Biocontrol Efficacy of *Cercospora rodmanii* on Waterhyacinth

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**ABSTRACT**


Increased disease intensities and faster epidemic development rates of leaf spot caused by *Cercospora rodmanii* occurred on waterhyacinth after one application of inoculum compared to naturally occurring endemics. Leaf production on waterhyacinth was stimulated after inoculation with *C. rodmanii*; but, due to higher leaf mortality on inoculated plants, the net effect of disease stress was a lower number of live leaves relative to controls. The overall rate of leaf production for uninoculated and inoculated original ramets increased as nutrients increased, to a maximum at 50% Hoagland's solution and then decreased at higher nutrient concentrations (the nutrient effect was significant, $P<0.01$). On secondary ramets, however, the rate of leaf production increased as nutrient concentration increased (the nutrient effect was significant at $P<0.01$), which resulted in lower disease severity on the secondary ramets than on the original ramets and a diminution of the level of disease stress on the whole plant. On inoculated plants, lowest disease-progression rates occurred at the highest nutrient concentrations, indicating that the biocontrol efficacy of this pathogen is conditioned by the rate of leaf turnover and the compensatory host growth. For practical levels of control of waterhyacinth by *C. rodmanii*, the fungus should be used under conditions that favor low to moderate host growth rates or in combinations with other biotic and abiotic agents, such as insect biocides and sublethal rates of chemical herbicides, that retard host growth.

Additional key words: aquatic weed, *Eichhornia crassipes*, epidemiology, microbial herbicide.

*Cercospora rodmanii* Conway, a leaf spot-inducing pathogen, is a candidate for mycoherbicidal control of waterhyacinth (*Eichhornia crassipes* [Mart.] Solms) (4–6). In terms of vegetative growth, waterhyacinth is one of the most productive photosynthetic organisms (8,10), and the biocontrol efficacy of *C. rodmanii* is related to the growth rate of its host. Under conditions favorable for growth, waterhyacinth was found to produce one new leaf every 5–6 days and thus was capable of outgrowing the disease caused by *C. rodmanii* (5). Conway et al. (5) hypothesized that when conditions favored disease development and limited leaf production to less than one leaf per 3 wk, *C. rodmanii* could kill leaves faster than the plant could produce new ones. The plant would then become debilitated and die unless conditions changed to stimulate its regrowth or conditions became less favorable for disease development.

Growth of waterhyacinth is directly related to the level of available nutrients in the water in which the plant is growing (2,15).

For example, the growth rate increases with increasing nitrogen levels from 1 to 25 ppm (9). Accordingly, the present study was to determine: the relationship between the disease caused by *C. rodmanii* and host growth rate at different nutrient levels; the level of disease stress and rate of disease progress required to kill waterhyacinth.

**MATERIALS AND METHODS**

Host. Waterhyacinth plants were collected from Orange Creek, at Rodman Reservoir, Marion County, FL, and maintained in tap water supplemented (1% v/v) with a solution containing 2 M MgSO$_4$·7H$_2$O and 2 M Fe(NH$_4$)$_2$(SO$_4$)$_2$·6H$_2$O. Plants were sprayed with malathion (O,O-dimethyl phosphorodithioate of diethyl mercaptosuccinate), maintained for 3 wk in a greenhouse, and transferred a day before the experiment to 35 cm-deep plastic buckets having 0.07 m water surface area. There were no daughter ramets (= offsets or clones) at the start of the test.

Plants were grown in the buckets at 5, 25, 50, 75, and 100% Hoagland's solution number 1 (7) in deionized water, which was modified to include half of the recommended concentration of MgSO$_4$·7H$_2$O plus the iron-magnesium supplement mentioned above (1%, v/v). The volume of the liquid in the buckets was maintained at the initial level by weekly additions of the respective solutions. The test was conducted in Gainesville, FL, between August and October in 1981. The disease occurs endemically at the test location.

