Evaluation of Cercospora rodmanii as a Biological Control of Waterhyacinths

Kenneth E. Conway

Assistant Research Scientist, Plant Pathology Department, University of Florida, Gainesville 32611.

The author thanks T. E. Freeman, R. Charudattan, and H. H. Luke for their valuable suggestions. The technical assistance of J. Dennis, S. Broos, S. Morey, and R. Cullen is acknowledged.

This investigation was supported in part by the U. S. Army Corps of Engineers Contract No. DACW 73-73-C-0049, Florida Department of Natural Resources; and by the U. S. Department of Interior, Office of Water Resources Research and Technology as authorized under the Water Resources Research Act as amended.

Florida Agricultural Experiment Station Journal Series Paper No. 8046.

Accepted for publication 20 February 1976.

ABSTRACT

CONWAY, K. E. 1976. Evaluation of Cercospora rodmanii as a biological control of waterhyacinths. Phytopathology 66: 914-917

A fungus, *Cercospora rodmanii* Conway, which was isolated from declining waterhyacinths in Rodman Reservoir in Florida, was evaluated for its biological control potential on waterhyacinths during two growing seasons (February-November). Results of greenhouse and field studies indicated that the fungus was responsible for the waterhyacinth decline. Infection was initiated on waterhyacinths in Lake Alice on the University of Florida campus during September and October, 1974. A combination of conidia and mycelia was applied to the plants. When the inoculum level was increased by a second similar spray application the disease spread rapidly. Browning of the waterhyacinths in the test pool was augmented in the later stages of the test by below-freezing temperatures. *Cercospora rodmanii* can be a virulent pathogen on waterhyacinth and its effect on field populations of waterhyacinth will be evaluated further to quantify damage to the plant.

The noxious waterhyacinth [*Eichhornia crassipes* (Mart.) Solms] was first introduced into the state of Florida in the 1890's (8). Since then, it has spread and now covers nearly 300,000 acres of waterways. The Florida Department of Natural Resources estimated that 10-15 million dollars are spent annually in Florida for aquatic weed control with a majority being spent on waterhyacinth control (7).

Control of waterhyacinths has been attempted by three techniques: mechanical removal, chemical control, and biological control (8). Waterhyacinth is a good candidate for biological control because its main means of reproduction and spread is asexual through offshoots. Insects have been the organisms used most in biological control of waterhyacinth (7). In 1970, a biological control program for aquatic weeds using plant pathogens was initiated at the University of Florida. As part of this program, foreign and domestic surveys were made on waterhyacinth and other target aquatic weeds (1, 4).

Particular interest has centered on a naturally occurring decline of waterhyacinth in the Rodman Reservoir, a large area of impounded water in the Cross-Florida Barge Canal. This decline was first evident in the spring of 1971; almost every waterhyacinth in the reservoir was affected. Symptoms of the disease included chlorosis of the plants, failure to produce offshoots, spindly petioles, and a root rot. These symptoms increased in severity over the growing season. It was assumed at that time that the root rot was the primary cause of the decline. Each year since 1971 the decline lessened in intensity until 1974 when very few affected plants were noted. A comprehensive survey was begun in 1973 of fungi occurring on waterhyacinth in the Rodman Reservoir (4). Among the fungi isolated was a *Cercospora* spp. that later was named *C. rodmanii* Conway (3).

The initial objective of this study was to determine if *C. rodmanii* was capable of infecting waterhyacinths under field conditions. This objective was expanded during the second year (February-November 1975) to continue the infection on the plants throughout the year and to determine the optimal time for application of the fungal inoculum.

MATERIALS AND METHODS

The fungal isolate was evaluated through a three-stage testing program:

Primary greenhouse testing.—*Cercospora rodmanii* (isolate WH-9) was grown on potato-dextrose agar with 0.5% yeast extract added (PDAY). Individual waterhyacinths were placed in plastic-lined pots that contained one liter of water with 5 ml of a solution of 0.2 M $Fe(NH_3)_2SO_4 \cdot H_2O$ and 0.2 M $MgSO_4 \cdot 7H_2O$ added.

Several areas on the waterhyacinth leaves and petioles were gently rubbed to break the cuticle. Mycelia and conidia were scraped from the agar surface and placed on these spots. A plastic bag then was placed over the entire plant for 2 weeks. This primary stage involved the mass screening and evaluation of >150 fungal cultures isolated from waterhyacinth. Isolates that caused disease were used in the second stage of evaluation.

