Biological control of neotropical aquatic weeds with fungi

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Abstract

Many of the world’s worst tropical aquatic weeds are native to the Neotropics. Besides, the majority of the most aggressive aquatic weeds that pose major invaders to aquatic habitats in other regions of the world have escaped from their natural distributions involved in the study of the neotropical mycobiota of some of these weeds: CABI Biosciences-Ascot, UK; Plant Protection Research Institute (PPRI), South Africa; Universidade Estadual de São Paulo-Jaboticabal (UNESP), Brazil; Universidade Federal de Viçosa (UFV), Brazil; University of Florida (UF), USA. Scientists of these institutions have often been working in formal or informal cooperation.

The pioneering survey of the mycobiota of a neotropical aquatic weed aimed at finding potential biocontrol agents through the pioneering effort of entomologists (reviewed in Room et al., 1981; Harley et al., 1984; Spencer and Coulson, 1976) but only in the late 1970s did plant pathologists start collecting fungal pathogens of aquatic weeds in the Neotropics to be evaluated for use as classical biocontrol agents or as mycoherbicides. We give herein an overview of the status of biocontrol with pathogenic fungi of the following selected Neotropical aquatic weeds: Alternanthera philoxeroides, Azolla filiculoides, Echinochloa polystachya, Eichhornia azurea, Eichhornia crassipes, Egeria densa, Myriophyllum aquaticum, Paspalum repens, Pistia stratiotes, Polygonum spectabile, Salvinia auriculata, Salvinia molesta and Typha domingensis. © 2000 Elsevier Science Ltd. All rights reserved.

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1. Introduction

The world’s worst tropical aquatic weeds are regarded native to the Neotropics, a vast biogeographic unit comprising South and Central America, the Caribbean and parts of Southern Mexico. Some of the species, such as alligator weed, Alternanthera philoxeroides (Mart.) Griseb. and Salvinia molesta D.S. Mitchell, despite being relatively minor problems in their native range, have become major invaders of aquatic habitats in other regions of the world after having escaped from their natural habitats. These species, together with water hyacinth (Eichhornia crassipes [Mart.] Solms) and water lettuce (Pistia stratiotes L.), have been the subject of extensive searches for arthropod biocontrol agents (e.g., Room et al., 1981; Harley, 1984; Harley et al., 1984; Cilliers, 1987; Laup, 1987; Harley, 1990) in the Neotropics, particularly in Brazil, Uruguay and Argentina. Besides, the majority of the most aggressive aquatic weeds in the Neotropical region are endemic, and therefore there is a need for explorations for biological control agents in that area. Some spectacular results have been achieved through the pioneering effort of entomologists (reviewed in Room et al., 1981; Harley et al., 1984; Spencer and Coulson, 1976) but only in the late 1970s did plant pathologists start collecting fungal pathogens of aquatic weeds in the Neotropics to be evaluated for use as classical biocontrol agents or as mycoherbicides.

Presently, there are at least five different research institutions involved in the study of the neotropical mycobiota of some of these weeds: CABI Biosciences-Ascot, UK; Plant Protection Research Institute (PPRI), South Africa; Universidade Estadual de São Paulo-Jaboticabal (UNESP), Brazil; Universidade Federal de Viçosa (UFV), Brazil; University of Florida (UF), USA. Scientists of these institutions have often been working in formal or informal cooperation.

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agents was undertaken by UF (reviewed by Freeman and Zettler, 1971). These authors have searched for pathogens of water hyacinth in Panama. Charudattan et al. (1976) undertook field research for a rust disease of water hyacinth in Argentina and southern Brazil. E. crassipes is by far the most important neotropical aquatic weed that has received the greatest attention of plant pathologists.

