Biological Control of Water Hyacinth by Using Pathogens: Opportunities, Challenges, and Recent Developments

R. Charudattan*

Abstract

There is good justification to renew concerted efforts to develop pathogens for biological control of water hyacinth (*Eichhornia crassipes*, (Mart.) Solms-Laub.). Among the most promising pathogens are *Uredo eichhorniae*, suitable as a classical biocontrol agent, and *Acremonium zonatum, Alternaria eichhorniae, Cercospora piaropi*, *Myrothecium roridum*, and *Rhizoctonia solani,* which are widely distributed in different continents, as bioherbicides. Other, less widely distributed pathogens, notably species of *Bipolaris, Drechslera,* and *Fusarium,* may hold promise, but further studies are needed to confirm their usefulness. Ongoing studies on the biology of *U. eichhorniae* are expected to help resolve the biocontrol potential of this rust fungus in the near future. It is also anticipated that recent advances in bioherbicide technology, and previous experience with pathogens of water hyacinth, will enable development of effective bioherbicides. Success in this effort will require the use of highly virulent pathogens and pathogen strains as well as novel formulations that help to counter water hyacinth's ability for rapid growth under different site conditions and assist the pathogen to overcome environmental limitations. In Florida, USA, our aim is to develop a bioherbicide that could be used in integration with the existing suite of introduced arthropod biocontrol agents and improve the overall effectiveness of the biological control strategy under different weed-control scenarios. In this attempt, unlike in previous unsuccessful attempts, we are aided by the availability of a large and diverse collection of highly virulent pathogen strains to choose from, the facility to formulate effective pathogens in newer materials to assure consistency of performance, the ability to integrate dual and multiple pathogens, and a 'biofriendly' posture of regulatory agencies toward the development and registration of bioherbicides. Results from our ongoing field trials suggest the feasibility and commercial potential of our current approach.

THERE are several good reasons to consider pathogens as biocontrol agents: pathogens can cause significant reductions in water hyacinth biomass, especially following natural disease outbreaks, after severe insect attacks, or when used as inundative bioherbicide agents (Charudattan et al. 1985; Shabana et al. 1995b). Published accounts of natural declines in water hyacinth populations following natural disease outbreaks further confirm the potential of pathogens in limiting water hyacinth populations (Martyn 1985; Morris 1990). Controlled experimental studies have confirmed the potential of *Acremonium zonatum, Alternaria eichhorniae,* and *Cercospora piaropi* to control (i.e. reduce weed biomass) water hyacinth (Martyn and Freeman 1978; Charudattan et al. 1985; Shabana et al. 1995b). In addition, it has been well proven that pathogens can be successful as classical or inundative (bioherbicide) agents. Currently, worldwide about 15–20 weeds are biologically controlled with pathogens (Rosskopf et al. 1999). Therefore, there is clear justi-

* Center for Aquatic and Invasive Plants, Plant Pathology Department, University of Florida, Gainesville, FL 32611-0680, USA. Email: rc@gnv.ifas.ufl.edu

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fication to explore pathogens fully and fairly as agents of biological control of water hyacinth. In this effort, we will be starting with the benefit of past attempts that have helped to lay the foundation through surveys and characterisation of host–pathogen systems. It is now imperative to take steps to turn this early empirical knowledge and success into practical utilisation of pathogens in weed-management programs. Clearly, with some pathogens, such as the rust fungus *Uredo eichhorniae,* further studies are needed to understand the biology and biocontrol potential of the agents before they can be used. Nonetheless, the current state of knowledge and experience give us the optimism to assert that pathogens can succeed in practice, especially when used in integrated weed control systems rather than as stand-alone control options.