Second-stage greenhouse testing.—Twenty-five to 30 waterhyacinths were grown in a large vat of water. The fungus was cultured in five Roux bottles containing 100

Fig. 1-8. Stages of infection of waterhyacinth by *Cercospora rodmanii*. **1)** The result of inoculation with *C. rodmanii* on waterhyacinth in a primary greenhouse test. Note the chlorosis of the inoculated leaf and the necrotic areas around the points of inoculum. **2)** The effect of inoculation with *C. rodmanii* on uninjuredwaterhyacinths in a second-stage greenhouse test. 3) Typical disease symptoms caused by *C. rodmanii* in the inoculated area in Lake Alice. Note the punctate spotting, chlorosis, and tip dieback on the leaves. **4)** The pool area in Lake Alice showing the brown dead and dying waterhyacinths on 21 November 1974. **5)** Aerial photograph of Lake Alice showing the original inoculation site (SS), the isolated pool (IP) and the main Lake Alice (LA). Note the brown strips of diseased plants along both sides of the main lake. **6)** Dead waterhyacinths in the inoculated area on December, 1974 due to a combination of both disease and frost. **7)** Regrowth of waterhyacinths in the inoculated area 18 February 1975. **8)** Waterhyacinths in main Lake Alice, 90-120 cm tall 18 February 1975. Fig. 7 and 8 contrast the amount of stress placed on the waterhyacinths by *C. rodmanii*.

ml of potato-dextrose broth with 0.5% yeast extract added. After 12 days, the mycelial mats were collected and comminuted in a Waring Blendor for 15 seconds. The resulting suspension was sprinkled onto nonwounded waterhyacinths, which more closely approached inoculation as it occurs under natural conditions. The plants were not covered following inoculation.

Field testing.—An isolated pool (1.7 ha) of Lake Alice on the University of Florida campus was chosen as a suitable site for field tests. The fungus was grown in 100 Roux bottles containing 100 ml PDY broth. The macerated mycelia and conidia were diluted with 38 liters of water and sprayed on the waterhyacinths. The fungus was applied to the waterhyacinths in the evening to utilize the cooler night temperatures and high relative humidity, thus promoting infection. The area sprayed represented an arc of 6.4 m radius from the shoreline for a coverage of 64.4 m^2. Approximately 1 kg (wet weight) of the fungus was used per application in this experiment. Two applications of the fungus were applied to the waterhyacinths, one on 4 September and the second on 3 October 1974.

RESULTS

Primary greenhouse testing.—After 3 weeks, the inoculated leaves of the waterhyacinths became chlorotic (Fig. 1) and necrotic spots were present on both the leaves and petioles of the plants where the inoculum had been placed. The spots on the leaves extended to the leaf tip.

Second-stage greenhouse testing.—Within 1 month after inoculation, the plants in the vat showed chlorosis, and many showed necrotic spots (Fig. 2). The plants were in an obvious state of decline. Spots extending to the leaf tip similar to those of the primary test were not noted. After several months, the plants became severely stunted, developed symptoms of root rot, and eventually died.

Field testing.—The first inoculation was applied to waterhyacinths in Lake Alice on 4 September 1974. Necrotic spots began to appear on the waterhyacinths in the treated area within 14 days and by the end of 28 days, some of the leaves showed a tip dieback (Fig. 3). A second application of the pathogen (3 October) resulted in still more necrotic spots and tip dieback within 7 days. The presence of *C. rodmanii* was confirmed by reisolation from the diseased plants in the inoculated area.

By 1 November, tip dieback was evident throughout the large pool area (Fig. 4). *Cercospora rodmanii* was isolated from diseased waterhyacinths on the opposite side of the pool from where it originally was applied. Symptoms of the disease were observed beyond a grass barrier surrounding the pool and on waterhyacinths in the main area of the lake. Aerial photography (Fig. 5) showed a gradient of disease incidence from the inoculation site to the main lake. Infection of plants by windborne conidia occurred on both sides of the grass barrier and on one edge of the tree barrier. Thus, a brown fringe of diseased plants was produced along both sides of the lake.

On 13 November a temperature of 0.5 C was recorded in fields near Lake Alice (3.3 C at water level). This is the first possible frost for the season and therefore could not have caused the tip burns noted earlier. Frost was recorded officially on 2, 4, 5, 10, 18, and 19 December and on 14 and 15 January.

All plants in the pool area were completely brown by 6 December (Fig. 6), owing to a combination of the disease and freezing temperatures. However, waterhyacinths in the middle of the main lake continued to show some green. The waterhyacinths in the inoculated pool were brown until 18 February 1975 when the crowns of some of the plants began to produce new leaves (Fig. 7). It was obvious that the stress effects of *C. rodmanii* persisted in the pool area for several months because plants normally 60 cm tall were less than 15 cm tall. In comparison to these small plants in the pool, the waterhyacinths in the main lake were 90-120 cm tall (Fig. 8).

Hydrocotyl umbellata L. (water pennywort), an aquatic weed common to Florida, encroached along the fringe of the pool during this period of stress on waterhyacinth. This encroachment also was evident in the main lake where the disease was present. *Hydrocotyl umbellata* was parasitized by *Cercospora hydrocotyles* Ellis and Everhart and also by *Puccinia hydrocotyles* (Link) Cooke. These fungi have potential as biological controls of *H. umbellata* where the weed is a problem, particularly in ditches and shallow ponds.