Barreto and Evans (1996) have surveyed the mycobiota of seven aquatic weeds (E. polystachya, E. crassipes, E. azurea, Paspalum repens, P. stratiotes, P. spectabile Mart. and T. domingensis) in the State of Rio de Janeiro (Brazil). This survey was later continued by Hanada (1998) in the Amazon Basin (state of Amazonas, Brazil). C.B. Maia (UFV) is finishing a similar survey (involving a large number of aquatic weed species) in the river basins of the Doce ans São Francisco rivers (states of Minas Gerais, Espírito Santo, Bahia, Pernambuco, Alagoas and Sergipe of Brazil). Ad hoc collections of fungal pathogens of Myriophyllum aquaticum (Vell.) Verdec. and A. philoxeroides have also been undertaken recently by UFV scientists. Intensive research activity has been undertaken recently by CABI, UF and PPRI recently, targeting mainly E. crassipes whereas UNESP work has been aimed mainly at the submerged aquatic weed E. densa (Nachtigal, 2000).

2. Alligatorweed: Alternanthera philoxeroides

Alligatorweed is an amphibious Amaranthaceae native to South America. Although recorded only as an occasional invader of irrigated rice plantations in Brazil (Lorenzi, 1991) it became a serious invader of aquatic habitats after it was introduced into Australia, Asia and North America. A search for insects attacking this host was undertaken by US scientists in southern South America. Two insect species (Agasicles hygrophila Selman and Vogt and Vogtia malloii Pastrana) were selected and released in the United States and later into Australia with great success (Spencer and Coulson, 1976; Julien and Chan, 1922). Worries concerning this weed are re-emerging in Australia, where the insects are not controlling the weed in terrestrial situations (Julien and Chan, 1922).

A study on the potential distribution of this plant in Australia (Julien and Bourne, 1988) has indicated that this weed still invades only a fraction of its potential distribution. Perhaps because of that, alligatorweed was recently included among the 20 weed species of national significance in Australia (W. Forno, CSIRO, personal communication). There are also worries concerning this weed in New Zealand, where introduction of the insect A. hygrophila did not limit its spread (Stewart et al., 1996).

Little attention was given to pathogens associated to this weed in its native range until recently. A preliminary search for alligatorweed fungal pathogens in Brazil yielded only two species of pathogenic fungi: Nimbya alternantherae (Holcomb and Antonopoulos) Simmons and Alcorn and Cercospora alternantherae Ellis and Langlois (Barreto and Torres, 1999). Both caused leaf spots that were not particularly damaging under field conditions around Vicosa (state of Minas Gerais, Brazil). Preliminary studies indicate that N. alternanthera may have potential for the development as a mycoherbicide. Although C. alternantherae causes only limited damage to its host in Vicosa, it was seen causing a much more severe disease at a higher latitude (State of Paraná) and should be studied as a possible option for future classical introductions. It is likely that future surveys conducted in Southern Brazil, Uruguay and Northern Argentina may yield a richer mycobiota.

3. Aquatic ferns: Azolla filiculoides, Salvinia auriculata and Salvinia molesta

Azolla is a genus of fast-growing water ferns intensively studied because it is a symbiotic host to nitrogen-fixing algae (Holm et al., 1997). Although several important uses have been found for plants in this genus, they are also regarded as troublesome weeds, particularly A. pinnata R.Br. an Old World species and A. filiculoides. The latter is the only species in the genus that has been a target of a biocontrol programme. In South Africa it is listed by Henderson (1995) as a “proposed declared weed.” Surveys of the associated insect fauna have been undertaken in the Neotropics and the weevil Stenopelminus rufinasus was imported into quarantine and its life history and host range were elucidated as prerequisites for its introduction in this country (Hill, 1998). Although there are several records of fungal pathogens of Azolla spp. in Asia and Oceania (e.g., Garcia, 1986; Arunyanart et al., 1983) there is no record in the literature of surveys of fungal pathogens of A. filiculoides, S. auriculata or S. molesta in the Neotropics. Muchovej and Kushalapa (1979) recorded Drecshlera sp on S. auriculata from the reservoir system of the Universidade Federal de Vicosa in Brazil where 95% of the plants were showing necrotic spots caused by this fungus. Collections were also made by these authors at other sites in the State of Minas Gerais, where the fungus was also present. The authors made no comment about the biocontrol potential of this fungus, but inoculum of the fungus was easily produced and pathogenicity was proven. Perhaps the great success that resulted from the introduction of Cyrtobagus salviniae Calder and Sands from Brazil into Australia and Africa has discouraged further studies on the mycobiota of this fern. There is nevertheless a need for a systematic survey of fungal pathogens of the invasive aquatic ferns at the center of origin because S. auriculata is still a troublesome invader in water habitats in the Neotropics (Lorenzi, 1991). Even though there may not be an immediate need for additional natural enemies of
S. molesta and A. filiculoides, surveys must continue at least as a precaution for any future need for complementary agents against these weeds.