Opportunities and Challenges

Development of a pathogen or pathogens for use in operational weed management systems is long overdue. In this regard, the present timing and opportunities may be the best we can ever expect. For instance, there is renewed interest in developing additional biological control agents to supplement those already in use. There is an urgent need for effective controls for water hyacinth in countries of Africa and Asia. Support, in the form of funding initiatives, is also evident, as in the case of the recently formed Pan-African mycoherbicide program. Despite these optimistic signs, the most important lesson learned from earlier attempts should not be forgotten: that foliar pathogens generally do not have the capacity to kill water hyacinth plants completely and quickly unless they can be used in conjunction with efficacy-enhancing formulations and adjuvants, low doses of chemical synergists, and/or insect biocontrol agents. Bioherbicides were regarded as biological substitutes for chemical herbicides and therefore as stand-alone products. There was little technological sophistication in the bioherbicide products; usually fresh fungal inoculum was used without formulations devised to protect the inoculum from adverse environmental conditions or to improve its performance. Improvements in efficacy of foliar pathogens are now possible through a number of recently developed formulations such as hydrophilic polymers, emulsions, surfactants etc. (Shabana et al. 1997; Green et al. 1998; Boyetchko et al. 1999). Several pathogens can be combined and used in a ‘multiple-pathogen strategy’ (Chandramohan et al. 2000) to improve the level of weed control, minimise or prevent development of host resistance, overcome age-related host resistance, assure consistency in bioherbicide performance, improve environmental latitude of activity, and so on. It is also possible to ‘customise’ the pathogen mixture depending on the types of pathogens available for use in a given country or region.

Among the challenges, we must address the pervasive perception among aquatic weed managers that biological control does not provide *quick and acceptable* levels of control and that biological control is inadequate under conditions that promote rapid expansion of water hyacinth mats. Given the general consensus of this working group that the overall effectiveness of biological control should be (could be) improved, we should aim to develop a pathogen or pathogens that can provide *quick and assured* levels of biological control. Accordingly, we should explore all potentially useful pathogens. In this respect, both classical and bioherbicide strategies should be pursued.

As we renew our efforts to develop pathogens, we should remember the following.

- Localised pathogens, fungi and bacteria that have not been previously explored, may be useful. Therefore, further surveys, especially in the African continent and the Neotropics, and thorough evaluations of the pathogens found, should be a part of our plans.
- Technological innovations should be developed to overcome micro-environmental conditions surrounding the plant that are non-conducive for disease development.
- Water hyacinth plants growing under conditions that promote rapid growth will result in the plant outgrowing the rate of progress of foliar diseases. Consequently, soon after a bioherbicide application, the disease pressure will wane, turning even a disease with polycyclic potential into a monocyclic disease. Therefore, the ability of water hyacinth plants to grow at rapid rates under different site conditions is a challenge that must be factored into the design of bioherbicides.

To address the dual challenges of rapid host growth rate and environmental constraints and to assure biocontrol effectiveness, the pathogen (specifically a bioherbicide) may be applied with low rates of a registered chemical herbicide. Alternatively, the bioherbicide formulation may contain an adjuvant (e.g. a phytotoxic compound or a registered chemical herbicide at low rates). Multiple applications of the bioherbicide may be also used, but the economics of multiple applications would have to be assessed. Also, bioherbicide applications may be timed to maximise the impacts of insect agents and thus increase the level of biotic stress. Novel formulations should be developed that help to prolong humidity on the leaf surface, protect the pathogen against solar irradiation, and/or promote leaf penetration by the pathogen. Combinations of two or three different pathogens may be used to increase the level of damage and consistency of performance, as has been tried under experimental conditions, most recently by Den Breeÿen (1999) and Vincent and Charudattan (2000). An approach that is quite applicable but has not been tried is the use of several strains of a pathogen (e.g. *C. piaropi*), each having different levels of virulence, fitness, phytotoxin production, and other desirable traits.

It is fair to say that, despite several field surveys in the past two decades, no *new and highly* promising pathogens have been added to the list of known, prospective biocontrol agents. However, this statement is not meant to imply that there may be no additional pathogen candidates left to discover, but rather it is an assessment of the current situation. For example, of the nearly 70 fungi and bacteria recorded on water hyacinth (Barreto and Evans 1996; Charudattan 1996), only about 15 have been adequately tested and confirmed to be highly virulent pathogens. Of these, three fungal pathogens, *Acremonium zonatum, Alternaria eichhorniae,* and *Cercospora piaropi (= C. rodmanii)*, have been studied intensively as biocontrol agents and shown to be effective in controlling water hyacinth under experimental conditions (Martyn and Freeman 1978; Charudattan et al. 1985; Shabana et al. 1995b). This leaves a large number of other reported fungi and bacteria that remain to be assessed for their biocontrol potential. Thus, for now, the choice of pathogens for biological control is limited to *Uredo eichhorniae,* as a classical biocontrol agent, and as bioherbicide agents, *A. zonatum, A. eichhorniae, C. piaropi (= C. rodmanii), Myrothecium roridum,* and *Rhizoctonia solani.* Several other, less widely distributed pathogens, such as species of *Bipolaris, Drechslera,* and *Fusarium,* may hold promise, but they need to be studied further to confirm their potential.

