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Survey and Evaluation of Mexican Native Fungi for Potential Biocontrol of Waterhyacinth

M. MARTINEZ JIMENEZ[1] AND R. CHARUDATTAN[2]

INTRODUCTION

In a study conducted in 1993, Gutiérrez et al. established that aquatic weeds infest approximately 62,000 ha of water resources in Mexico (Gutiérrez et al. 1994). The most prevalent species is waterhyacinth (*Eichhornia crassipes* (Mart.) Solms), which is present in 40,000 ha of the infested waters, followed by pondweed (*Potamogeton* sp.), cattail (*Typha* spp.), hydrilla (*Hydrilla verticillata* (L.) Royle), waterlettuce *(Pistia stratiotes* L.), and duckweed (*Lemna* spp.).

Chemical and mechanical controls have been used in Mexico since 1958 to manage waterhyacinth (Gutiérrez et al. 1994). However, these methods provide only temporary relief, and for sustainable, long-term solution it appears necessary to employ an integrated approach in which biological control agents play a key role (Gutiérrez López et al. 1996). Presently, five waterhyacinth insects, *Neochetina eichhorniae* Warner, *N. bruchi* Hustache (Coleoptera: Curculionidae), *Sameodes albiguttalis* Warren (Lepidoptera: Pyralidae), *Cornops aquaticum* Bruner (Orthoptera: Acrididae), and *Orthogalumna terebrantis* Wallwork (Acarina: Galumnidae) are well established in Mexico, but additional agents are needed to supplement the current levels of biological control (Gutiérrez López et al. 1996). Use of plant pathogens as bioherbicides, which has been recommended for integrated management of waterhyacinth (Charudattan 1986), is being considered in Mexico, but little is known about the occurrence and impacts of native pathogens in this region of North America.

Several fungal pathogens have been reported to attack waterhyacinth in various parts of the world. Among them are: *Acremonium* (= *Cephalosporium*) *zonatum* (Saw.) Gams, *Alternaria eichhorniae* Nag Raj & Ponnappa, *Cercospora piaropi* Tharp, *C. rodmanii* Conway, *Myrothecium roridum* Tode ex Fr., *Rhizoctonia solani* Kühn, and *Uredo eichhorniae* Gonz.-Frag. & Cif. *Cercospora rodmanii* and *C. piaropi* have been shown to be capable of decreasing waterhyacinth biomass, and in some instances have caused substantial decline of waterhyacinth populations (Charudattan et al. 1985, Freeman and Charudattan 1984, Martyn 1985, Morris 1990). In addition to their capacity to inflict debilitating foliar diseases, both species of *Cercospora* produce the diffusible phytotoxic pigment cercosporin in culture media, and this phytotoxin can be used

[1]Instituto Mexicano de Tecnología del Agua, Subcoordinación de Impacto Ambiental, Paseo Cuaunháhuac 8532, Jiutepec, Morelos, Mexico. C.P. 62550. Apdo. Postal No. 22 CIVAC, Morelos. E-mail: mmartine@chac.imta.mx.

[2]Center for Aquatic Plants, Plant Pathology Department, 1453 Fifield Hall, Box 110680, University of Florida, Gainesville, Florida 32611-0680. E-mail: rc@gnv.ifas.ufl.edu. Received for publication on March 14, 1998 and in revised form July 7, 1998.

as a chemical adjuvant to promote rapid disease development (Charudattan, unpublished).

In 1993, the Mexican Institute for Water Technology (IMTA) began an aquatic weed control program with the aim to develop and use methods of waterhyacinth management that are most suitable to the ecosystem and water uses (Gutiérrez López et al. 1996). Under this program, a study was initiated to survey and identify indigenous plant pathogens of waterhyacinth in Mexico that could be developed as bioherbicides. The objective of this paper is to report the occurrence and pathogenicity of significant fungal pathogens of waterhyacinth that we have found so far.