4. Cattail: Typha domingensis

*Typha domingensis* is a native species in the Neotropics that can become an aggressive invader causing serious problems in its native range. Although useful and desirable in many instances, it is often considered a noxious plant included in the main lists of Brazilian weeds (Kissman, 1991; Lorenzi, 1991). In Australia, where it is also thought to be native, *T. domingensis* is of increasing economic importance in irrigated areas as well as in recreational areas of lakes (Finlayson et al., 1983). Barreto (1991) presented a check-list of fungal pathogens in recreational areas of lakes (Finlayson et al., 1983). Economic importance in irrigated areas as well as being noxious, is considered the worst aquatic weed in the world. Barreto and Evans (1996) based on observations of *E. azurea* in the Paraiba do Sul basin warned that “its introduction to other parts of the globe would be a disaster.” That observation could be extended to *P. repens*. These authors gave an account of fungal pathogens collected in the state of Rio de Janeiro (Brazil) on *E. polystachya* and *P. repens*. Three fungal species were found on the former (*Phaeoramularia* sp., *Phoma* sp. and *Uredo uromicoides* Speg.) and two on the latter (*Colletotrichum falcatum* Went. and *Bipolaris* sp.). In addition to these species, *Phyllachora* sp. was also collected on *P. repens* by Hanada (1998) in the Amazon and more recently the same author collected another *Phyllachora* sp. on *E. polystachya* (Hanada, personal communication). Preliminary results of studies on the potential of *C. falcatum* as a mycoherbicide for *P. repens* were disappointing. Disease severity after inoculations under a variety of conditions were always low. Although Barreto and Evans (1996) considered *Phaeoramularia* sp. as “sufficiently severe to encourage further studies to evaluate its biological control potential” for *E. polystachya*, no additional studies were made on this fungus. The survey completed by C.B. Maia (UFV) in the basins of the Doce and São Francisco rivers did not contribute to additional records for the mycobiota of these two grasses.

6. Egeria: Egeria densa

*Egeria densa* is the worst submerged aquatic weed in most neotropical areas. In Southeast Brazil this species, together with *Egeria najas*, causes great annual losses to the hydroelectric companies. Interruptions of electricity generation and damage to grids and equipment are common in reservoirs belonging to CESP (Centrais Elétricas do Estado de São Paulo) in São Paulo (particularly at the power plants at 3 Irmãos, Salto Grande and Júpia) and Light (the electricity company of Rio de Janeiro) in Rio de Janeiro (particularly at Santana Reservoir) (R. Pitelli, personal communication). R.A. Pitelli at UNESP has been working on the biocontrol of egeria on a project funded by CESP for several years. After a series of field surveys and obtaining isolates from other institutions, a collection of hundreds of fungal isolates were formed. Laboratory tests have shown that eight *Fusarium* sp.
isolates appeared to have potential for the development as biocontrol agents. One isolates of *Fusarium graminearum* Schw. was the most pathogenic and the easiest to be manipulated. Interestingly, the most promising isolate was neither originally isolated either from egeria nor from an aquatic habitat. Plants of both species of *Egeria* developed progressive chlorosis followed by necrosis and complete tissue disintegration after being exposed to inoculum of this isolate. A series of in vitro and in vivo studies were carried out. These comprised: mass production, formulation, shelf-life, optimum inoculum concentration, adequate water condition for fungus effectiveness against the weed and host-range (Nachtigal, 2000). This possible product was provisionally called FUSGRA and may become a pioneering mycoherbicide for egerias.