**Pathogen.** The isolate WI-99R of *C. rodmanii* was used. For inoculum, the fungus was grown for 3 wk in Roux bottles on potato-dextrose broth containing 5% yeast extract (both from Difco, Detroit, MI). Cultures and broth from several bottles were blended for about 10 sec in a Waring blender and used at the rate of 1.1 g wet weight of mycelium per square meter. Triton X-100 (a polyethylene ether; Sigma, St. Louis, MO) was used (0.05% v/v) as a wetting agent. Inoculum was applied to plants in buckets with a hand-operated, low-pressure, pump sprayer. Plants sprayed with 0.05% Triton X-100 without the fungus or broth were used as controls. Both inoculated and control plants were incubated in a dew chamber in darkness for 16 hr at 100% RH and 28 ± 1 C. The plants were maintained outdoors, and the buckets were arranged in a completely random design with three replicates. Each bucket (replicate) had three plants. Plants were trimmed to four leaves per plant on the day of inoculation. The root biomasses were comparable since the plants were collected from a homogenous population and maintained under uniform conditions.
Measurements, calculations, and analyses. Disease severity (DS) was quantified as the proportion of leaf area necrosed, and disease incidence (DI) as the proportion of leaves with necrotic spots. The DS, which was measured on all leaves, was assessed with a pictorial rating scale developed for this pathosystem (6), and the values were transformed to proportions.

The following disease and host growth variables were quantified weekly per plant for 6 wk: number of green (= live) original leaves (NGOL); number of dead original leaves (NOL); disease severity on original leaves (DSOL); disease severity on new leaves of original ramet (DNLS); number of new, live leaves produced on original ramet (NNL); number of new leaves that became diseased on original ramet (NNLR); disease severity on new leaves of original ramet (DNLS); number of new, secondary ramets (NNR); number of live leaves on new ramet (NLLR); number of diseased leaves on new ramet (NNLR); disease severity on leaves of new ramet (DNL); number of dead nonoriginal leaves (NDL). Disease incidence on new leaves of original ramet (DINL) and on leaves of new ramets (DILNR) were derived from NNL/NL and NNL/NL, respectively. Average DS and DI for whole plants were calculated as:

\[
\text{Mean DS} = \left(4^*\text{DSOL} + (\text{NNL}^*\text{DSNL}) + (\text{NNLR}^*\text{DNL})\right)/4
\]

\[
\text{Mean DI} = (\text{NOL} + \text{NNL} + \text{NNLR} + \text{NDL})/(4 + \text{NNL} + \text{NNLR} + \text{NDL})
\]

Cumulative host growth was calculated as:

- Green (live) leaves = NGOL + NNL + NLNR
- Total leaves = 4 + NNL + NLNR + NDL

in which 4 = the initial number of leaves. The area under the disease progress curve (AUDPC) was calculated according to Shaner and Finney (13).

Logistic (16) and Gompertz (1) transformation models were tested to determine which was more suitable for linearizing DS and DI progress data (1). Simple linear regression analysis was used to test for statistical fitness of the models based on correlation coefficients and standard residual sums of squares (11).

The effects of inoculation, nutrient, and the inoculation-nutrient interaction on plant growth and disease variables, disease progress rates, leaf production rates, and AUDPC were determined by analysis of variance. All statistical analyses were performed by using SAS (12) and the computing facilities of the Northeast Regional Data Center and the IFAS Computer Network at the University of Florida.

RESULTS

Effect of inoculation of C. rodmatii on disease progress and intensity. Fifteen to 33% of the leaf area of the original leaves was diseased by 2 wk after inoculation with C. rodmatii (Fig. 1A). The disease spread to 98 to 100% of the leaf area of original inoculated leaves by 6 wk. At 100% DS, these leaves were dead at the end of 6 wk (Fig. 1A). The original leaves on control plants developed disease (presumably through naturally disseminated conidia of C. rodmatii) gradually during 6 wk; DS was never more than 50% on these leaves (Fig. 1A). The DS on new leaves of original and secondary ramets was much higher for inoculated plants relative to controls (Figs. 1B and C). By week 6, maximum values for DNL and DNLN on inoculated plants were 55 and 28%, respectively. The DS on whole plants (Fig. 1D) was between 43 and 67% for inoculated plants and 4 and 12% for control plants by week 6. On inoculated plants, the highest DS was on plants kept in 50% Hoagland's solution, and the lowest DS was on plants in 100% Hoagland's solution (Fig. 1D). The DI on original leaves of inoculated plants was 100% by week 1 (Fig. 2A). On the control plants, DI reached 92-100% by week 4 (Fig. 2A). The DI on new leaves of original and secondary ramets, and for whole plants, (Figs. 2B to D) was also higher on inoculated plants than on controls. By week 6, the highest DI on inoculated whole plants occurred on those in 50% Hoagland's solution and the least DI on those in 100% Hoagland's solution (Fig. 2D). The number of dead leaves was 5 to 12 times higher on inoculated than on control plants by week 6 (Fig. 3).

