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Biological Control of Water Weeds With Plant Pathogens

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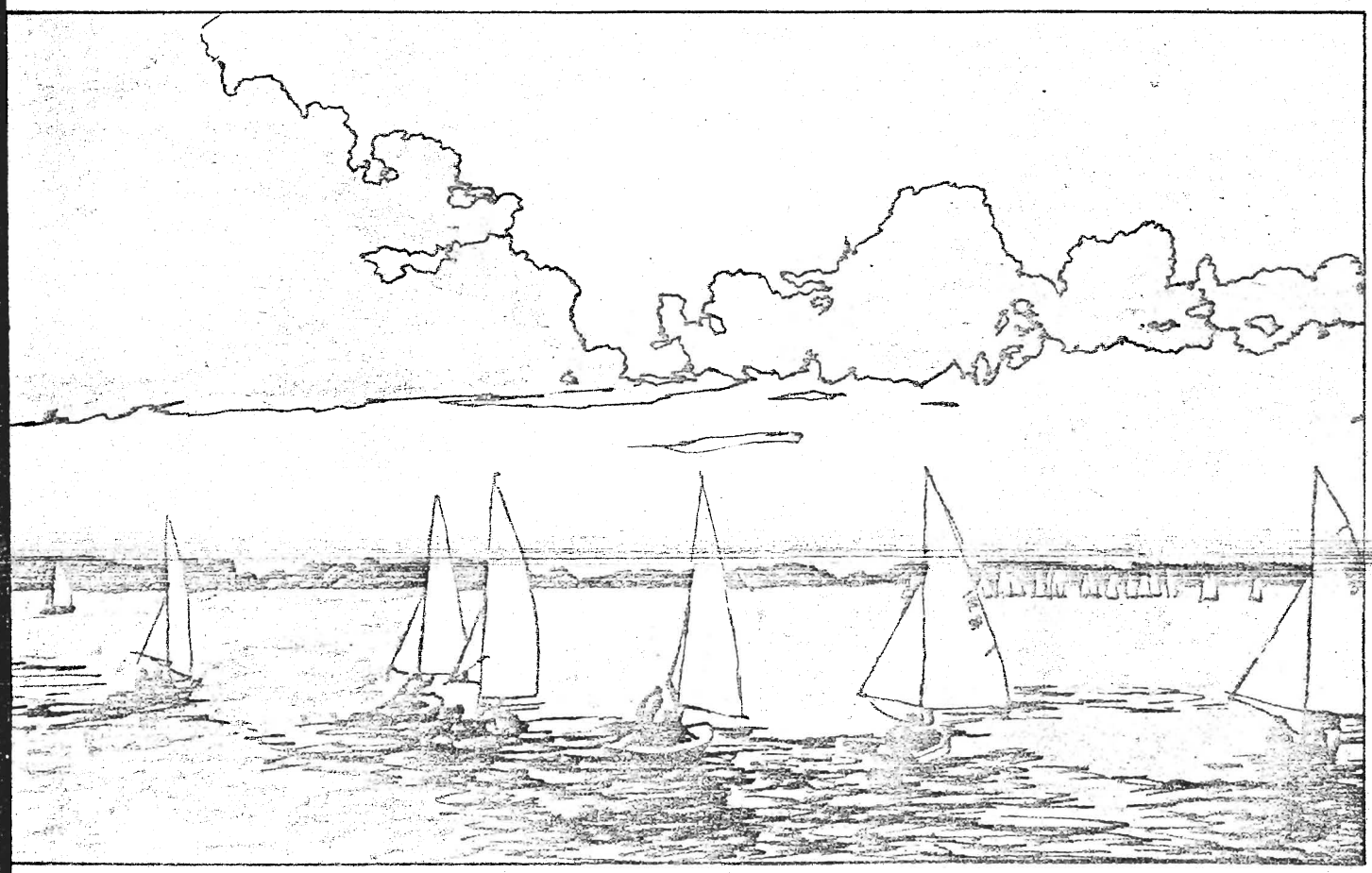

UNIVERSITY OF FLORIDA

Biological Control of Water Weeds With Plant Pathogens

By

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BIOLOGICAL CONTROL OF WATER WEEDS WITH PLANT PATHOGENS

Introduction

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 normally 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 Resources Research, 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.

The program 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 at least one noxious aquatic plant is nearing fruitition. We have reached the stage where large-scale field evaluation of the fungus Cercospora rodmanii for control of waterhyacinth is warranted. An additional three or four organisms also should soon reach this point. We have also attempted to find and research diseases with biocontrol potential for other aquatic weeds.

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 occuring 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 20 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 to include virtually all of Florida. Within the last 5 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, alligator weed, 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 fruitition 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 entomologist 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 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. Publications Number 23 and 30 of the Florida Office of Water Resources Research summarizes the first six years of our research work.

During the past two and one-half years, our efforts have been directed primarily toward those pathogens with definite biocontrol potential. These are: the endemic pathogens of waterhyacinth, Acremonium zonatum and Cercospora rodmanii and two exotic ones, Uredo eichhorniae and Fusarium culmorum for hydrilla control. We have carried out extensive cultural studies in the laboratory, greenhouse studies and, in the case of the endemic pathogens, various small scale field tests. These latter tests have shown A. zonatum and C. rodmanii to have considerable potential as biocontrols. We have tested both of these at locations in Florida and in Lake Concordia in Louisiana. In this latter test, the two pathogens were combined with two insects (Neochetina eichhorniae and Arzama densa) in all possible combinations. This test was 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). Laboratory and greenhouse studies with A. Zonatum on waterhyacinth have elucidated a general resistant mechanism in this plant that accounts for its disease reaction.

Work with the two 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-033-Fla. For more detail on specific points, the reader is referred to published articles. The project is being continued.

DEVELOPMENT OF CERCOSPORA RODMANII AS A BIOLOGICAL CONTROL FOR EICHHORNIA CRASSIPES [1]

K. E. Conway, T. E. Freeman, and R. Charudattan

Introduction

A decline of populations of waterhyacinth was first noticed in 1970 in Rodman Reservoir, a large impoundment of water (3,491 ha) near Orange Springs, Florida. Symptoms associated with waterhyacinth during this decline were a yellowing of the plant, formation of spindly petioles and a rot of the root portion of the plant. It was estimated that this decline saved approximately $20,000 for weed control in this reservoir for one season. Unfortunately, this decline was less severe in each succeeding year and allowed the waterhyacinth to almost completely reestablish in the reservoir. A survey of fungi associated with waterhyacinth in this reservoir (Conway, et al. 1974) resulted in the isolation of a Cercospora that was later determined to be a new species, Cercospora rodmanii Conway (Conway 1976a).

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[1] From Proc. EWRS 5th symposium on aquatic weeds. 1978.