MATERIAL AND METHODS

Waterhyacinth plants or leaves with disease symptoms were collected during the rainy season in 1994 from 52 weed-infested sites in the southern to the central parts of Mexico. The plant and leaf specimens were collected, stored in polyethylene bags in an ice chest and transported to the laboratory where isolation of pathogens was attempted, usually on the same day of collection. Leaf pieces (2 mm^2) were cut from the margins of necrotic or chlorotic lesions on the leaves, surface disinfected in 0.26% sodium hypochlorite solution, and rinsed thrice with sterile water to remove traces of the disinfectant. Five or six leaf pieces were placed on potato dextrose agar (PDA; Difco Laboratories, Detroit, MI) in petri plates and incubated at 25 C. All emergent fungi were isolated and pure cultures were obtained by the single-spore or hyphal-tip technique depending on the type of fungus. To observe spore development, microcultures of the fungi were prepared as follows: Fresh cultures of the fungi were grown on PDA and four pieces of the culture (4 mm^2) were cut from the leading edges of the fungal growth and placed on a microscope slide supported on a glass triangle (made of a 5-mm-diameter glass rod) placed in the lower dish of a petri plate. The PDA squares were covered with a cover glass, a shallow layer of water was added to the dish to provide high humidity, covered with the lid, and maintained under sterile condition. Replicate plates were prepared for each of the fungal isolates and the plates were incubated at 25 C and ambient light in the laboratory until the cover glass was filled with mycelia. The slides were then observed with a microscope for spores and spore producing structures. Fungi were identified according to descriptions provided by Tharp (1917), Chupp (1953), Barnett and Hunter (1972), and Ainsworth et al. (1973).

Fungi that had distinctive characteristics of saprophytes (i.e., very rapid growth rate on PDA plates and isolates of *Penicillium, Aspergillus,* and *Trichoderma,* which were easily identified) were excluded from further consideration after their initial isolation. Other isolates were grouped according to their generic identification and one representative isolate of each genus was tested to establish its pathogenicity to waterhyacinth by fulfilling Koch's postulates.

For pathogenicity trials, healthy waterhyacinth plants collected in the vicinity of IMTA were washed with tap water, sprayed with malathion (O,O-dimethyl phosphorodithioate of diethyl mercaptosuccinate) to eliminate insect infestations, maintained for 4 weeks in 50% Hoagland's solution (Hoagland and Arnon, 1950), and transferred a day before the experiment to plastic pots (20 cm diameter by 30 cm depth) filled with 50% Hoagland's solution. The isolates were tested by placing PDA pieces containing fungal growth (mycelia and/or spores) on 30 leaves per isolate that had been rubbed with carborundum powder to create small surface abrasions. The inoculum piece was placed with the fungal side in contact with the wound. Thirty control leaves per isolate were similarly abraded and a drop of water was placed on the abrasion. After inoculation, the plants were placed in a plastic chamber (2 × 2 × 1 m: w × l × ht) at 24-27 C and misted with an Ultrasonic Humidifier (Orwave Corp.,Taipei, Taiwan) to maintain relative humidity near saturation. The Hoagland's solution was replaced once a week.

Five days after inoculation, isolates were rated for their ability to cause disease by the following criteria: presence or absence of disease symptoms such as leaf spot, chlorosis, and necrotic blight. The isolates were ranked on the basis of the severity of disease damage they inflicted. Disease severity was assessed as follows: O = leaves healthy, with no damage; the isolate is not pathogenic and not virulent: 1 = a few spots or slight necrosis found; isolate mildly virulent; 2 = leaves moderately susceptible; several leaf spots or noticeable necrosis present but the leaf is not dead; isolate moderately virulent; and 3 = heavy leaf-spotting or necrosis present; leaves highly susceptible and died from the inoculation. Isolates rated at 3 are considered highly virulent and aggressive and therefore capable biocontrol agents. The plants were maintained for 4 weeks, to allow for the full expression of symptoms. The pathogenicity trials were repeated at least once with each isolate.

RESULTS AND DISCUSSION

This is the first known extensive survey for pathogens attacking waterhyacinth in Mexico, and none of the fungi discussed in this paper appears to have been previously reported from this host in Mexico. To date we have surveyed 52 sites in 10 of the 14 Mexican states where waterhyacinth has been reported as a serious problem, and collected 50 fungal cultures. These fungi were identified to belong to 17 known genera (Table 1) on the basis of morphology and development of conidia. The conidial characteristics enabled us also to identify some of the fungi to the species level.

Acremonium zonatum, Alternaria sp., *Cercospora piaropi, Fusarium* sp., and *Verticillium* sp. were highly virulent and severely damaged inoculated waterhyacinth leaves. On the basis of the highly susceptible host reaction (rating of 3), these five fungi are considered to be potential bioherbicide agents of waterhyacinth (Table 1). The remaining 12 fungi are not considered to have any potential as bioherbicides.

Among the highly virulent fungi were two known pathogens of waterhyacinth: *Acremonium zonatum* and *Cercospora piaropi.* In our survey, these two were the most frequently seen pathogens in the field. *Acremonium zonatum* causes a zonate leaf-spot disease characterized by concentric dark-brown rings. In pathogenicity trials, spots first appeared 8-10 days after inoculation. Spores were produced on infected area on the underside of the leaves, 2 weeks after inoculation. The *C. piaropi* caused small ovate leaf spots (2 × 4 mm), 15-20 days after inoculation. The spots were dark brown with whitish centers

TABLE 1. FUNGI ISOLATED FROM WATERHYACINTH IN MEXICO.