The relationship between disease and time in this pathosystem was more effectively linearized by the Gompertz than by the logistic transformation model as determined by statistical fitness (11). Therefore, as proposed by Berger (1) and Plaut and Berger (11), gompit values were used for further analyses.

Inoculated plants had significantly ($P < 0.0001$) higher disease progress rates (k values) than the control plants (Table 1). A fungus-nutrient interaction was significant for rates of both DS ($P < 0.01$) and DI ($P < 0.05$). On control plants, the DS rate on whole plants increased as nutrients increased, but for inoculated plants the opposite occurred, and DS rate tended to decrease as nutrients increased.

Chronic disease stress. The AUDPC values, measures of chronic disease stress (14, page 130), were calculated for the DS and DI components (Table 2). The values were significantly higher for the inoculated relative to the control plants for all DS and DI variables ($P < 0.0001$). Therefore, the application of inoculum to plants caused a higher amount of disease stress than that which resulted from natural influx of the pathogen on controls. A nutrient effect was significant only for DSOL ($P < 0.05$), DIL, DIL, and DILN ($P < 0.01$).

Level of chronic stress and rate of disease progress needed for biocron. On inoculated plants, the AUDPC values for DS on original leaves (DSOL) ranged between 21.62 and 25.16 (Table 2) and nearly all of these leaves were dead by week 6 (Fig. 1A). In comparison, the AUDPC values for DS on new leaves of inoculated plants (Table 2) ranged between 3.20 and 6.83 for DSNL and 1.53 to 2.78 for DSNL. Thus, there was a drastic reduction in the amount of chronic stress from the original to the newer leaves (DSOL > DSNL > DSNL). This was because the disease curve began much higher on original inoculated leaves compared to new leaves, for which $y = 0$ at the start. AUDPC values for DI were patterned similarly, with much higher values on original leaves relative to new leaves (Table 2).

Based on the observation of original inoculated leaves that developed lethal levels of DS corresponding to 21.62 to 25.16 AUDPC units, it is predicted that a similar level of stress to the entire plant would result in total kill. The overall observed AUDPC values for DS on new growth, however, were far below these values (Table 2). Hence, it is clear that waterhyacinth could not be killed by C. rodmatii under these disease conditions.

Epidemic rates (k values) based on Gompertz transformation of DS on inoculated original leaves ranged between 0.159 and 0.174 (Table 1). At these rates, the disease caused leaf mortality in 6 wk (Fig. 1A). Hence, an overall k value $\geq 0.16$ on new growth may be needed to achieve plant death. However, in the time frame and under the conditions of this study, the k values for DS on new growth (DSNL and DSNL; Table 1) seldom reached these rates.

Effect of nutrient levels on leaf production. The rate of leaf production on original ramets (NNL per week) increased as nutrients increased, to a maximum in 50% Hoagland's solution, and decreased at higher nutrient concentrations (Fig. 4A). The rate of leaf production on secondary ramets (NNLR per week), however, increased as nutrient concentration increased (Fig. 4B). The mean rates of leaf production for whole plants (on the basis of both green leaves and total leaves) and those for leaves on original ramets were patterned similarly, with maximum production rates at 50% Hoagland's solution (Figs. 4C and D).