Pathogenicity Evaluations

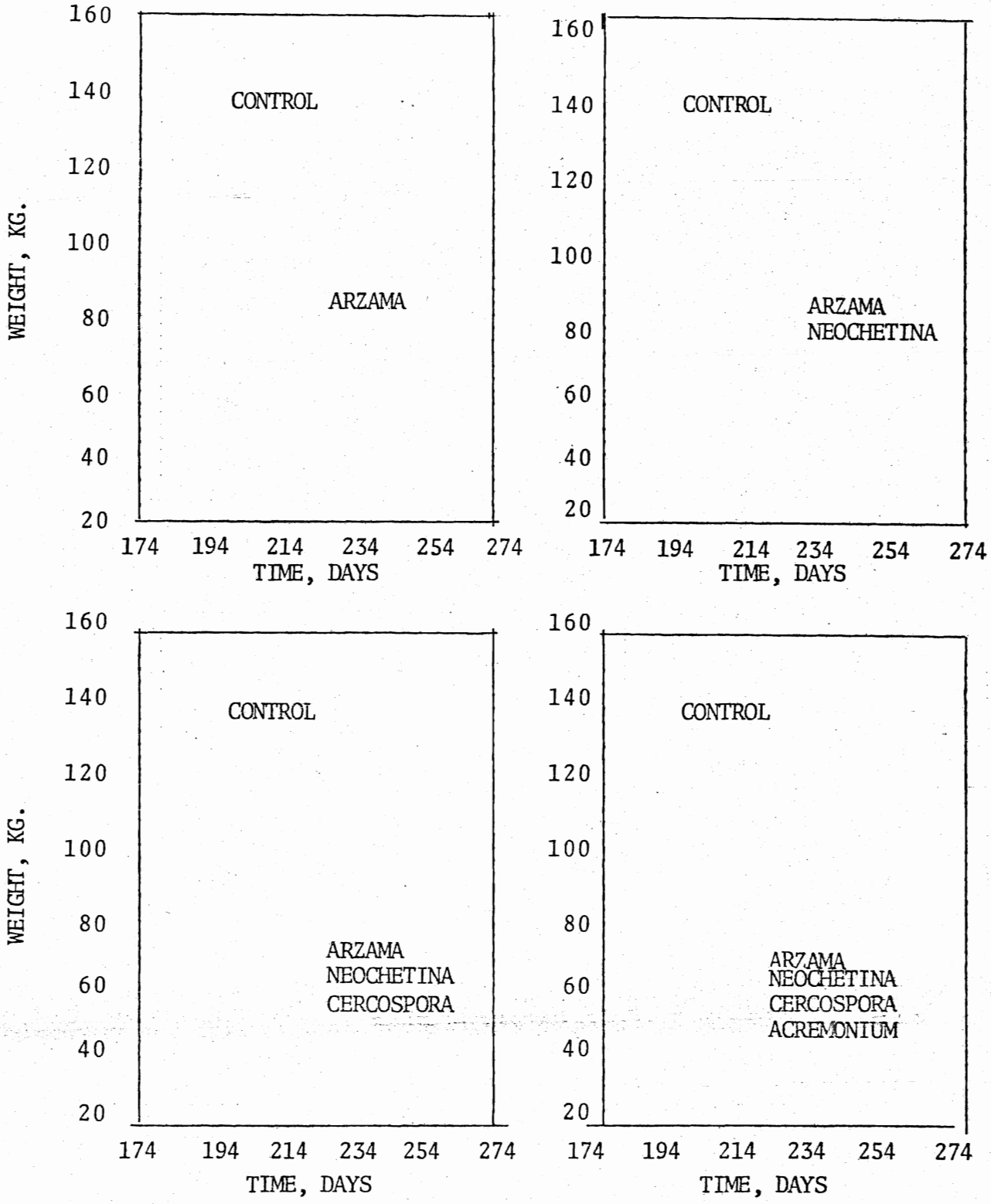
The pathogenicity of the fungus was evaluated in several preliminary tests (Conway 1975) which indicated that the fungus was capable of producing lesions and chlorosis on leaves and petioles of waterhyacinth. This testing culminated in a small-scale field test (Conway 1976b) in a small isolated pool of Lake Alice, located on the campus of the University of Florida. Procedures for the preparation and application of the fungus have been published (Conway 1976b). Approximately 1 Kg of a mycelial suspension was inoculated onto waterhyacinth in an area of 65 M^2. Infection was evident on the inoculated plants within two weeks. Although the fungus was applied to a small area of waterhyacinth, the results from this test indicated that once the disease was established on plants it was capable of spreading via windborne conidia to infect most of the waterhyacinths in the pool (1.7 ha). Even though the populations of waterhyacinth regrew in the spring, there were indications that the new populations had been severely stressed, based on their slower growth rates when compared to noninoculated plants on the other side of the lake. In addition, the fungus was capable of overwintering on the plants and initiated the disease cycle again in the spring.

Host specificity of C. rodmanii was tested (Conway and Freeman 1977) on over 85 economically and ecologically important plants that are either grown commercially or occur

naturally in Florida. A modified centrifugal (related plants) and varietal (economic plants) strategy was used to determine which plants would be included for testing. Plants tested represented 22 families of higher plants and were evaluated in both the greenhouse and the field. The results indicated that C. rodmanii was pathogenic only to waterhyacinth and was safe to use as a biological control in Florida. In a recent publication of preliminary results concerning an attempt to control waterhyacinth with an integrated pathogen-insect combination (Addor 1977a), it was erroneously stated that C. rodmanii had a wide host range. Thus, this publication should be disregarded in favor of Addor's (1977b) later publication of the completed results in which C. rodmanii was correctly noted as being host specific for waterhyacinth.

As integrated pathogen-insect field test which include the pathogens, C. rodmanii and Acremonium zonatum (Sawada) Gams, and two insects, Neochetina eichhorniae Warner and Arzama densa Walker, was conduced in Lake Concordia, Louisiana (Addor 1977). These organisms were evaluated alone and in combination on waterhyacinths confined in frames enclosed in an area of approximately 3.25 M^2. C. rodmanii was applied as a mycelial suspension at a rate of 48 gm/M^2 and A. zonatum was applied at a rate of 96 gm/M^2. The stocking rates for the insects N. eichhorniae and A. densa were 150 and 50 insects per frame, respectively. The

FIGURE 1



THE EFFECT OF COMBINATIONS OF PLANT PATHOGENS AND INSECTS ON THE WEIGHT OF WATERHYACINTH

INTEGRATED CONTROL OF WATERHYACINTH
LAKE CONCORDIA, LOUISIANA, USA
23 JUNE - 30 SEPTEMBER

frames were weighed at two week intervals from 19 June to 30 September 1975. The criterion used for damage to these populations of waterhyacinth was the difference in weight of the treated plants vs. the untreated plants. The important result of this study was in indication that damage to waterhyacinth increased with the number of organisms used to stress the plants. The additive effect of combinations of plant pathogens and insects is illustrated in Figure 1. The reduction in the weights of the waterhyacinth in the frames by each organism as well as in various combinations of each organism were determined. Acremonium zonatum caused the least reduction in weight of the waterhyacinths when compared to each of the other organisms. However, greater reductions in the weight of waterhyacinths were achieved as more organisms were applied to these plots. The greatest reduction of weight was achieved at the end of the test period when all four organisms were used to stress the plants.

