional project has been initiated through our department in cooperation with the Department of Animal Science Nutrition Laboratory and the Department of Veterinary Science to study mycotoxins present in waterhyacinths and their effect on animals. Thus, our efforts in determining mycoflora that may have biological control potential may also provide valuable information for workers concerned with other methods of control and utilization of waterhyacinth.

Other studies: Less extensive studies have been conducted with several other endemic pathogens of aquatic plants. These were concerned primarily with minor pathogens of waterhyacinth and the alligatorweed stunt virus (see Hill and Zettler, Phytopath. 63:433.)

Repeated attempts to transmit the alligatorweed stunt virus by mechanical means and insects were unsuccessful. However, it was transmitted by grafting of healthy and diseased stock. Such a method of transmission limits this agent's usefulness as an effective biocontrol. It does prove conclusively that the malady is viral induced and not a genetic abnormality. Dr. Zettler is convinced that the virus belongs in the beet yellows group and may be actually a strain of beet yellows. Being in this group could present problems in clearing this virus for use as a biocontrol since tristeza virus of citrus also belongs to the beet yellows complex.

Two other minor pathogens have been reported affecting waterhyacinth in Florida. There are Sigmoidea sp. (Lin and Charudattan, Proc. Phytopath. Soc. 2:137) and Mycoleptodiscus terrestris (Charudattan and Conway, Plant Dis. Reptr. 66:77-80.) Both appear to be weak pathogens of little potential in biological control. A new non-pathogenic fungus of the genus Doratomyces was also recovered from waterhyacinth foliage (Conway and Kimbrough, Mycotaxon 2:127-131).

Exotic Pathogens

Dr. Charudattan has traveled extensively in search of exotic pathogens on waterhyacinth and hydrilla. In 1973, he spent 90 days in India (supported by the Fla. Dept. of Natural Resources) collecting pathogens on these two plants. The regions in India surveyed included Sringar (State of Kashmir), the surroundings of Delhi and Calcutta, Dehra Dan Utter Preadesh, Kota (Rajasthan) and several areas in the state of Orissa. These areas were not covered during his previous tour in India from November 1971, to February 1972. In addition, several previously surveyed areas around Madras were revisited. One hundred and eighty-two fungal and bacterial isolates were obtained from hydrilla, waterhyacinth, water lettuce and miscellaneous submersed aquatic plants. Ninty-five were from hydrilla and the remainder from other plants.

Twenty-four of the Indian hydrilla isolates proved to be pathogenic to hydrilla. These were primarily species of Penicillium, Aspergillus and Trichoderma. These were pathogenic through the production of toxic metabolites (Charudattan and Lin, Hyacinth Contr. J. 11: 44-48.) These toxic agents were identified as oxalic acid and penicillic acid. They are general toxic agents not specific for hydrilla. Thus, their direct use in a biocontrol program appears limited.

Fourteen of the remaining isolates were found to be pathogenic to waterhyacinth. Among these were isolates of Myrothecium roridum, Alternaria eichhorniae and Cephalosporium sp. The others have not been identified because they are weakly pathogenic and are of academic interest only. The three pathogens have been evaluated and rejected as biocontrol agents in India. Never-the-less, we are maintaining them for a possible future role here in the United States.

Dr. R. Charudattan undertook two survey tours during the first quarter of 1974. The first trip, to the Dominican Republic, was from February 11th to 16th. On this survey tour, about 82 fungal and bacterial isolates from diseased waterhyacinth were collected. The genera of fungi collected included, Helminthosporium, Phoma, Macrophoma, Colletotrichum, Fusarium, Chaetomium, Pestalotia and Alternaria. Unfortunately the rust and smut diseases of waterhyacinth reported to occur in this country were not found. The second trip to Venezuela, between March 3rd and 12th yielded a collection of approximately 87 cultures. The majority of these are fungi, but they are yet to be identified. Pathogenicities of about 35 isolates from the Dominican Republic have been tested on waterhyacinth. So far, a species of Phoma and two species of Helminthosporium have been found to be virulent on waterhyacinth. One of the species of Helminthosproium is extremely pathogenic on waterhyacinth. This appears to be a hither to unreported pathogen of waterhyacinth. Preliminary tests indicate it to be comparable in pathogenicity to Cephalosporium zonatum and Rhizoctonia solani. Among the diseases found in Venezuela, was a necrotic leaf spot surrounding each feeding injury caused by Neochetina. Diseases of alligatorweed of Pistia were not encountered, though these plants were found in these countries. Several new and useful contacts were established in the two countries.