Fungal isolate	Location and Isolate designations	Host reaction/Disease severity[1]
Acladium sp.	Veracruz (Mx-WH-1.6); Jalisco (Mx-WH-34.2, Mx-WH-35.2)	1
Acremonium zonatum (Saw.) Gams	Tabasco (Mx-WH-26); Veracruz (Mx-WH-12)	3
Alternaria sp.	Puebla (Mx-WH-43.1); Sinaloa (Mx-WH-51); Hidalgo (Mx-WH-46); Michoacán (Mx-WH-40, Mx-WH-41)	3
Basipetospora sp.	Edo. de México (Mx-WH-38.3)	1
Bipolaris sp.	Sinaloa (Mx-WH-52.2)	1
Blastomyces sp.	Puebla (Mx-WH-42.2)	1
Cercospora piaropi Tharp	Jalisco (Mx-WH-30, Mx-WH-31, Mx-WH-37); Michoacán (Mx-WH-40.1); Morelos (Mx-WH-44, Mx-WH-45); Veracruz (Mx-WH-15.1)	3
Curvularia sp.	Tabasco (Mx-WH-17, Mx-WH-20, Mx-WH-22); Veracruz (Mx-WH-1.2); Sinaloa (Mx-WH-52.3)	1
Cylindrocladium sp.	Tabasco (Mx-WH-28.1, Mx-WH-17.3); Veracruz (Mx-WH-6.1, Mx-WH-14.1)	1
Epicoccum sp.	Guanajuato (Mx-WH-50.1)	1
Fusarium sp.	Tabasco (Mx-WH-18, Mx-WH-26); Veracruz (Mx-WH-4, Mx-WH-5, Mx-WH-12); Jalisco (Mx-WH-31.1)	3
Monilia sp.	Edo. de México (Mx-WH-38.2); Hidalgo (Mx-WH-47); Puebla (Mx-WH.42.1)	1
Nigrospora sp.	Sinaloa (Mx-WH-52.4); Tabasco (Mx-WH-20.4, Mx-WH-28); Veracruz (Mx-WH-12.2)	1
Periconia sp.	Veracruz (Mx-WH-14, Mx-WH-11.1)	1
Pestalotia sp.	Tabasco (Mx-WH-17.4); Veracruz (Mx-WH-4, Mx-WH-6, Mx-WH-3)	1
Verticillium sp.	Sinaloa (Mx-WH-52.5)	3
Stemphylium sp.	Veracruz (Mx-WH-1.5); Hidalgo (Mx-WH-48.1)	1

[1]Based on a disease assessment scale of 0 = leaves healthy, with no damage; the isolate is not pathogenic and not virulent; 1 = a few spots or slight necrosis found; isolate mildly virulent; 2 = leaves moderately susceptible; several few spots or noticeable necrosis present but the leaf is not dead; isolate moderately virulent; and 3 = heavy leaf-spotting or necrosis present; isolate highly virulent and damaging.

Because *A. zonatum* and *C. piaropi* are among the most widespread and commonly found pathogens of waterhyacinth worldwide (Charudattan 1990), and are capable of inflicting severe damage on waterhyacinth plants, we studied these fungi in greater depth. Only one species of *Acremonium is* known to attack waterhyacinth, whereas two species of *Cercospora, C. piaropi* and *C. rodmanii,* have been described as pathogens of this host. *Cercospora rodmanii* has been extensively researched as a bioherbicide in the United States (Charudattan et al. 1985, Charudattan 1986, 1990, Freeman and Charudattan 1984) and attempts to develop *A. zonatum* as a bioherbicide were halted even though preliminary studies appeared promising (Galbraith 1987, Martyn and Freeman 1978). In our study, both *A. zonatum* and *C. piaropi* caused substantial damage on waterhyacinth plants under greenhouse conditions. The symptoms we observed with both species were identical to those previously reported (Freeman and Charudattan 1984, Rintz 1973, Galbraith 1987).

Based on our observations of the severity of field infections and in greenhouse inoculations, *A. zonatum* and *C. piaropi* appear to possess the level of virulence and aggressiveness necessary to be effective bioherbicide agents. Hence, we consider these fungi to be suitable biological control agents for development and use in Mexico. Therefore, we are continuing to evaluate them as biocontrol agents, alone and in combination with *Neochetina* spp.

The other pathogenic fungi found in this study, namely, *Alternaria* sp., *Fusarium* sp., and *Verticillium* sp., are being studied to identify them to the species level, to compare them with previously reported species (Charudattan 1990, Nag Raj and Ponnappa 1970, Shabana et al. 1995), and to estimate their efficacy as biological control agents.

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