Following the Lake Alice study, the ability of C. rodmanii to stress waterhyacinth populations was tested further in Florida. The fungus was re-introduced onto waterhyacinths in the area of Rodman Reservoir where the disease had been isolated originally. Five applications, each consisting of approximately 1 kg of mycelial inoculum, were applied every two weeks to a small area (65 M^2) of waterhyacinth along the shoreline beginning in February 1975. The disease became established in this area within two weeks of the first application. During the next six months the

disease spread to infect most of the waterhyacinth in the area (approximately 15.0 ha). At that time (July), some of the individual waterhyacinth plants had died and sunk beneath the surface of the water. Waterlettuce (Pistia stratiotes L.) and yellow cow lily (Nuphar luteum (L.) Sibthorp and Smith) had replaced these dead plants in the waterhyacinth mats. By August, the enitre population of waterhyacinth was under severe stress from the pathogen and there was approximately 7.0 ha of open water where originally there had been complete coverage of waterhyacinth. During the Fall of 1975, the area of open water reached a maximum of 10.0 ha before cold winter temperatures limited the spread of the disease. No additional inoculations of the fungus have been applied to waterhyacinth in this area and each year since the original inoculation, the disease has overwintered and initiated epidemics the following spring. The disease spread to infect waterhyacinth in most of the reservoir and as a result of the stress placed on the plants by the disease it has not been necessary to expend either large sums of money or energy for waterhyacinth control in the reservoir during 1975-77. A complete elimination of waterhyacinth is not possible using the disease alone and fluctuations in the waterhyacinth populations have occurred which are influenced by environmental factors which condition the host-pathogen balance.

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 depending on environmental conditions and during unfavorable periods this rate may decline to less than one leaf produced over a three week period. Therefore, the success of the epidemic will depend upon the rate at which the pathogen can infect and kill these new leaves. In order to determine if there was an optimal concentration of inoculum necessary to initiate disease, an inoculum rate experiment was begun in a small lake southeast of Gainesville, Florida. Waterhyacinths were confined in 9 M^2 frames and treated at three mycelial inoculum concentrations: 48 gm/M^2, 96 gm/M^2, and 192 gm/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 gm/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 three weeks. Therefore, when conditions exist which favor disease development and which limit leaf production to less than one leaf per three 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.

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, Chicago, Illinois, U.S.A., to produce a commercial product of the fungus. Evaluation of a product has already begun.

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DISEASE RESISTANCE MECHANISMS IN WATERHYACINTHS AND THEIR SIGNIFICANCE IN BIOCONTROL PROGRAMS WITH PHYTOPATHOGENS [1]

R. D. Martyn

The waterhyacinth [Eichhornia crassipes (Mart.) Solms.] is a free-floating vascular hydrophyte that has colonized much of Florida's inland waters. 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 (Sawada) Gams. It causes severe spotting on both leaves and petioles of this plant under conditions of high humidity.

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.

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[1] From Ph.D. Dissertation, University of Florida.

Waterhyacinths displayed various degrees of resistance to A. zonatum depending on their morphotypic state of development. Results of this study indicate that these differences in resistance are due to the variations in phenol chemistry among plants of different sizes and to subsequent changes induced by infection (Table S-1).

Small plants are more resistant to fungal attack than are medium or large plants, based upon the number of lesions/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 in plant morphotypes and is coincident with the observed differences in resistance.

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 three-fold 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.

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%). 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.

The total phenol content decreased 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 of small plants. Large plants, on the other hand, retain their total phenol content and at the same time exhibit a three-fold 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.

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

TABLE 1. DIFFERENCES AND SIMILARITIES AMONG HEALTHY AND A. ZONATUM-INFECTED WATERHYACINTH MORPHOTYPES.

ASSESSMENT CRITERIA	MORPHOTYPE		
	SMALL	MEDIUM	LARGE
MEAN # LESIONS/LEAF	3.7	12.8	18.3
% TOTAL DISEASE	41.3	37.0	39.5
MEAN # 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[A]	3 CELL TYPES[A]	3 CELL TYPES[A]
PPO LOCALIZATION (DISEASED)	ALL CELLS[B]	ALL CELLS[B]	ALL CELLS[B]
TYPE OF PHENOLIC ACIDS (HEALTHY)	6	6	9
TYPE OF PHENOLIC ACIDS (DISEASED)	7	7	9
TOTAL PHENOLS (HEALTHY)	92 µG/G	106 µG/G	104 µG/G
TOTAL PHENOLS (DISEASED)	80 µG/G	96 µG/G	105 µG/G
FUNGAL GROWTH (HEALTHY)	STIMULATIVE[C]	STIMULATIVE[C]	STIMULATIVE[C]
FUNGAL GROWTH (DISEASED)	STIMULATIVE[D]	STIMULATIVE[D]	STIMULATIVE[D]
LEAF REGENERATION (HEALTHY)	27.3%	28.5%	46.1%
LEAF REGENERATION (DISEASED)	21.6%	33.9%	93.3%

[A]VASCULAR PARENCHYMA, BUNDLE SHEATH, AND PHENOL CELLS: [B]ALL CELLS WHICH CONTAIN CHLOROPLASTS; [C]$P=0.05$; GROWTH INCREASED OVER CONTROLS: [D]$P=0.05$; GROWTH INCREASED OVER HEALTHY COUNTERPART

However, because each morphotype responds to infection differently (in most cases in contrast to each other) disease severity balances among the plant sizes at approximately 40% of the leaf-surface area.

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 becomes 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.

It has been shown that A. zonatum is capable of synthesizing indoleacetic acid 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 the 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 observed in large plants by inhibition of the IAA oxidase system.

Perhaps the most significant data supporting a positive role for phenols in disease resistance comes 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.

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 enzyems.

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 yeast extract and extracts from diseased leaves over that on media containing healthy leaf extracts.

Several cytological changes were observed in the cells from infected waterhyacinth leaves. First, chloroplasts in cells of healthy leaves have an abundance of starch granules which disappear after infection. Second, there are only a few plastoglobuli in chloroplasts in healthy cells, but after infection, they increase both in size and in number.

Third, there is a noticeable increase in microbodies in the cytosol of infected cells. It is believed that each of these cytological changes is the result of a shift in host metabolism induced by infection.

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 which, 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.

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

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 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.

A DUTCH ISOLATE OF FUSARIUM ROSEUM 'CULMORUM' MAY CONTROL HYDRILLA VERTICILLATA IN FLORIDA[1]

R. Charudattan and D. E. McKinney

Introduction

It is estimated that a fifth of all fresh water ponds, lakes and rivers in Florida is infested with Hydrilla verticillata L. F. Royle (Hydrocharitaceae), and the weed is spreading rapidly. Since its introduction into Florida waters around 1960, this weed has moved to several other states in the U.S.A. Serious economic losses and ecologic damages resulting from this submerged weed have spurred research on biological, chemical, and mechanical controls. Among biological agents researched are plant pathogens (Charudattan, et al., 1974; Freeman, 1977); however, very few disease of submerged weeds are known (Zettler and Freeman, 1972) and those found on Hydrilla (Charudattan, 1973; Charudattan and Lin, 1974; Freeman, 1977) have not been sufficiently damaging or specific to this host to promote their use in the field.

In 1974, a disease of Stratiotes aloides L. (Hydrocharitaceae) was discovered near Wageningen by Dr. J. C. J. van Zon who brought it to our attention. Mature plants had

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[1]From Proc. EWRS 5th Symposium on aquatic weeds.