Four additional applications of *C. rodmanii* were placed on the waterhyacinths in the same area of the pool during March to July. In April most of the waterhyacinths in the pool area were infected by *C. rodmanii,* however, as conditions became more favorable to rapid waterhyacinth growth, the new leaves outgrew the disease and produced a "canopy effect" (6) with the disease symptoms of *C. rodmanii* confined to the older lower leaves while the canopy looked clean. This condition prevailed throughout the summer until September when the growth of waterhyacinth was slowed by cooler night temperatures. Increased infection was apparent on the new leaves as small discrete spots and as tip dieback. By October, definite disease symptoms were present on all plants throughout the pool. Cold night temperatures (0.6 C) occurred in mid-November and caused the waterhyacinths to turn brown in the pool. The plants in the main lake remained green except for those portions that had been infected with *C. rodmanii.*

DISCUSSION

The purpose of a biological control of waterhyacinths is to increase the stress on the target organism and not necessarily to eliminate the organism from the environment. Ecological balance is important in the aquatic ecosystem because the elimination of one plant species will open the system to invasion by other weeds which may be more difficult to control such as the submerged weed *Hydrilla verticillata* Royle. It has been shown in this study that *C. rodmanii* is easily cultured and that infection can take place from conidial or mycelial fragments, it is wind disseminated, it is host-specific (2), and it can severely stress the target weed. Therefore, *C. rodmanii* fits all the criteria desirable in a biological control organism (5, 8).

The field test of *C. rodmanii* in Lake Alice corresponds well to the natural outbreak of disease in the Rodman Reservoir, and thus provides evidence that this organism was responsible for the disease observed (7) in Rodman Reservoir. One difference is that the disorder in Rodman Reservoir was reported as a slow or more progressive

disease that began in April and culminated in November (versus September to November). However, all the symptoms reported for plants in the reservoir were observed on waterhyacinths in both greenhouse and field experiments in the current study, except that the damage seen in Lake Alice was augmented by cold temperatures in the latter stages of the test. The difference in rate of disease progression may result from the fact that Lake Alice receives the run-off from the University's sewage treatment plant and is in a highly eutrophic condition. This may impart a higher degree of immunity to the waterhyacinths either through an increased growth rate or an altered metabolism.

The waterhyacinths appeared to be killed by the pathogen in both Rodman Reservoir and Lake Alice. However, in Lake Alice the apical meristems of some of the plants survived and produced small reduced plants that continued to spread in the pool. *Cercospora rodmanii* sporulated abundantly on the surfaces of dead leaves. These leaves characteristically are submerged in water when the entire leaf dies. If the inoculum concentration of the fungus is high enough to reinfect and kill the new leaves and petioles produced by the plant, the disease will gradually weaken the plant. Plants that have undergone a period of disease stress become spindly. The root region where dead petioles are attached becomes invaded and rotted by secondary organisms. A reduction in disease incidence due to the loss of inoculum can result under natural conditions when the inoculum concentration is not sufficient to continue infection due to the submergence of dead leaves and plants. Such a condition could account for the reduction in disease severity in Rodman Reservoir from 1971 to 1974.

The effect of a continuous stress on waterhyacinth due to disease holds much promise in controlling waterhyacinth populations. Once the pathogen becomes established in the ecosystem it should exert stress on the waterhyacinth to give control of the population without re-inoculation. Further tests are planned to quantify the effect of *C. rodmanii* on field populations of waterhyacinth in several lakes in Florida and Louisiana.

LITERATURE CITED

1. CHARUDATTAN, R. 1973. Pathogenicity of fungi and bacteria from India to hydrilla and waterhyacinth. Hyacinth Control J. 11:44-48.
2. CONWAY, K. E. 1975. Procedures used to test endemic plant pathogens for biological control of waterhyacinth. Proc. Amer. Phytopathol. Soc. 2: 31. (Abstr.).
3. CONWAY, K. E. 1976. Cercospora rodmanii a new pathogen of waterhyacinth with biological control potential. Can. J. Bot. 54: 1079-1083.
4. CONWAY, K. E., T. E. FREEMAN, and R. CHARUDATTAN. 1974. The fungal flora of waterhyacinth in Florida, Part I. Water Resources Research Center Publ. No. 30. University of Florida, Gainesville. 11 p.
5. DANIEL, J. T., G. E. TEMPLETON, R. J. SMITH, JR., and W. T. FOX. 1973. Biological control of northern jointvetch in rice with an endemic fungal disease. Weed Sci. 21:303-307.
6. DINOOR, A. 1974. Role of wild and cultivated plants in the epidemiology of plant diseases in Israel. Annu. Rev. Phytopathol. 12:413-436.
7. FREEMAN, T. E., R. CHARUDATTAN, and F. W. ZETTLER. 1973. Biological control of water weeds with plant pathogens. Water Resources Research Center Publ. No. 23. University of Florida, Gainesville. 52 p.
8. ZETTLER, F. W., and T. E. FREEMAN. 1972. Plant pathogens as biocontrols of aquatic weeds. Annu. Rev. Phytopathol. 10:455-470.