Dr. Charudattan traveled to Argentina and Uruguay during the last half of April, 1974, in search of diseases on waterhyacinth and alligatorweed. On this trip he was successful in finding the rust disease on waterhyacinth, but was unsuccessful in locating rust of alligatorweed. The rust on waterhyacinth was present in Argentina but not in Uruguay, where it had been reported to flourish. He was able to bring back adequate material for critical study in our isolation greenhouse.

Morphologically the rust fits the description of Uredo eichhorniae. This fungus was originally recorded on waterhyacinth in the Dominican Republic in 1927. The organism has been loosely referred to as Puccinia eichhorniae. No basis for this name has been established, since only the uredial stage of this rust is known. The material from Argentina did not have teliospores and they have not formed in material brought back to Gainesville. We have been able to infect plants in the isolation culture and increase it for further study.

Considerable work remains to be done before we can determine if this disease has biocontrol potential. It did not appear to be causing significant damage on waterhyacinth in Argentina. However, this doesn't rule out the possibility that it will cause significant damage on the host in Florida. A different host gene pool may exist here that has not had the selection pressure of the disease exerted upon it. This we will soon determine. The big advantage of rust diseases is their host specificity. However, some rust species can affect related plant species and many have alternate hosts in non-related plant groups. Presently we do not have information concerning additional related hosts or alternate hosts of U. eichhorniae. Dr. Charudattan did find a similar appearing rust on Pontederia sp. growing in close proximity to infected waterhyacinth. A similar rust also occurs on Pontederia in Florida (Charudattan and Conway, Mycologia 653-657.) Cross inoculation studies with these two rusts are presently in progress.

One additional exotic pathogen was added to our collection during 1974. This was a species of Alternaria isolated from leaf spots on alligatorweed from Puerto Rico. Diseased material was collected and transferred to us by Mr. Chuck Zeiger of the Army Corps and Dr. Dave Perkins of the USDA. We had received unofficial reports of an Alternaria affecting waterhyacinth in California as well as other locations. This represents our first collection of the fungus. Its pathogenic potential is still under evaluation.

With the completion of the preceeding trips we now have a fair idea of the disease picture as it occurs on waterhyacinth. Interestingly, the widest varieties of disease have been found in the area of Puerto Rico and the Dominican Republic.

The two exotic pathogens that appear to have the most biocontrol potential are the waterhyacinth rust and the Helminthosporium (Bipolaris) on waterhyacinth. These have been studied the most extensively and are still being actively investigated.

Initial attempts to inoculate waterhyacinth with the rust pathogen, U. eichhorniae were unsuccessful, possibly due to lack of proper environment. A variety of treatments to stimulate the germination of uredospores of waterhyacinth rust were tried. They were: hydration of spores for 24 hours, freezing (-5^{o}C for 24 hours) or heating (for 3 minutes at 55^{o}C) prior to hydration, hydration in various concentrations of a mineral salts medium, in specialized media for growing rusts (obtained from Dr. C. A. Hollis), and in 0.1% solution of Tween 20. All these treatments failed to stimulate spores of *U. eichhornia* to germinate. Hydration in dilute (0.0025 to 0.025 percent) aqueous solutions of nonanol and octanol stimulated less than 1% of the spores to germinate.

After this initial failure, Charudattan again visited Argentina, Uruguay, Paraguay and Brazil during October 12th through November 8th, 1975, to collect spore samples of waterhyacinth rust, *Uredo eichhorniae*. As part of the general information he is seeking on rusts of *Pontederiaceae*, he also made collections of *Uromyces pontederiae* on *Pontederia cordata*, *P. lanceolata* and *Eichhornia azurea*. An apparently new rust on *Reussia subovata* was also discovered in the Missiones province of Argentina. Its relation to *U. eichhorniae*, *U. pontederiae* and *Uromyces heterantherae* is being studied. The taxonomic study of these rusts would be vital to the host-specificity tests on *U. eichhorniae*.

A sample of *U. eichhorniae* from Dique Lujan, Campana, Argentina was successfully inoculated onto waterhyacinth from Florida in greenhouse tests. Several additional technics of spore germination were tried on *U. eichhorniae*. These included, varying periods of hydration of uredospores, heat-shock, various temperatures and incubation with volatile alcohols, synthetic media or host leaf-extracts. Freezing spores for periods up to 24 hours prior to hydration seems to trigger spore germination and subsequent infection of host. Use of very freshly collected uredospores also seemed vital to successful germination and host-infection.