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, where a group of fungi were cultured from them including predominantly a Fusarium roseum 'Culmorum' (Lk. ex Fr.) Synd. & Hans. 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 to us. Among the fungal isolates obtained from S. aloides, only 'Culmorum' was capable of killing Hydrilla (Charudattan and McKinney, 1977). Results presented here will prove that the Dutch 'Culmorum' is a virulent pathogen of Hydrilla unlike most other fusaria tested on this host, and that it may help control of Hydrilla in Florida.

Experimental

The effects of the 'Culmorum' isolate 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 X 15 cm glass tubes with 40 ml of sterile water to which were added dense macroconidial suspensions. Control tubes were without conidia. Fungal inocula, consisting of filtered macroconidial suspension obtained from potato dextrose agar cultures, were quantitated with a hemacytometer. Inoculum levels between 2500 and 250,000 conidia per ml (100,000 and 10 million conidia per tube containing 40 ml of water) were set up my mixing suitable concentrations of conidial suspensions. Inoculated and control Hydrilla tubes

were incubated under diffuse light at 22 ± 2 C for several weeks. Damage to Hydrilla from the Dutch 'Culmorum' was usually evident as chlorosis and discoloration of inoculated shoots 10 to 14 days after inoculation. In 3 weeks, death and lysis or regrowth of partially damaged Hydrilla were observed. 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 while at higher inoculum levels the effects were drastic and lethal.

In the second system, 20 liter aquarium tanks were layered with river sand, filled with 14 liters of water, and planted with 100 terminal ends of Hydrilla shoots, each with an active growing bud. After two days, the tanks were inoculated with conidial suspensions of 'Culmorum' at approximately 80,000 or 90,000 conidia per ml of water in tanks. Three weeks after inoculation, Hydrilla shoots started to discolor and developed signs of rotting. In about 5 weeks, the shoots broke down completely, and some that were still green were defoliated and uprooted, and floated to the water surface.

In the third system, the fungus was grown for two weeks on a sterilized mixture of 9 parts sand, 1 part oat meal and 3 parts water, and 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 per inoculated and control tubes. After a week, the inoculated plants turned pale and were dead by the end of 14 days.

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

In order to decide that the effects of the 'Culmorum' isolate 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, three unidentified Fusarium spp., isolated from Hydrilla in Florida, a F. roseum from Ficus elastica Roxb. and a F. roseum 'Graminearum' from Eichhornia crassipes (Mart.) Solms in Florida were included. The test tube procedure described first was used, with inoculum densities between 2500 and 250,000 conidia per ml of treated water.

The results confirmed that the Dutch '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 'Graminearum' from E. crassipes was capable of damaging Hydrilla, inciting similar symptoms as the Dutch 'Culmorum'. However, the threshold of inoculum needed to cause damage by this isolate was approximately 60,000 conidia per ml, or 2.4 times higher than that of 'Culmorum'. The Dutch 'Culmorum' isolate hence was not only pathogenic to Hydrilla but also was more virulent than any Fusarium tested.

In another experiment, conidia and mycelial fragments of the Florida isolate of 'Graminearum' from E. crassipes were applied either as suspension or was injected into bottom sand around 25 rooted Hydrilla shoots maintained in 4 liter glass jars under 2.5 liters of water. For inoculum, the fungus was grown on potato dextrose broth for a week. About 30 g of wet, filtered mycelium and conidia were blended in 125 ml of sterile water. The resulting slurry was applied with an 100 ml hypodermic syringe, fitted with a blunt needle, at 10, 20, and 40 ml portions consisting of 0.96 g, 1.92 g and 3.84 g of conidia and mycelium per liter. The inoculum was suspended over Hydrilla in water or injected into the soil. Control plants received equal amounts of sterile water. Inoculum applied as suspension caused considerable turbidity to 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 were visible, but mostly the plants were healthy, similar to the

controls.

Since the Dutch isolate is still maintained under quarantine due to its foreign origin, the effects of the local 'Graminearum' isolate was tested in an outdoor, large scale test. Plastic swimming pools of 3.04 m diameter and 0.76 m height were layered with river sand, each was planted with 45 kg of fresh Hydrilla, and filled with irrigation water. After five weeks, pools were inoculated with mycelial homogenates. One pool was inoculated with a suspension of approximately 0.18 g/liter of conidia and mycelium and a second pool at 1 g/liter. Control pools were maintained. There were isolated patches of dead Hydrilla a month following inoculation, but no appreciable control of this plant was achieved in pools. This lack of field efficacy may be due to insufficient levels of inoculum used or poor virulence of 'Graminearum' or both. Test with higher inoculum levels of 'Graminearum' as well as with other 'Culmorum' isolated from U.S.A. are in progress.

Host range of the Dutch 'Culmorum' to a few common aquatic plants of Florida and a limited number of crop hosts has been tested. Rooted aquatic plants in glass containers were screened, using inoculum 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 quadalupensis (Spreng.) Magnus (Najadaceae). On E. crassipes, it

caused severe root rot. Alternanthera philoxeroides (Mart.) Griseb. (Amaranthaceae); Nuphar luteum (L.) Sibthorp. and Smith (Nymphaeaceae); and Ruppia maritima L. (Ruppiaceae) were not affected by this isolate.

In preemergence infectivity trials using ca. 38,000 conidia/g of soil, the 'Culmorum' did not depress germination of seeds or cause seedling blights on bean (Phaseolus vulgaris L. var. Pole, Blue Lake.); celery (Apium graveolens L., var. dulce DC., var. Pascal); corn (Zea mays L., var. Silver Queen); lettuce (Lactuca sativa L., var. Bibb); pepper (Capsicum annuum var. Yolo); sorghum Sorghum vulgare Pers., var. unknown); and soybean (Glycine max Merr., var. Forrest). Other crop hosts are under testing. When complete, the host range test will have included most of the economic crop plants grown in Florida and several ecologically important plants.

Since 1971 several hundred fungi and bacteria have been tested for pathogenicity to Hydrilla (Charudattan, Freeman, unpublished). To date no other F. roseum 'Culmorum' or another pathogen possessing virulence comparable to 'Culmorum' has been discovered in the U.S.A. or elsewhere. The Dutch 'Culmorum' appears to be a significant pathogen of Hydrilla.

Conclusions

Results of our tests with a Dutch isolate of R. roseum 'Culmorum' from S. aloides up to now have been encouraging

with regard to its potential as a biological control. The outcome of studies on its field efficacy and safety based on host range testing will determine if this foreign pathogen could be released for control of Hydrilla in Florida and elsewhere.

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GERMINATION AND STORAGE OF AND INFECTION BY UREDOSPORES OF UROMYCES PONTEDERIAE[1]

D. E. McKinney

Collection. - Uredospores of Uromyces pontederiae may be obtained from field-collected detached leaves of Pontederia cordata or from infected plants maintained in the greenhouse using a cyclone spore collector. Spores produced under conditions of 100% RH and temperatures above 30 C will have reduced germination. A three-day interval is necessary between spore collections from infected plants in the greenhouse, to obtain uredospores with good germinability.