A Synopsis of Highly Virulent and Useful Pathogens

The following are brief descriptions of virulent pathogens that are leading candidates for further development.

Acremonium zonatum

This fungus causes an easily identified necrotic zonate leaf spot characterised by spreading lesions, most noticeable on the upper laminar surface (Fig. 1). On the lower surface, which is normally protected from direct sunlight, the area directly under the spot may have a sparse, spreading layer of white fungal (mycelial) growth. Each spot may be small (2 mm diameter) to large (> 3 cm diameter) and the spots may coalesce, covering most of the lamina. The zonate pattern may not be evident in new infections when most spots are small. This disease has been reported from Australia, USA, and many countries of Asia, Central America, and South America. It is often associated in the field with infestations of the water hyacinth mite *Orthogalumna terebrantis*. This pathogen is represented by several highly virulent strains such as the ones found in Mexico by Martinez Jimenez and Charudattan (1998).

Alternaria eichhorniae

Two species of *Alternaria, A. eichhorniae* and *A. alternata,* have been recorded on water hyacinth. One or both of these species have been reported from Australia, Bangladesh, Egypt, India, Indonesia, and South Africa. *Alternaria alternata* appears to be a weak, opportunistic parasite, whereas *A. eichhorniae* is a highly virulent, host-specific pathogen of water hyacinth. *Alternaria eichhorniae* has been shown to have good potential as a bioherbicide agent (Shabana et al. 1995a,b; these proceedings). It causes discrete necrotic foliar spots (oblong, 2–4 mm long) surrounded by a bright yellow halo. Blighting of the entire leaf lamina can be induced by using mycelial inoculum and providing prolonged, 100% relative humidity (Fig. 2). In culture, *A. eichhorniae* produces several bright red compounds in culture, including bostrycin and deoxybostrycin that are phytotoxic to water hyacinth leaves (Charudattan and Rao 1982). The extent of naturally occurring variability in virulence in this pathogen is not clear. More details about this pathogen are given by Shabana (2001).

Cercospora piaropi (= *C. rodmanii*)

Symptoms caused by *Cercospora* spp. may be easily confused with those of many other foliar pathogens, including many opportunistic, weak parasites. Until now, two species of *Cercospora, C. piaropi* and *C. rodmanii*, have been recognised as pathogens of water hyacinth, but recently Tessmann et al. (2001) have merged the two species into an emended *C. piaropi. Cercospora piaropi* has been reported on water hyacinth from throughout the present range of the weed. This pathogen causes small (2–4 mm diameter)

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necrotic spots on laminae and petioles (Fig. 3). The spots are characterised by pale centres surrounded by darker necrotic regions. Occasionally, the spots may appear in the shape of 'teardrops' that coalesce as the leaf matures, causing the entire leaf to turn necrotic and senescent. In fact, the senescence is accelerated by the *Cercospora* disease, and the disease can rapidly spread across water hyacinth infestations, causing large areas of the weed mat to turn brown and necrotic. Under severe infections, the plant may be physiologically stressed, lose its ability to regenerate, become waterlogged, and sink or disintegrate.

Tessmann et al. (2000) compared 60 isolates of *Cercospora* species isolated from water hyacinth leaves showing symptoms of *Cercospora* infection which were collected from the USA, Mexico, Venezuela, Brazil, South Africa, and Zambia. They found the isolates to be variable in pigmentation in culture, spore morphology, and virulence. Virulence of the isolates was also variable: isolates ranged from being nearly avirulent to highly virulent and capable of causing leaf death. These traits were independent of the geographic origin of isolates. The isolates were then tested to see if the species concept based on conidial and cultural morphology and virulence, as used by Conway (1976) in his designation of *C. rodmanii* into a separate species, might agree with a species concept developed with the help of molecular markers.

Accordingly, members of a collection of isolates representing acquisitions from the USA (Florida and Texas), Mexico, Venezuela, Brazil, South Africa, and Zambia were compared on the basis of the DNA sequences of gene segments for beta-tubulin (*TUB2*), histone-3 (*H3*), and elongation factor-1-alpha (*EF1a*), corresponding to 380, 309, and 431 base pairs (bp), respectively. Eight of the isolates were also compared for the rDNA regions containing ITS1, ITS2, and the 5.8S gene. Extracted DNA was amplified by polymerase chain reaction using TUB2, H3, and ITS primer pairs selected from the literature. The combined phylogenetic relationships of *TUB2*, *H3*, and *EF1a* sequences done with phylogenetic analysis using parsimony did not support the species distinction between *C. piaropi* and *C. rodmanii*. Isolates representative of both species grouped into a single, well-supported clade (Tessmann et al. 2000).