7. Parrot’s feather: *Myriophyllum aquaticum*

This submerged weed with attractive terminal leafy shoots of up to 50 cm above the water is popular as an aquarium plant. As such, it was spread to many different parts of the world becoming invasive. In South Africa it is a “declared weed” and a subject of biocontrol investigation (Henderson, 1995). Its spread and establishment is a cause for concern in Portugal (Teles and Silva, 1974) and France (Chimits, 1978). In its native range in Brazil, this species is never seen forming dense monotypic infestations as in the Old World. Fungal collection work based at UFV has yielded three different species associated with severe blights of parrot’s feather (Hanada, 1998). These are: *Chalometella raphigera* Swift, *Cercospora* sp. and *Mycosphaerella* sp. Preliminary attempts to reproduce the disease failed. It is suspected that a combination of adjuvants may be necessary to allow the fungi to overcome the thick wax layer deposited on the above-water parts of *M. aquaticum*. Work on these associations is still progressing.

8. Smartweed: *Polygonum spectabile*

Among the troublesome weeds forming floating mats in the Neotropics there are two particularly aggressive species of *Polygonum*. According to Kissman and Groth (1995), these species, *Polygonum ferrugineum* Weddell and *Polygonum lapathifolium* L., are easily confused and have been treated together under the name *P. spectabile* (a later synonym for *P. ferrugineum*). An example of such a mistake is in Loreniz (1991). This particular problem is of significance for bicontrol as one of the species is considered native (*P. ferrugineum*) whereas the other (*P. lapathifolium*) is native to Europe. This may provide an explanation for the poor mycobiota found on smartweed in Brazil. Initial surveys in Rio de Janeiro (Barreto and Evans, 1996) and recent surveys in other Brazilian states yielded only one new species belonging to *Mycosphaerella* spp. and its anamorph (*Pseudocercospora*). Confirmation of the center of origin and additional collections aimed at classical introductions are necessary for this target-weed.

9. Water hyacinth — *Eichhornia crassipes* and anchored water hyacinth — *Eichhornia azurea*

Water hyacinth is a floating plant native from the Neotropics that has become widely distributed as a pantropical weed recognized as the world’s worst aquatic weed (Holm et al., 1977). Arthropods collected in South America have been playing an important role as biocontrol agents of water hyacinth (Harley, 1990). Nevertheless, a consensus was reached among water hyacinth biocontrol workers during a meeting (Stellenbosch, South Africa, in 1996) that “there is a need for wide ranging surveys for pathogens.” Additional pathogen and arthropod agents were regarded as necessary in order to complement the effect of the arthropods presently used. A renewed collecting effort was started involving several institutions. Results of such efforts are still being evaluated. One difficulty nevertheless, is easily recognizable for the successful accomplishment of these searches. The exact center of origin of *E. crassipes* within the Neotropics remains unknown. Intensive searches are often difficult to operate in remote, but promising areas. Intensive collections of fungal pathogens in selected river basins were started in the early 1970s by Charudattan et al. (1976). Promising results were obtained with the use of *Cercospora pioropi* Tharp, a pantropical pathogen of water hyacinth, during studies conducted in UF, and much has been published about this fungus (Sanders and Theriot, 1980; Charudattan, 1984; Freeman and Charudattan, 1984; Charudattan et al., 1985, among others). Perhaps because of the great expectations concerning the mycoherbicide approach for control of water hyacinth with this fungus, searches for other novel pathogens of *E. crassipes* were slowed. Unfortunately, although this fungus was one of the first pathogens to be patented as a mycoherbicide, practical problems related to mass production of inoculum and the need of combining it with chemical sprays to achieve an acceptable level of control resulted in the abandonment of commercial development.