Germination. - Both U. pontederiae and Uredo eichhorniae uredospores were stimulated to germinate by ∝- and β-ionone, 2-heptanone, 5-methyl-2-hexanone and 2-hexanone, better than by 1-octanol, 1-nonanol and n-nonanal. Spores of U. pontederiae were best germinated under stimulation by chemicals in vapor - or liquid phase in Conway diffusion cells. Stimulation was less effective when spores were seeded on 2.0% water agar containing concentrations of these chemicals. Chemical pretreatment of U. pontederiae uredospores prior to their contact with H_2O was best accomplished by 20 min. exposure of spores to chemicals in a hydrated atmosphere. An apparent source of stimulus for germination of U.

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[1]From M.S. Thesis, University of Florida.

pontederiae uredospores to the host plant, P. cordata.

Germination of U. pontederiae uredospores may be inhibited in dense concentrations by the presence of an endogenous, water soluble, self-inhibitor. A second endogenous metabolite may be involved in stimulation of germ tube growth. Uromyces pontederiae spores seemed to be inhibited by light, but not irreversibly so. Uromyces pontederiae spores germinated between 10 and 30 C when stimulated, and if not, between 15 and 30 C.

Storage. - Uredospores of U. pontederiae are best stored at -196 C in liquid nitrogen, which stops all metabolic processes and freezes residual spore water. Storage under temperatures of -12 C is best when spores have been vacuum-dried for three hours, which decreases damage by residual liquid solutes. Rapid viability losses were observed with U. pontederiae spores at temperatures of -12 C or less under conditions of no treatment, or storage over desiccants. Humidities of 35, 52, and 61% were beneficial to spore survival at 5 C. Spores did not exhibit cold-dormancy.

Inoculation. - Of the inoculation media tested, best results were obtained with spore-talc mixtures and 0.27% water agar. Stimulation of inoculated spores on the host was best accomplished by vapor stimulation with 2-heptanone in a dew chamber. Incorporation of a stimulant in 0.27% water agar containing U. pontederiae uredospores was less efficient in stimulation of germination on laminae.

Pretreatment of uredospores of U. pontederiae by vapors of stimulants prior to inoculation was not successful in stimulation of spores on inoculated laminae, although this technique worked in Conway diffusion cells.

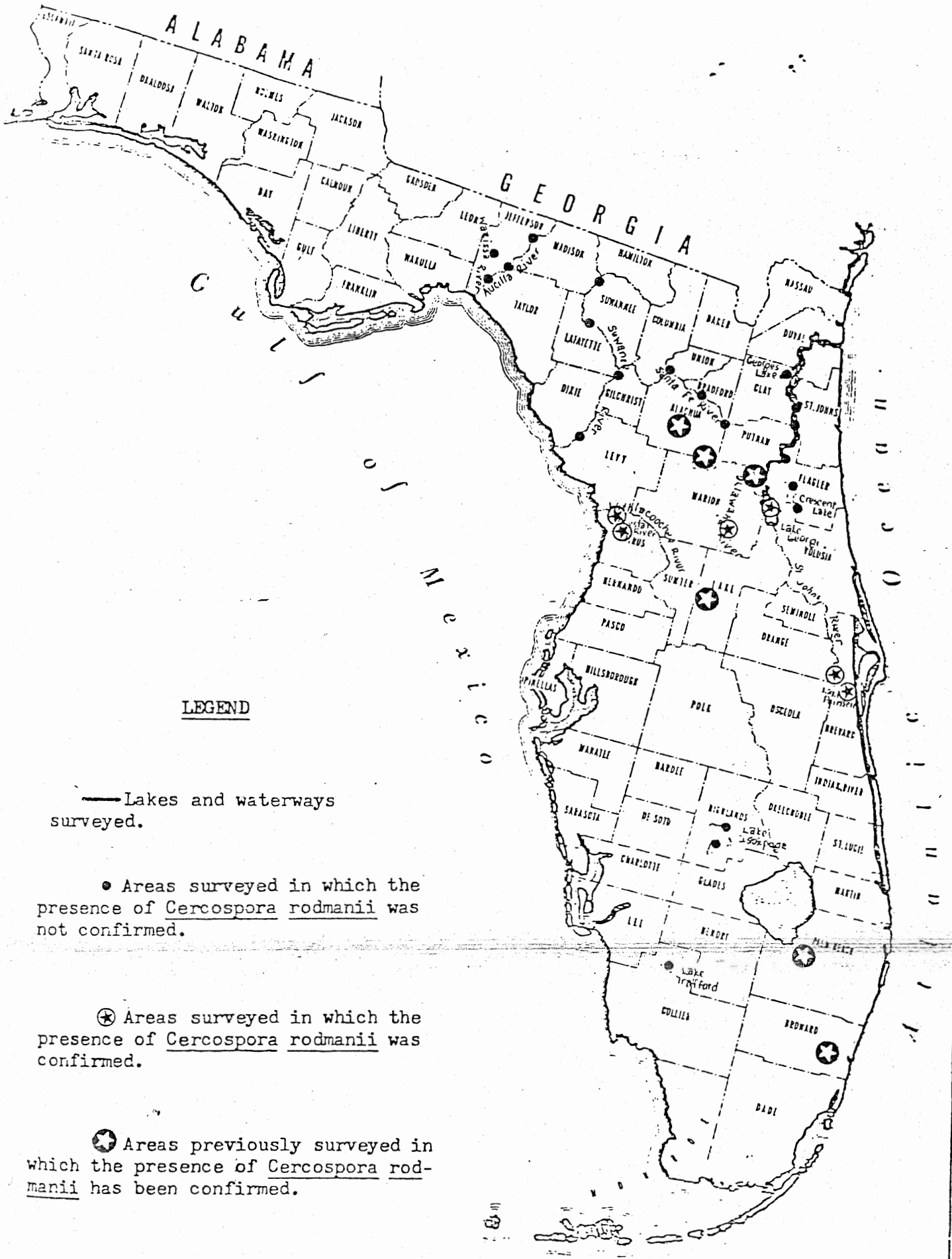
Infection. - Infection of P. cordata by U. pontederiae occurs at temperatures of 15, 20, and 25 C. Apparently due to static growth rate of the host, uredosori were not produced on P. cordata at 15 C, even after 5 wk incubation, but were produced 1 wk after plants were transferred to warmer temperatures in the greenhouse. Uredosori were produced at 16 and 17 days at 20 and 25 C, respectively. Spores germinated poorly and under stress on leaves at 30 C and did not infect. No spores germinated at 25 C. Uredospores of U. pontederiae germinated equally at 25 C on leaves of pickerelweed and waterhyacinth, but penetrated and formed uredosori only on the former host.

OTHER STUDIES

In addition to those summarized in the previous sections, several additional short-term studies have been conducted. These studies are summarized in this section. Two of these concerning a comparison of spray nozzles for application of C. rodmanii and an evaluation of the role of bacteria in the decline of hydrilla were special projects assigned to and conducted by students. Their final reports are included as the final portion of this section.