Influence of C. rodmatii on host growth. On the basis of total number of leaves produced (Table 3), the mean rate of growth was higher for inoculated plants than for controls ($P < 0.0001$). However, leaf mortality was greater for inoculated plants (Fig. 3). Therefore, on the basis of number of green (live) leaves present (Table 3), the rate of net growth was higher for control plants ($P < 0.0001$). Thus, the effect of disease stress on inoculated plants was a significant reduction in the net rate of leaf production.
Fig. 1. The severity of disease (DS), as a proportion of diseased tissue, on waterhyacinth plants either inoculated with *Cercospora rodmanii* or uninoculated (control) and grown at five nutrient levels. DS on: A, original, inoculated, and control leaves; B, new leaves that developed on the original ramet; C, leaves of new, secondary ramets; and D, whole plants. Nutrient levels: —— O —— 5%; —■—— O 25%; ▲/Δ—— ▲/Δ 50%; —— O —— 75%; and —— O —— 100%. Hoagland's solution. Disease progress rates were calculated from these data by Gompertz transformation (1).
Fig. 2. Disease incidence (DI), as a proportion of waterhyacinth leaves diseased with leaf spot caused by *Cercospora rodmani* on plants grown at five nutrient levels. DI on: A, original leaves; B, all leaves of the original ramet; C, new ramet; and D, whole plants. Nutrient levels: ☐ 0; ☐ 5%; ☐ 25%; ☐ 50%; ☐ 75%; and ☒ 100%. Hoagland's solution. Disease progress rates were calculated from these data by Gompertz transformation (1).
The rate of leaf production on original ramets (Table 3) was significantly higher for inoculated plants than for controls ($P < 0.05$). However, the rate of leaf production on new ramets of inoculated and control plants was not significantly different. Therefore, inoculation had no deleterious effect on production of new leaves on daughter plants. The reduction in net rate of leaf production on whole plants after inoculation was due to the death of the original four inoculated leaves. When the original four inoculated leaves were excluded from analyses, no difference in net leaf-production rate was observed between inoculated and control plants (unpublished).

Reduced net growth rate on inoculated plants (whole plant, green leaves; Table 3) was observed for all nutrient levels except 100% Hoagland’s solution, in which a higher net growth rate was observed for inoculated plants compared to control plants. This was due to the reduced growth rates of control plants that occurred at this nutrient level (Fig. 4C) and the decreasing disease severity at increasing nutrient levels for inoculated plants in later weeks, especially on leaves of new ramets (Fig. 1C). Apparently, at high nutrient levels, the rate of growth of waterhyacinth was faster than the epidemic rate.

**DISCUSSION**

*Cercospora rodmanii* has been shown to be capable of reducing waterhyacinth biomass (3,5,6). However, control of waterhyacinth in practice often requires the total or nearly total elimination of the weed, and this is generally accomplished with chemical herbicides. For *C. rodmanii* to be useful in waterhyacinth control, it must be shown to kill or significantly decrease plant populations under

<table>
<thead>
<tr>
<th>Nutrient level</th>
<th>Original leaves (DSOL)</th>
<th>New leaves on original ramets (DSNL)</th>
<th>New ramets (DSLRN)</th>
<th>Whole plant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control plants</td>
<td>5</td>
<td>0.037 (0.007)</td>
<td>0.022 (0.001)</td>
<td>0.015 (0.006)</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>0.048 (0.002)</td>
<td>0.026 (0.003)</td>
<td>0.017 (0.001)</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>0.065 (0.019)</td>
<td>0.020 (0.004)</td>
<td>0.015 (0.002)</td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>0.059 (0.005)</td>
<td>0.023 (0.003)</td>
<td>0.019 (0.003)</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>0.065 (0.023)</td>
<td>0.018 (0.005)</td>
<td>0.022 (0.004)</td>
</tr>
</tbody>
</table>

**Table 1. Rates of progress of disease (gompit k values) incited by *Cercospora rodmanii* on waterhyacinth**

<table>
<thead>
<tr>
<th>Nutrient level</th>
<th>Original leaves (DIOL)</th>
<th>New leaves on original ramets (DINL)</th>
<th>New ramets (DILRN)</th>
<th>Whole plant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control plants</td>
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<td>0.275 (0.048)</td>
<td>0.066 (0.001)</td>
<td>0.066 (0.006)</td>
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<tr>
<td></td>
<td>25</td>
<td>0.292 (0.017)</td>
<td>0.063 (0.007)</td>
<td>0.061 (0.011)</td>
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<tr>
<td></td>
<td>50</td>
<td>0.321 (0.014)</td>
<td>0.075 (0.008)</td>
<td>0.064 (0.002)</td>
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<tr>
<td></td>
<td>75</td>
<td>0.345 (0.005)</td>
<td>0.062 (0.005)</td>
<td>0.062 (0.003)</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>0.332 (0.021)</td>
<td>0.160 (0.066)</td>
<td>0.156 (0.003)</td>
</tr>
</tbody>
</table>