Barreto and Evans (1996) undertook the first systematic survey of fungal pathogens of water hyacinth in Brazil during 1988–1989 (State of Rio de Janeiro) but found a very scarce mycobiota associated to this weed (limited to a single species — *C. pioropi* Tharp). Barreto (1991) considered this poor mycobiota as an indication that the area covered in his survey was not within the center of origin of this species. Other authors (Barret and
Hanada (1998) also found a mycobiota restricted to *C. piaropi* in his survey in the state of Amazonas. The only truly obligate, highly specialized fungal pathogen found on *E. crassipes* in Brazil is *Uredo eichhorniae*. Fragoso and Ciferri. This fungus is known to occur in Argentina, southern Brazil (Charudattan and Conway, 1975; Charudattan et al., 1976) and the Dominican Republic (Ciferri and Fragoso, 1927). In South America this fungus is found only in subtropical latitudes. Recently a collection of this fungus was made in southeastern Brazil (Poços de Caldas, state of Minas Gerais) (Hanada, 1998) but this was at a high altitude (1200 m). The absence of this fungus from other northern areas in Brazil would suggest that this fungus is adapted to lower temperature regimes. It is nevertheless, interesting to note that the fungus was first described from Dominican Republic, and the type collection was from the River Jaina, not a subtropical site. There is another fungal pathogen of water hyacinth which would also deserve the status of “obligate—highly specialized.” This is the smut fungus *Doassansia eichhorniae* Ciferri. Coincidentally this fungus was also described from the Dominican Republic. This could be regarded as an indication of the center of origin of the weed. In December 1998, R.W. Barreto undertook a brief survey trip to the Dominican Republic aimed at searching potential biocontrol agents for weedy species of the Melastomataceae. A search for pathogens of water hyacinth was also carried out along the river Jaina and other collecting sites recorded at Bonao by Ciferri. Unfortunately a combination of great hurricane damage to the river system (with complete destruction of most marginal lakes temporarily connected with the river) and a program of drainage of pools, lakes and ponds by the Dominican government (to fight disease vectors) has virtually eliminated all accessible habitats for water hyacinth. Very few *E. crassipes* plants were collected along the margins of the river Jaina showing symptoms of a fungus later identified as *Myrothecium roridum* Tode ex Fr. In other areas of Dominican Republic where *E. crassipes* was found (e.g., Hatillo Dam and the river Gran Arceo), the plants were generally quite healthy and were aggressively invading those habitats—an unlikely scenario if this were the center of origin for this weed.

R. Charudattan (UF), along with M. Morris and A. den Breéyn (PPRI), and R. Pitelli (UNESP), with support form the USDA-Foreign Agricultural Sciences and cooperation from UNESP, Jaboticabal, have made several surveys in southeastern Brazil, Uruguay, and Argentina to map the distribution of *U. eichhorniae*. The presence of *U. eichhorniae*-infected waterhyacinth plants was confirmed at several new locations in the states of São Paulo, Paraná, and Santa Catarina. Samples of rust-infected plants were collected for studies at Jaboticabal and under quarantine at Stellenbosch, South Africa, and Gainesville, Florida. Inoculation and spor germination studies were conducted with uredospores collected from these plants, and methods of propagation of the rust under quarantine conditions were established. The rust survived in its uredial stage for approximately 4 and 12 weeks on infected plants in Gainesville and Stellenbosch, yielding uredospores that could be used for inoculation of new, healthy leaves. Subsequent inoculations made from uredospores harvested from these plants were successful, but the rust infections did not keep up with the new growth. Consequently, the plants out-grew rust infections. Similar results were independently obtained at UFV with infected material brought alive from Poços de Caldas which was maintained either in controlled temperature room (18, 22 and 25°C) or in a greenhouse.

So far, these surveys have not confirmed the presence of teliospores in the field, but efforts are continuing. Studies are also underway to determine the cross-infectivity of *U. eichhorniae* and *U. pontederiae* from *Eichhornia azurea* and *Pontederia* sp. A list of plants that co-occur with rust-infected waterhyacinth plants is being made to search for possible alternate host(s). Until now the *U. eichhorniae*-E. crassipes pathosystem is puzzling. Some aspects requiring explanation are: (a) the general absence of a telial stage in the field and the inability of its production under controlled conditions; (b) its apparently very discontinuous distribution (i.e., southern South America and the Caribbean, the Caribbean record (Ciferri and Fragoso, 1927) has not been validated (Charudattan and Conway 1970); (c) its apparent restriction to subtropical habitats in South America together with its first record in the tropical Dominican Republic lowlands; (d) the great difficulty of keeping the infection in plants outside the habitat where the fungus occurs; (e) the morphological similarity of the anamorph of *Uromyces pontederiae* on *E. azurea* and *Pontederia lanceolata* Nutt. paralleling the absence of natural infections of water hyacinth in places of high rust incidence on anchored water hyacinth; and (f) the inability (so far) of promoting cross-infections under controlled conditions. All these aspects represent challenges to a better understanding of the water hyacinth rust and its full development as a classical biocontrol agent.