Survey for C. rodmanii in Florida and Louisiana:

During July and August of 1978, M. T. Olexa surveyed several locations in the state of Florida for the presence of C. rodmanii. The survey was prompted by two factors; 1) there had been several unconfirmed reports of the disease at various locations and 2) we needed a more complete record of the distribution of the fungus in state prior to the establishment of large scale field tests. During the survey, numerous sites ranging from near Tallahassee in the north to Ft. Lauderdale in the South were visited (see Figure 2). Suspected diseased plants were collected and transported to Gainesville where the presence or absence of C. rodmanii was verified by R. Cullen. He based his verification on both microscopic examination and isolations of the fungus from diseased tissue. The fungus was found to be present in 6 of 22 sites surveyed. These six coupled with the six



LEGEND

——Lakes and waterways surveyed.

• Areas surveyed in which the presence of Cercospora rodmanii was not confirmed.

⊛ Areas surveyed in which the presence of Cercospora rodmanii was confirmed.

✪ Areas previously surveyed in which the presence of Cercospora rodmanii has been confirmed.

prior known locations bring . to 12 sites in which the fungus is now known to occur. See Figure 2 for present distribution of C. rodmanii in Florida.

In attempts to evaluate the potential of C. rodmanii for waterhyacinth control in Louisiana the, Army Corps of Engineers has transferred diseased plants from the Lake Concordia experiment to locations in South Louisiana. In September of 1978 we accompanied Mr. E. Addor of the Corps on a survey of several of these sites. Plants were collecte and returned to Gainesville for examination. Cercospora rodmanii was present on plants from 4 of 9 locations visited. The fungus was present on plants from a site North of Hayes LA; sites I and II on Pecan Island, LA; and site I in Bayou Manchac. It was not present at either Sorrento II, Hayes West, Centerville C, Bayou Louise or Manchac IV sites.

Results of these surveys indicate that C. rodmanii is becoming widespread in both Florida and Louisiana. There are indications based on observations that in several of these areas it is beginning to exert a degree of control.

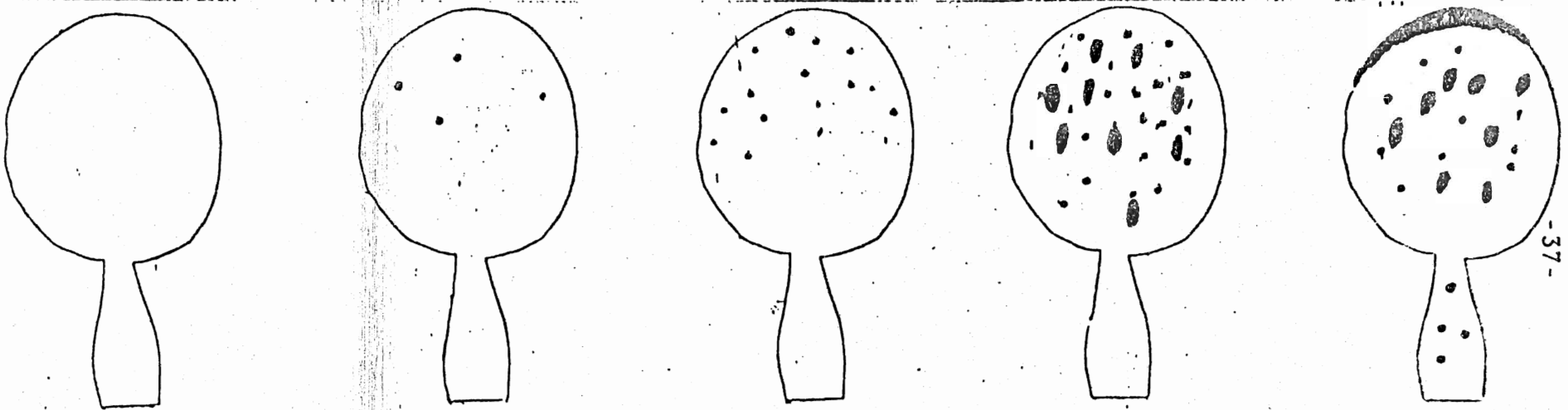
Rating scale for C. rodmanii damage.

One of the problems encountered in plant disease work is that of evaluation of disease damage on a quantitative basis. Based on his years of experience with the disease, Dr. Conway was able to devise a rating scale system for C. rodmanii damage based on individual leaf damage. This system is shown in Figure 3. The system worked exceptional

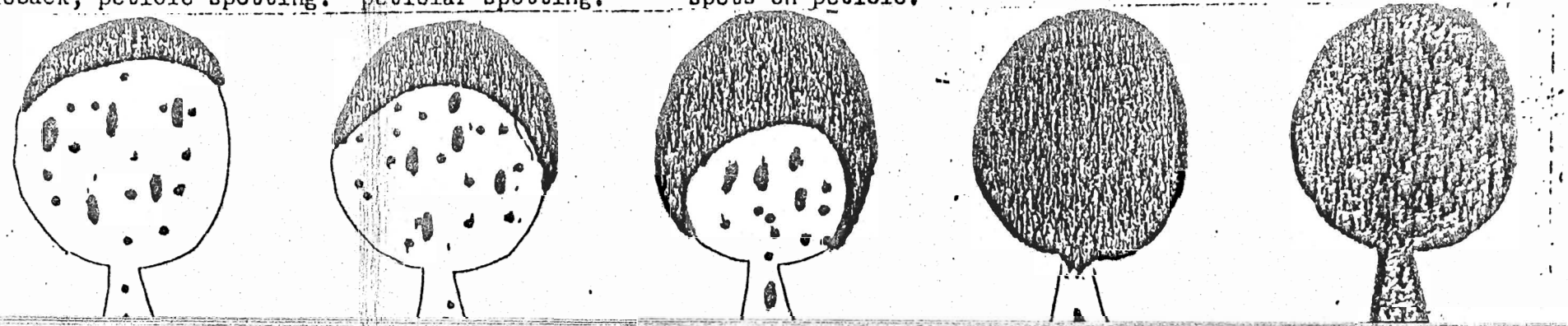
Figure 3

RATING SCALE SYSTEM FOR DAMAGE TO LEAVES OF WATERHYACINTH BY CERCOSPORA RODMANII.

NUMERICAL RATING	0	1	2	3	4
SYMPTOMS	No spots on leaf of petiole.	1-4 spots on leaf, no petiolar spotting.	Less than 25% of leaf surface with spots, no coalescence or petiolar spotting.	Less than 50% of leaf surface with spots, some coalescence, no petiolar spotting.	Less than 25% of leaf surface with spots, coalescence, some tip-dieback and petiolar spottin



NUMERICAL RATING	5	6	7	8	9
SYMPTOMS	Less than 50% of leaf surface with spots, coalescence, 10% tip-dieback, petiole spotting.	Less than 75% spots, coalescence,(30%)tip-dieback, increasing petiolar spotting.	Greater than 75% spots, coalescence,(60%) tip-dieback, coalescing spots on petiole.	Dead leaf Blade, Petiole green,but heavily spotted.	Dead leaf blade and petiole (submerged).



well in the Fish Prairie studies where inoculum concentration and subsequent spread were variable being evaluated. It is, therefore, recommended that this system be used in all future work with C. rodmanii. It is readily adaptable to rating entire plant damage by simply rating all the leaves on a given plant and dividing the total rating by number of leaves per plant.