Thus, the molecular evidence pointed strongly to a common phylogeny of *Cercospora* pathogenic to water hyacinth. This raises some important questions relevant to the use of *C. piaropi* for water hyacinth control. For instance, given the worldwide distribution of this pathogen and the molecular evidence pointing to a common origin of *Cercospora* isolates pathogenic to water hyacinth, should this pathogen be subject to plant quarantine restrictions? Would it not be advantageous to import and supplement native *C. piaropi* strains with more highly virulent strains from whichever continent or country they could be found? Perhaps a search for highly virulent strains (of any pathogen) should be an inherent priority for all mycoherbicide programs in order to ensure that only the best strains are used. These questions deserve to be considered.

Myrothecium roridum

This fungus causes a teardrop-shaped leaf spot (up to 1 × 5 cm), rounded on the side facing the petiole and tapering to a narrow point in the direction of the laminar tip (Fig. 4). Older leaf spots turn necrotic with dark brown margins, with the centre of the spot covered with discrete white and black conidial masses. Myrothecium disease of water hyacinth has been reported to occur in India, Malaysia, Indonesia, possibly Mexico, and some western African countries. Although this species is worldwide in distribution, the typical myrothecium disease has not been recorded on water hyacinth in the Americas. The occurrence of variability in virulence in this pathogen is therefore not clear. Some recent studies suggest that some *Myrothecium* species can be used as broad-spectrum bioherbicides against several weeds (Walker and Tilley 1997), a finding that has implications for the development of *M. roridum* for water hyacinth control.

Rhizoctonia solani

Disease symptoms caused by this fungus may resemble damage caused by a desiccant type of chemical herbicide (e.g. diquat). Symptoms consist of irregular, necrotic spots, and broad lesions (Fig. 5). Unlike chemical damage, the brown necrotic areas are usually surrounded by noticeable, thin, water-soaked margins of darker brown colour than the rest of the lesion. Rhizoctonia disease has been reported on water hyacinth from the southeastern United States, Brazil, Mexico, Panama, Puerto Rico, India, Malaysia, and Indonesia. This fungus is usually very aggressive and destructive, capable of rapidly killing water hyacinth plants. The extent of variability in virulence of *R. solani* pathogenic to water hyacinth is not clear, but isolates collected in the USA, Panama, and Brazil have been found to be extremely virulent (R. Charudattan, unpublished data; R.A. Pitelli, University of the State of Sao Paulo, Jaboticabal, Brazil, pers. comm.).

Figure 1. Zonate leaf spots caused by *Acremonium zonatum* (left) and naturally infected plants in the field (right)

Figure 2. Discrete necrotic leaf spots surrounded by yellow halo (left), symptoms developed when inoculated with spores (middle) or mycelium and kept under high humidity (right)

Figure 3. Leaf spot symptoms caused *Cercospora piaropi*

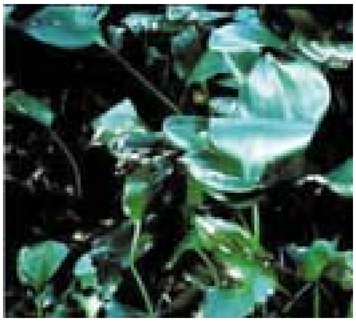

Figure 4. Teardrop-shaped foliar lesions and the extent of damage caused by *Myrothecium roridum*

Figure 5. Blighting symptoms caused by *Rhizoctonia solani* on leaves (left) and whole plants (right). Picture on the right shows fungus-infected and uninfected control plants

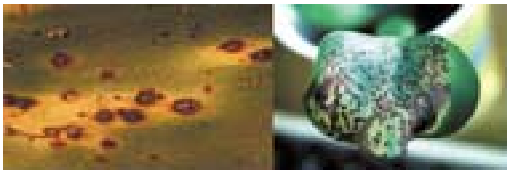

Figure 6. Water hyacinth leaves showing uredial pustules of *Uredo eichhorniae*

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Despite its high virulence and destructive capabilities, *R. solani* has never been seriously considered as a bioherbicide agent because of its reputed wide host range. However, this should not be a deterrent in today's regulatory environment which has permitted registration and commercial use of pathogens that do not possess high levels of host specificity (e.g. *Colletotrichum gloeosporioides* f.sp. *aeschynomene, Phytophthora palmivora*, and *Chondrostereum purpureum*).