The possible lack of prolonged contact between uredospores and the shiny-waxy leaves of waterhyacinth was considered partly responsible for failure of infections. Hence, undiluted glycerine and three different oils, namely heavy paraffin oil, citrus oil and Visko-Rhap mineral oil were tested as sticking agents in inoculations with U. eichhorniae. The oils were less desirable than glycerine. The less viscous citrus oil and Visko-Rhap proved toxic to waterhyacinth leaves, causing burn damage. While mineral oil was non-toxic, it was suspected of creating a hydrophobic phase between spores and leaves and thus preventing spore germination. Glycerine proved to be a successful medium for spore inoculation.

Close to one hundred percent relative humidity following deposition of uredospores on leaves was necessary for infections. This was achieved by maintaining spores and plants in chambers containing saturated $CaSO_4 \cdot 2H_2O$ solution.

Rust infection on inoculated waterhyacinths were visible in about two to three weeks after spore deposition. Development of uredosori was complete after the third week. During a two week period following eruption of uredosori, several crops of spores have been observed.

Inoculations of waterhyacinth with U. pontederiae from Pontederia spp. and E. azurea are being attempted to determine cross-infectivity among rusts of Pontederiaceae.

The isolate of Bipolaris identified as B. stenospila (Charudattan, Freeman, Conway, Proc. Phytopath. 2: 65) from the Dominican Republic was compared for its virulence with an isolate of Helminthosporium stenospilum Drechs. (=B. stenospila) from the American Type Culture Collection. The former was significantly more virulent and destructive to the waterhyacinth than the latter which was moderately pathogenic. In comparison, H. cynodontis, H. sacchari and H. sativum were weakly pathogenic while the following were non-pathogenic to waterhyacinth: H. maydis (O), H. maydis (T),

H. victoriae, H. setariae and H. carbonum. Both H. sacchari and H. stenospila are sugarcane pathogens found in Florida and other sugarcane regions of the southeastern U.S.A. Therefore, it is likely that we may find an isolate of B. stenospila in Florida that is more virulent on waterhyacinth than the type specimen of H. stenospila (see Table 4.)

Integrated Control

In cooperation with Dr. Perkins at Ft. Lauderdale, limited integrated control tests using Acremonium zonatum and the insect Neochetinia eichhorniae were conducted. These tests indicated, though inconclusively, that increased damage resulted from the use of the two biotic agents together.

A more extensive integrated test was set in Lake Concordia, La. the week of June 23, 1975. This test which is still in progress is a cooperative effort with William Rushing of the Waterways Experiment Station in Vicksburg and Neal Spencer of the USDA in Gainesville. The object is to test the effectiveness of two plant pathogens and two insects, alone and in all possible combinations. Treatments are as follows: a=Arzama densa (insect); b=Neochetinia eichhorniae (insect); c=Cercospora rodmanii (plant pathogen); and d=Acremonium zonatum (plant pathogen). Rates of treatment were: A. densa = 40 per cage; N. eichhorniae = 50 per cage; Cercospora = 80 grams wet weight mycelium and spores; and A. zonatum = 160 grams wet weight mycelium and spores. Plant pathogens were put on in split applications June 24 and 25. One half of each inoculum batch was put on in approximately two liters of water per plot June 24, beginning at the west end of replication number 1. The following day the other half was put on beginning at the eastern edge of replication number IV. Insects were released two weeks later (July 10). At this time infection was noted by both pathogens

Table 4 Virulence of *Bipolaris stenospila* from the Dominican Republic (353), American Type Culture Collection (13447) and Florida (14 b) to waterhyacinth and four graminaceous plants.

Isolate	Waterhyacinth	Bermudagrass	Sugarcane	Corn[1]	Oat[1]
353	4+[2]	3+	2+	0	0
13447	2+	2+	2+	not tested	not tested
14 b	2+	3+	2+	not tested	not tested

1 Varieties resistant and susceptible to Helminthosporium were tested.

2 Rating of pathogenicity was based on the degree of damage. Replicated 3 times (plant). Experiment repeated twice with grasses and 4 times with waterhyacinth.

(personal communication with Neal Spencer and Sam Shirley).

The Lake Concordia experiment has been monitored at regular intervals during the growing season and will continue through the 1976 season.

Preliminary weighing data show that the four agents in combination cause a significant decrease in biomass accumulation. By mid-September, this weight of plants attached by all four biotic agents was less than 50% of that of the check plants. Complete results of this test will be published upon its completion in 1976. The preliminary results are very encouraging.