Pathogenicity of Phycomycetes to Hydrilla.

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 these latter are soil inhabiting. We tested the susceptibility of hydrilla to 25 such pathogens belonging to three genera. Out of these, 3 would consistently attack hydrilla under conditions of this test (sprigs of hydrilla in test tubes of distilled water). Results are shown in Table 2. However, larger scale tests in gallon jugs and 5 gallon aquaria yeilded unconsistant pathogenicity results. Similar results (inconsistent) were also obtained with unidentified species of Pythium and Phytophthora isolated from declining hydrilla in Orange Lake, FL. Despite this inconsistency, the search for pathogens of hydrilla in the Phycomycete group should be continued because of the adaption of this group of fungi to an aquatic mode of existense.

Table 2. Reaction of Hydrilla following inoculation with various Phycomycetes.

Phycomycete	Original host	Hydrilla reaction[a]
Aphanomyces cochliodes	Beta vulgaris	-
A. euteiches	Pisum sp.	-
Phytophthora cinnamoni	Persea americana	-
P. citrophthora	Citrus sp.	-
P. cryptogea	Aster sp.	-
P. dreschleri	Citrus sp.	-
P. erythroseptica	Solanum tuberosum	++
P. palmivora	Ficus sp.	-
P. parasitica	Lycopersicon esculentum	+++
P. parasitica	Nicotiana tabacum	-(?)
P. stellata	unknown	-
Pythium acanthicum	unknown	-
P. aphanidermatum	Chrysanthemum sp.	-
P. carolinianum	unknown	
P. deboryanum	unknown	-
P. graminicolum	unknown	
P. herbicoides	unknown	-
P. irregulare	unknown	-
P. irregulare	Caladium sp.	-
P. myriotylum	unknown	-
P. paroecandrum	Zea mays	-
P. polytylum	unknown	+

P. splendens	unknown	-
P. ultimum	unknown	-
P. vexans	unknown	-

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[a] +, ++, +++ = slight, moderate, and high degree of susceptibility indicated, - = not susceptible, -(?) = indefinite.

Other fungi isolated from Hydrilla.

During the summer of 1977, Dr. Olexa isolated over 175 cultures of fungi from declining hydrilla primarily from Remuda Canal in Southwest Florida and Orange Lake and Rodman Reservoir in North Central Florida. Only one of these, tentatively identified as a microconidial Fusarium sp. was consistently pathogenic on hydrilla. However, pathogenicity level was considered too low to be of value in a biological control program. Therefore, the search for hydrilla pathogens with biocontrol potential must be continued.

Effect of C. rodmanii on fish.

One of the major concerns in the use of plant pathogens for biological control of aquatic weeds is that they will harm fish. To determine if C. rodmanii was detrimental, it was tested against the fish Gambusia affinis in a standard 96 hr. bioassy. Ground up mycelium and spores of C. rodmanii was placed in the fish containers at rates ranging from 0.4 gm/liter to 6.34 gl liter. The lowest rate corresponded to an inoculum level of 48 gm/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 4 times higher than the highest rate we have ever used to inoculated waterhyacinth with C. rodmanii. None of the fish in any of the treatments were adversely affected. In fact at the highest rate, the fish ate the fungus which was subsequently isolated from their

feces. Therefore based on this limited test, we feel that C. rodmanii poses no threat to fish but other species need to be tested.

SURVEY OF HYDRILLA UNDERGOING ANNUAL DECLINE FOR PATHOGENIC BACTERIA

Daryl E. McKinney

PT 621

RESEARCH PROJECT

Introduction

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 to Florida from South America around 1940 (4). Since its introduction, it 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 body of water so infested.

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 it grows similar to an annual plant.

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 (6). It grows towards the surface of the water from depths as great as 40 to 50 feet. The hydrilla may then form dense, entangled mats that can reduce light penetration to less than 5% at a 30 cm depth (3). Throughout the summer, hydrilla stores starch in its stems, stolons, and underground rhizomes. By late summer the mat is at a maximum density. An increase in epiphytic growth is commonly observed at this time (2). As the summer passes into fall, hydrilla begins to undergo what

is known as annual decline. At this time of the year the weather is the warmest, with the growing season and light intensity near maximum. Plants undergoing annual decline exhibit 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.

There are several possible explanations for annual decline. It is possible that hydrilla loses an excessive amount of photosynthate, as dissolved organic matter (DOM), through its leaves and epiphytes then use the DOM. This would correlate with the observed growth increase of epiphytes (1). These epiphytes (bacteria and algae) may then interfere with CO_2 diffusion or photosynthesis by the hydrilla (5). When photosynthetic processes are reduced below equilibrium with the respiration processes the plant would begin to die. The epiphytes may produce toxic metabolites that damage hydrilla tissue. Another theory is that plant pathogens are involved in annual decline (2).

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) inoculated on healthy hydrilla where it would have to cause symptoms associated with annual decline, and (d) the

bacterium would have to be reisolated from the test plants.

Materials and Methods

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

At the site isolations were made by cutting necrotic hydrilla stem and leaf tissue into approximately ¼" pieces and surface sterilizing 20 of these pieces. Sterilization was accomplished by rinsing the hydrilla pieces with a 10% clorox solution in a petri plate for one minute 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 one liter of deionized water), nutrient agar, and potato dextrose agar. Plates were then incubated at 25 C for three days.

Lab isolations were made from hydrilla spigs 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 three days.

Seven apparently different bacteria were then 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 - Two liter flasks containing 500 ml of nutrient broth were inoculated from stock cultures and shake cultures at approximately 60 strokes per minute for 24 hours. Isolates six and seven 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.

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

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

Two inoculum systems were used. The first consisted of incubating an 80 to 100 mm long growing hydrilla shoot

in a 30 X 150 mm glass tube in a bacterial suspension. The suspension was prepared by adding 4 ml of a 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.

The second inoculation method was similar to the first except tubes were vacuum infiltrated two mintues at 25 mm Hg vacuum after inoculation.

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

Disease assessment after three 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 1).

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

The seven colony types are listed in Table 1. The majority (4/7) of the colonies were gray or white. This

agrees with Berg's (2) finding of three white colony types commonly isolated from hydrilla undergoing annual decline. Colony types 2 and 3 may be the same bacterium, with 3 being a rough mutant. All bacterial types grew similarly on NA as they did on hydrilla infusion agar. This indicates a lack of specific growth requirements.

Results from inoculated hydrilla sprigs show that the bacteria tested were not pathogenic to hydrilla (Table 2). The chlorosis observed was probably due to a nutrient deficiency and not a pathogenic response.

Reisolations (Table 3) from the inoculated and control hydrilla resulted in a random pattern of bacteria reisolated. Many of the reisolations contained bacteria that had not been inocualted on that particular hydrilla sprig. These bacteria were compared to stock tubes and related visually on the basis of their simularities in gross morphology.

Discussion

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 from such tissue expecially if more than one reisolation is made. 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 inocualtions.

Berg (2) previously isolated three white bacterial

TABLE 1. Description of the 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 2. Visual comparison of hydrilla sprigs three weeds 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; N= Necrotic

TABLE 3. Reisolations (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)	---

*( ) contain bacterial type reisolated, compared with stock cultures.