CABI Bioscience has been concentrating on potential centers of origin of water hyacinth in the Upper Amazon region: an area that until now has received little attention for surveys. In conjunction with entomologists from the USDA and PPRI, a survey was conducted along the Amazon and Ucayali rivers in Peru in April–May 1999. Of the pathogens found, a target-spot caused by *Leptosphaeria* sp. was the most common disease, but a complex of pathogens is associated with decline and death of water hyacinth following insect damage (notably by *Taosa* and *Thripticus* sp.) Most of the fungi are still
awaiting identification and pathogenicity screening but at least one represents a new genus. In sharp contrast to the Peruvian Amazon, water hyacinth is an uncommon member of the aquatic flora in the Ecuadorian Amazon. A survey along the Napo river in September 1999 revealed that the plant is not present in the main river and appears to be restricted to isolated lagoons (‘cochas’). An insect–pathogen association, similar to the above, was also noted and isolates are being evaluated. The rust, Uredo eichhorniae was found to be common on E. azurea, 1999 but, once again, it was not collected on E. crassipes.

Other pathogens have been investigated for the biocontrol of E. crassipes and the status of these was reviewed by Charudattan (1996, 1999). He listed 10 species and considered six of them as having practical possible use. One of the species which has been studied in great detail and is outstanding for its potential for mycoherbicide development is Alternaria eichhorniae Nag Raj and Ponnappa, a species having a wide distribution in the Old World and Oceania but that has not been recorded from the Neotropics. Intensive work based at Mansoura University (Egypt) and UF has been made since the late 1980s (Elwakil et al., 1989; Shabana et al., 1995, 1997a, b; Shabana, 1997a, b).

10. Water lettuce: Pistia stratiotes

Water lettuce is another floating pantropical weed that is considered either native from the Neotropics (Laup, 1987; Cilliers et al., 1996) or of uncertain origin (Holm et al., 1977). It is considered as the third aquatic weed of world importance (after E. crassipes and S. molesta) (Bennett, 1975). Also for this weed, a highly successful biocontrol program was developed in Australia, New Guinea and South Africa with a weevil imported (Neohydronomous affinis Hustache) from Brazil (Laup, 1987; Harley, 1984; Cilliers et al., 1996; Cilliers, 1987). Barreto (1991) undertook an extensive survey of the mycobiotic of water lettuce in the state of Rio de Janeiro (Brazil). The only fungus collected during this survey was Cercospora pistiae Nag Raj, Govindu and Thirumalachar. This fungus was restricted to a single ecosystem (two sites at interconnected reservoirs—Vigário and Santana) in Rio de Janeiro. Later surveys of the mycobiotic of aquatic weeds demonstrated the presence of this fungus also in other Brazilian states (basins of Rio Doce and Rio São Francisco rivers). Barreto et al. (1999) provided a list of fungal pathogens recorded in the literature and herbarium accessions on P. stratiotes. This list had only eight species (which could be reduced to six, because of misidentification in other records of Cercospora). Three of these are well-known plurivorous pathogens. These authors regarded the mycobiotic of P. stratiotes as scarce, perhaps indicating that until now the surveys did not include the center of origin of the plant. Recent surveys in the Brazilian states of Minas Gerais, Espirito Santo, Bahia, Pernambuco, Alagoas, Sergipe and Amazonas, did not provide any new additions to this list. Although until now the success attained with the use of N. affinis have been considered as sufficient for the biocontrol of water lettuce, additional surveys for fungal biocontrol agents are needed. Complementary agents have often been necessary in the history of biocontrol, even when after a successful agent had been found.

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