Uredo eichhorniae

This rust fungus occurs in southern Brazil, Argentina, and Uruguay. It is known only in its uredial spore stage (Fig. 6). Because it is a rust fungus, it is likely to be highly host-specific and therefore a desirable classical biological control agent, but several aspects of its biology remain to be fully understood. For instance, since this fungus was first described from the Dominican Republic, a tropical island, it is unclear why it does not occur beyond its present range of distribution in the subtropical to temperate regions of South America. It is possible that it is adapted to slower growing plants of the temperate fringes of water hyacinth's distribution and its original finding in the Dominican Republic is an anomaly. Nevertheless, the occurrence of this pathogen only inside the native range of water hyacinth, but not outside (e.g. Asia, Africa, or Australia), is to be expected on the basis of ecological theory of coevolution of rust fungi.

Our earlier attempt to import *U. eichhorniae* into Florida was disallowed by U.S. regulatory agencies on the grounds that the full life cycle of this fungus was unknown. Stimulated by the current interest in deploying additional biocontrol agents, we have recently restarted research on the life cycle and biology of this fungus in collaboration with scientists from the Plant Protection Research Institute, Stellenbosch, South Africa and the University of the State of Sao Paulo at Jaboticabal, Brazil. The objective is to import the rust into quarantine at two locations outside its native range, one in the southern hemisphere (Stellenbosch, South Africa), the other in the northern hemisphere (Gainesville, Florida), to initiate epidemio-logical studies under controlled conditions. In this regard, South Africa, with its climatic, latitudinal, and hemispheric similarity to temperate South America, offers an eminently suitable location to conduct these studies.

As a first step in the evaluation of its biocontrol potential, we have initiated studies on the life cycle (i.e. spore stages, particularly the aeciospores and teliospores) and disease cycle of *U. eichhorniae* under field conditions. The biocontrol potential of this fungus will be determined by using fungicide(s) to block the effects of the rust disease in natural field populations of water hyacinth and comparing the growth rates of such fungicide-protected plants with rust-infected plants. These studies should help to establish the suitability of *U. eichhorniae* as a classical biocontrol agent and set the stage for its eventual release into the USA, South Africa, and elsewhere. The addition of this rust pathogen to the existing suite of biocontrol agents is likely to improve the prospects for a sustainable, long-term biological control of water hyacinth.

Recent Developments in Progress

With the above-mentioned concepts in mind, our current efforts in Florida are aimed at the development of a bioherbicide that can be used in combination with existing insect biocontrol agents. Our goal is a bioherbicide that will help to improve the overall effectiveness of the biological control system under different control scenarios. Our emphasis is on a knock back (reduce biomass) rather than a knock down (weed kill and biomass elimination) strategy. Specifically, we are evaluating two pathogens, *C. piaropi* and an isolate of *M. roridum* (isolated from begonia since no virulent isolate of this pathogen has been found on water hyacinth in the United States). The pathogens are being tested individually and in combination and applied with a surfactant, an invert emulsion, and/or a humectant gel. Applications are made to water hyacinth plants with and without natural populations of *Neochetina* spp. Results from field trials in progress indicate that a treatment with *C. piaropi* applied in the surfactant Silwet L-77 provided the best levels of biomass reduction and damage severity. The combination of the two pathogens was not significantly better than *C. piaropi* plus Silwet L-77, possibly because the isolate of *M. roridum* used is not a true pathogen of water hyacinth (Vincent and Charudattan 2000). Further, large-scale field trials are under way to develop a commercially feasible bioherbicide formulation.

Conclusions

It is a challenge to develop an effective bioherbicide that is acceptable for use in practical water hyacinth management programs. The challenge can be met, especially now, given the bio-friendly regulatory climate in the United States and other countries, and the availability of newer, innovative approaches to bioherbicide development and deployment. An assessment of the potential usefulness of *Uredo eichhorniae* as a classical biocontrol agent is in progress and the prospects for using this rust fungus on a global scale should be known in the near future.

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