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 non-toxic metabolites produced by the bacteria became concentrated to such an extent that they were toxic. His tests with the toxins found them to be non-specific on other aquatic plants, this supports the idea that they are super-concentrated metabolites and not toxins per se.

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 3) of inoculated and non-inoculated hydrilla which indicate the presence of these bacteria on healthy hydrilla.

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COMPARISON OF THREE NOZZLE SYSTEMS FOR SPRAYING CERCOSPORA RODMANII

by

Deborah F. Reese

As a senior research project I undertook the assignment of determining if nozzle systems have any effect on the efficacy of biological control organisms. Under the direction of Dr. Kenneth E. Conway and with the technical assistance of Richard Cullen, a field test was carried out on plots in Thrasher Pond on Fish Prairie, south of Micanopy, FL.

The objectives of this experiment were to see if nozzel types have any affect on the size of inoculum particles and pathogenicity of Cercospora rodmanii, Conway, a biological control organism currently being experimented with for waterhyacinth control.

Materials and Methods

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

Each nozzle was used in conjunction with a Spray Systems gun jet #12GH, adapted with a Delevan gun #3160 and two 14 inch extensions for ease in covering the plots from a boat. A portable spray power rotary pump with a modified even flow tank was used to deliver the spray. Each nozzle was calibrated to determine the amount of flow/second. The raindrop nozzle delivered 300 ml/sec., the mister 200 ml/sec.,

Inoculum, amount used and method of application: Isolate

WH 9 of C. rodmanii, was grown on potato-dextrose broth with 0.5 % yeast extract (PDBY) for approximately two and one-half weeks. A concentration of 48 b/M^2 wet weight mycelium was used. The mycelium was diluted with water to give the proper spray volume.

Each plot received the same amount of inoculum, 6000 ml. To insure an even inoculation, the plots were sprayed in the following series; 3000 ml of inoculum was applied to waterhyacinths in plot #33, 34, and 35. Then the remaining 3000 ml of the total 6000 ml was applied in series to plots #35, 34, and 33.

Plots - Frames (made from PVC pipe) 9 m^2 were floated on the pond. Each frame contained approximately the same number, age, and degree of previous infection (this variable was unavoidable as field test with C. rodmanii for rate and effect is an ongoing experiment at this pond). The frames were numbered #33, 34, and 35. The inoculum was applied to waterhyacinths in frame #33 using the raindrop nozzle. Waterhyacinths in frame #34 were inoculated using the mister nozzle. And waterhyacinths in frame #35 were inoculated using the hollow cone nozzle. Each frame was rated for damage before inoculation and at two and seven weeks after inoculation.

Results

Immediately after spraying, ten leaves per plot were

removed. Each leaf was examined for average size of inoculum particles and average number of particles/unit area 2.83 cm^2) using a binocular microscope. Dimensions of ten particles per leaf were recorded and the area of an elispe formula was used to calculate the area of each particle. The unit area was chosen at random on each leaf. The following is a breakdown of data on particle size and number of particles/unit area:

Nozzle	Average Area (mm^2)	Range (mm)	Particles/ Unit area
raindrop	0.99	0.62-2.2	8.3
mister	1.6	0.61-5.6	13.1
hollow cone	1.3	0.46-2.8	10.2

The leaves of ten waterhyacinth plants from each of the three plots were rated using a system of numbers 0.9. Zero being no infection and nine being death and/or submersion of the leaf. For the purpose of this experiment, we limited the data collected to inoculum sprayed out for this test. Therefore, only the leaves with damage of 0-6 were counted. The following is a breakdown of damage caused by inoculum sprayed using the three nozzle types:

	March 31	April 26	May 31
Raindrop: (Plot #33)			
Total Damage	106	170	158
Average damage/plant	10.6	17.0	15.8
Average damage/leaf	2.0	2.2	2.2
Mister: (Plot #34)			
Total Damage	102	165	162
Average damage/plant	10.2	16.5	16.2
Average damage/leaf	1.8	2.3	2.3
Hollow cone: (Plot #35)			
Total Damage	107	146	122
Average damage/plant	10.7	14.6	12.2
Average damage/leaf	1.9	2.2	2.0

Following inoculation plants in plot #33 had the greatest amount of total damage and average damage/plant. Plants in plot #34 had intermediate amount of total damage and average damage/plant. Plants in #35 had the least total damage and average damage/plant. But plants in plot #34 had the greatest amount of average damage/leaf, plot #33 intermediate and plot #35 the least. The average damage/leaf was studied because each plant did not have the same amount of leaves.

Visually on April 26, the plants in plot #33 had the most spotting. Plants in plot #35 ahd intermediate spotting and plants in plot #34 had the least spotting.

On May 31, the plots were rated again to see if nozzle type had any affect on the long-term pathogenicity of the inoculum. The plants in plot #34 had the greatest total damage, average damage/plant, and average damage/leaf. The

plants in plot #33 had intermediate total damage, average damage/plant and average damage/leaf. The plants in plot #35 had the least amount of total damage, average damage/plant and average damage/leaf. Visually it was hard to tell which plots had been sprayed with inoculum from different nozzles.

Conclusions and Recommendations

Using the premise that the larger particle size of inoculum and the more inoculum particles/leaf is best, I concluded that the mister nozzle would be the best nozzle for spraying C. rodmanii. The nozzle delivered the largest particle and the most particles/leaf, although infection got a slower start. During the course of this test, inoculum sprayed by the mister nozzle produced the most damage on the plants.

I believe the calculation of data concerning total damage had too many variables. Not knowing exactly which leaves were inoculated and which leaves were already infected with C. rodmanii satistically affected the data results. We should have tagged the oldest living leaf and the youngest leaf. I recommend that this test be repeated tagging the youngest and oldest leaf before inoculation. Then we will rate those leaves between the tags at the beginning and end of the test to insure that only those leaves inoculated directly with C. rodmanii are rated for statistical analysis.

SUMMARY AND CONCLUSIONS

During the past two and one-half years, considerable additional progress has been made in reaching our goal of the utilization of plant pathogens in biological control programs for aquatic weeds.

The pathogen Cercospora rodmanii has been shown to be effective against waterhyacinth in tests in both Louisiana and Florida. Methods of culturing and dissemination of this fungus for biocontrol purposes have been developed and considerable basic information concerning the host parasite relationship has been elucidated. This fungus shows so much promise that the University has decided to apply for a patent for its use in biocontrol programs and Abbott Laboratories has entered into an agreement with the University to develop it into a marketable product form for possible worldwide distribution.

Two exotic pathogens also show biocontrol potential in our tests. The rust fungus, Uredo eichhorniae, from Argentina appears to have potential in biocontrol programs for waterhyacinths. A Dutch fungus, Fusarium roseum 'Culmorum' shows promise for hydrilla control. Research with both of these fungi has been slowed because of the necessity of conducting research on them in quarantine.

In addition to the above studies, several other investigations have been conducted with other pathogens and potential pathogens on various weed host. As a result, we

feel our program has advanced faster than anticipated and is nearing our goal, at least in some areas. Based on this work, we can conclude that our original proposition is correct - plant pathogens are viable candidates as biocontrol agents for aquatic weeds.

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