Conclusion

Significant advances have been made in realizing the goal of the utilization of plant pathogens in biocontrol programs for aquatic weeds. It is anticipated that the results of this study will help bring this goal to fruition within the next few years. The support of the Office of Water Research and Technology has been a significant contributing factor in making these efforts possible.

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Plate I

The damage resulting from inoculation of waterhyacinth with a *Cercospora* sp. in an isolated area of Lake Alice. 1. Application of fungus to healthy waterhyacinth October 3, 1974. 2. Area of immediate spray (arrow) showing initial damage October 15, 1974. 3. Overall area viewed from inoculation site November 13, 1974. 4. Same view as 3 on November 21, 1974. 5. Same area on December 17, 1974, after three light frosts. 6. Closeup of diseased and frosted plants on December 6, 1974.

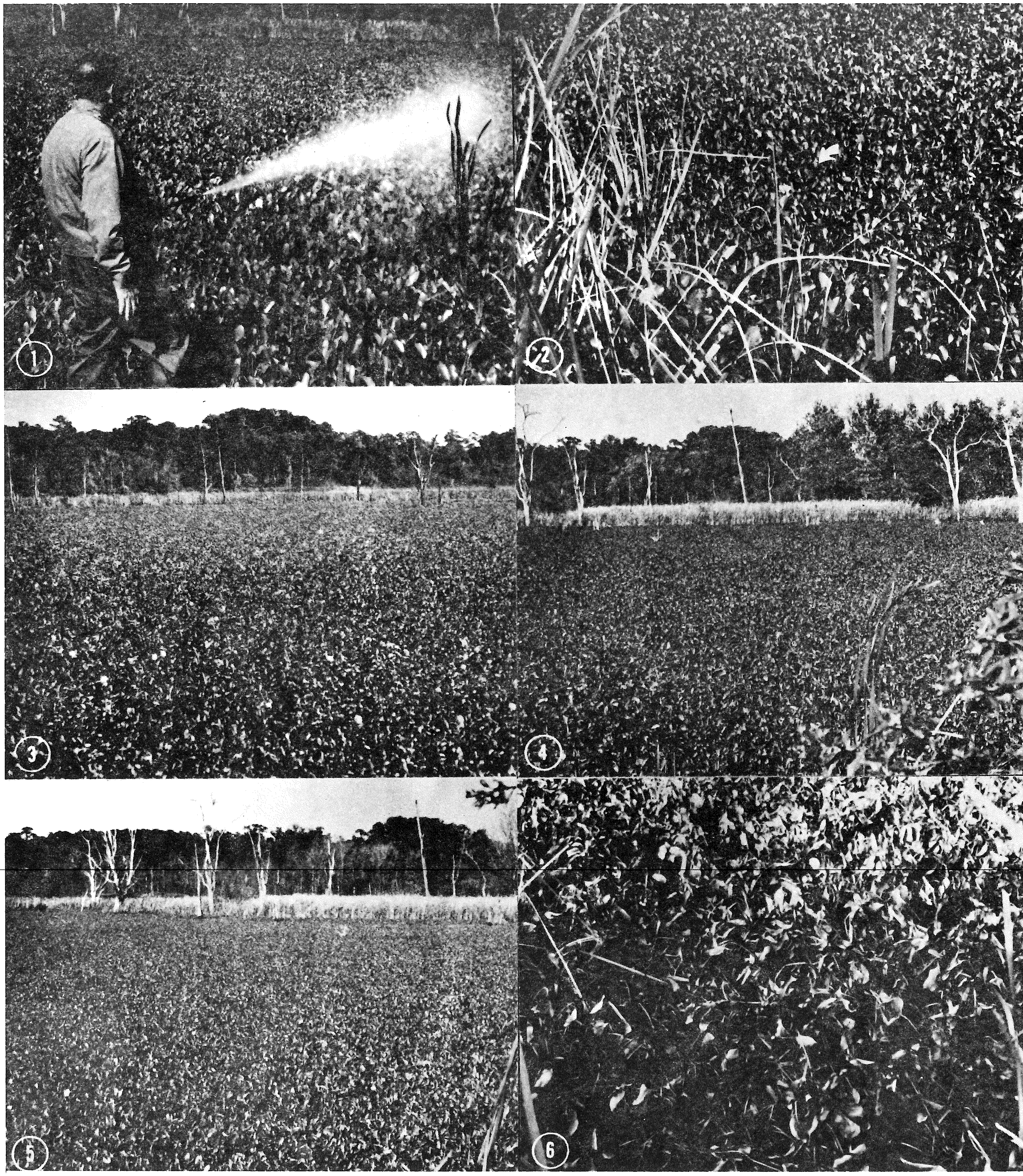
1
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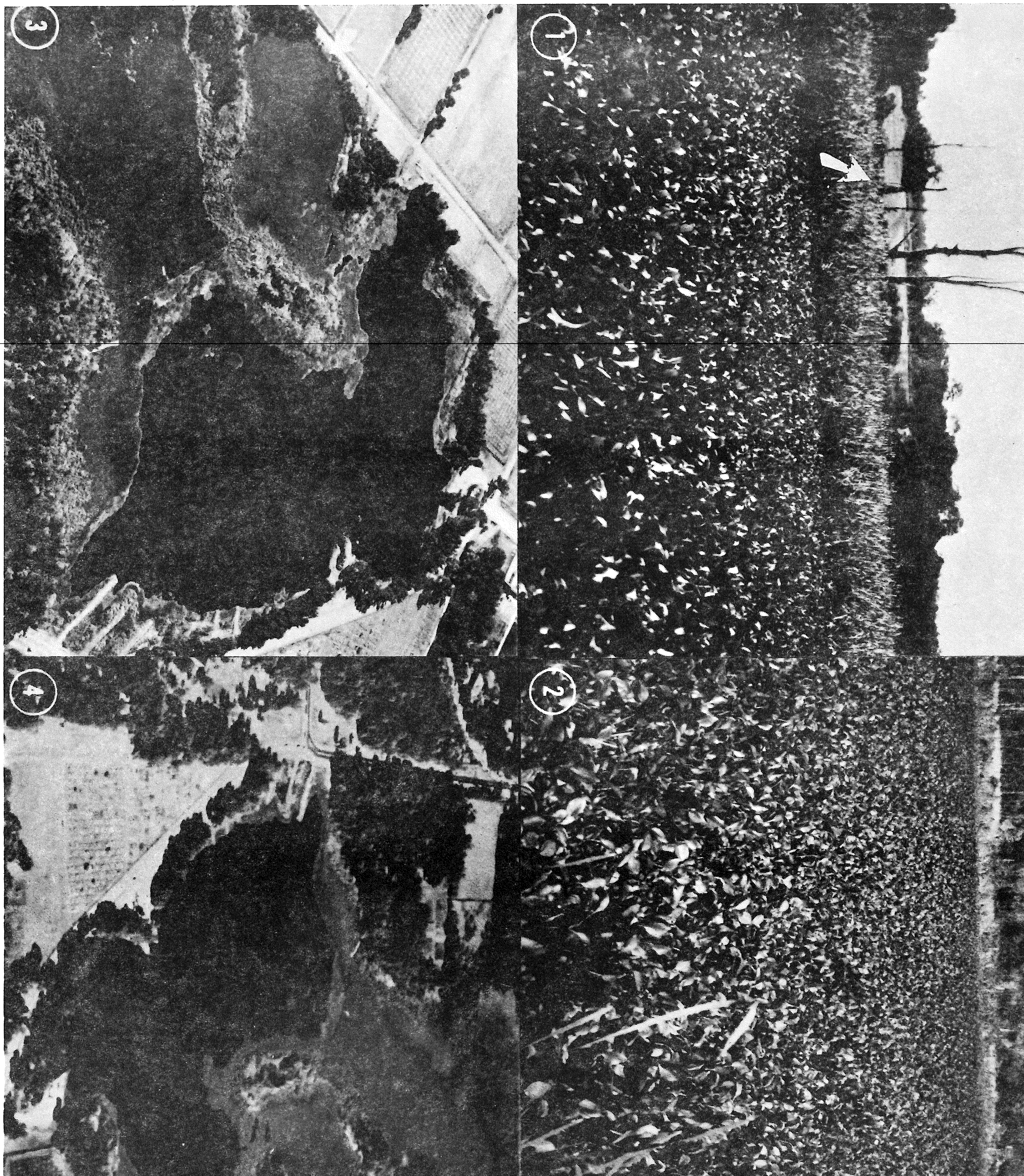
Plate II

Spread of Cercospora sp. on waterhyacinth in Lake Alice after October 3, 1974, application. 1. Initial spread from inoculation site (arrow) through sawgrass barrier into main body of lake November 13, 1974. 2. View from sawgrass barrier looking towards main body of lake November 18, 1974. 3. Aerial view showing spread of disease from initial inoculation site (arrow) around entire perimeter of lake November 21, 1974. 4. IR photograph of lake on November 21, 1974. Compare with view in 3.


1
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