**Table 2. Areas under the disease progress curves (AUDPC) for the *Cercospora rodmanii*—waterhyacinth pathosystem**

**Fig. 3. Influence of inoculation with *Cercospora rodmanii* and nutrient levels on the number of dead leaves on waterhyacinth plants. Nutrient levels:**

- **Control:**
  - 0% Hoagland's solution
  - 25% Hoagland's solution
  - 50% Hoagland's solution
  - 75% Hoagland's solution
  - 100% Hoagland's solution

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1. The k (gompits per day) values were calculated according to Berger (1). Values in brackets are standard deviations. The data were analyzed by using the analysis of variance procedure. The k values were significantly higher for inoculated versus control plants ($P < 0.0001$). A fungus-nutrient interaction was significant for all DS ($P < 0.01$) and DI ($P < 0.05$) variables.

2. Five to 100% Hoagland’s solution number 1 (7), modified as described under Materials and Methods.

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3. Calculated according to Shaner and Finney (13) for data presented in Figs. A and B. Values in brackets are standard deviations. The data were analyzed by using the analysis of variance procedure. The AUDPC values were significantly higher ($P < 0.0001$) for inoculated plants versus the controls. A nutrient effect was significant for DSOL ($P < 0.05$), DIOL, DINL, and DILRN ($P < 0.01$).

4. Five to 100% Hoagland’s solution number 1 (7), modified as explained under Materials and Methods.
Fig. 4. Influence of nutrient levels and inoculation with *Cercospora rodmanii* on the number of leaves on waterhyacinth plants. Number of leaves A, on the original ramet; and B, on new ramets. C, Total number of green leaves; and D, all leaves (including dead ones) on the plant. Nutrient levels: • 0 0.0%; ● 0 5%; ■ 0 25%; ▲ 0 50%; ○ 0 75%; and ◆ 0 100% Hoagland's solution.
defined conditions. However, the efficacy of \textit{C. rodmani} is often complicated by varying growth rates of waterhyacinth. This study, therefore, describes the relationship of waterhyacinth growth rate to \textit{C. rodmani} efficacy as a predictive tool in this biocontrol system. Based on determinations of host growth rates, it should be possible to predict the disease intensity and the disease progress rates, and thus the potential effectiveness, of \textit{C. rodmani} in a given situation.

The DS and DI rates reported here are for the visible levels of disease. If not diluted by host growth, the actual epidemic development rates will be much faster. Nevertheless, although the disease intensity and disease progress rates obtained in this study where insufficient to kill waterhyacinth, significant reductions in host growth rates (green leaves) were obtained with just one application of \textit{C. rodmani} when host growth was limited by low nutrient levels (Table 3). However, when nutrient concentration was highest, waterhyacinth grew at a rate faster than the epidemic rate.

Growth causes a centrifugal spread of waterhyacinth. In the free-floating condition of the plant, new leaves emerge in the center of the ramet and older leaves are pushed out and into the water. When the plant is in crowded stands, older leaves are pushed out and towards lower foliar strata. In this study, plants were free floating. Apparently, in the process of growth, the host compensated for diseased and dying leaves with a rapid turnover of leaves. As a result, there was a significantly higher number of dead leaves on inoculated plants (Fig. 3). Reduction of the subsequent inoculum by this rapid turnover of infected leaves may have a sanitizing effect; it appears to have been directly related to the observed diminution of DS from the original to the newer leaves.

This study was conducted in an area where disease caused by \textit{C. rodmani} is endemic. Disease caused by endemic inoculum was indeed observed on control plants. To minimize the effect of natural influx of the pathogen on the results, the study was limited to 6 wk. During the study, the pathogen killed only the leaves present at the time of inoculation and the inoculum on these leaves appeared to be unavailable for subsequent infection cycles due to rapid turnover of leaves. Therefore, long-term biocontrol with single applications of \textit{C. rodmani} is unlikely when the host growth is rapid.

To counter constraints on the biocontrol efficacy of \textit{C. rodmani} in the field, it would be necessary to: use multiple applications of inoculum when waterhyacinth is in the seasonal early growth phase (i.e., late spring in the southeastern United States) and to combine the pathogen with other biotic or abiotic agents capable of retarding the growth rate of waterhyacinth. Combinations of the pathogen and insect biocontrol agents or the pathogen and sublethal rates of chemical herbicides are potentially useful approaches that are under evaluation.

LITERATURE CITED