

IMPECCA¹: an International, Collaborative Program to Investigate the Development of a Mycoherbicide for Use against Water Hyacinth in Africa

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Abstract

The IMPECCA Programme has been established to develop a mycoherbicide for the control of water hyacinth, using fungal isolates that have been found in Africa. Such a mycoherbicide could replace the use of broad-spectrum herbicides, which are used routinely at present, but have caused concerns about contamination to fish and degradation of water quality. In addition, a mycoherbicide might be more compatible with the use of insect biological control agents. The project will build upon existing studies of formulating water hyacinth fungi into mycoherbicides which have been carried out in Egypt and Zimbabwe, and expertise gained by CABI Bioscience during the development and commercialisation of mycoinsecticides.

One of the key outputs will be the strengthening of technical capacity and linkages within African national programs to undertake biological control of weeds. Scientists will carry out extensive exploration for pathogens of water hyacinth already present in Africa. These will be identified, characterised and assessed for suitability as the basis for a mycoherbicide; these studies will include molecular and chemotaxonomic identification of both fungal isolates and water hyacinth biotypes. Characteristics of a fungus isolate suitable for mycoherbicide development include: high pathogenicity, acceptable host specificity, low mammalian toxicity, and capacity for mass production and formulation. Candidate products will be laboratory and field tested for efficacy and compatibility with other (especially biological) control options. A water hyacinth management strategy will be proposed appropriate for local needs.

WATER hyacinth is perhaps the most pernicious aquatic weed in the world. Water hyacinth is generally the dominant plant when it occurs outside of its native

range and is capable of suppressing or eliminating other species. It forms dense mats of vegetation in lakes and dams, and irrigation and flood channels, where it impedes boat traffic, increases eutrophication and harbours the mosquito vectors of malaria, encephalitis and filariasis (Forno and Wright 1981). The problems are most severe in developing countries, where human activities and livelihoods are closely linked to the water systems. Conventional methods of control rely mainly on mechanical/manual removal and chemical herbicides, which have generally been found to be inadequate and expensive measures to apply on a large scale. Herbicides have the added dis-

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advantage that they might have adverse environmental effects, and must be applied carefully and selectively. They also can interfere with or nullify the action of biological control agents present (Charudattan 1995). In recent years, attention has been given to using natural enemies to control the weed. Indeed, the only logical, long-term and sustainable solution to managing the weed is to employ an integrated approach, with special emphasis on biological control agents (Charudattan 1995).

As described in other chapters of these proceedings, biological control using insects such as *Neochetina* spp. has been highly successful in reducing water hyacinth populations in several countries in northern and central America and parts of Africa. The need for a range of effective management tools against this weed, including mycoherbicides, is illustrated by the waterways where water hyacinth remains a problem despite attempts to introduce insects for biological control (Hill and Olckers 2001; Charudattan 2001). Furthermore, several studies have demonstrated possible insect-pathogen interactions combining to cause a decline in water hyacinth populations (Galbraith 1987; Charudattan et al. 1978; Kasno et al. 1999). Mycoherbicides might therefore be used to augment other natural enemies.

Stages Required in the Development of a Mycoherbicide

Several research groups have identified promising microbial agents that might be used as biopesticides. However, this research has rarely resulted in the development of biopesticide products, which currently account for only a fraction of one percent of the global crop protection market (which is approximately US\$3 × 10¹⁰). Although there is a long history of research on microbial control agents, it is not always appreciated that obtaining an active isolate is only the beginning of a series of activities necessary for implementing the use of a new mycoherbicide (O'Connell and Zoschke 1996). There are important issues to consider including: mass production (see e.g. Jenkins et al. 1998), delivery systems and 'laboratory to field' studies (Bateman 1998), strategies for use, registration and commercialisation.

Although focusing on 'downstream' processes of mycoherbicide development, the IMPECCA Programme will also carry out further surveys for pathogens for a limited period. Several isolates have already been collected that will act as standard isolates for

comparison with newly collected material. The criteria for the selection of fungi were that:

- they must have been isolated in Africa;
- they must be widely distributed across the regions of Africa where water hyacinth is found; and
- they must be shown to have good pathogenicity to water hyacinth in laboratory or field studies.

In contrast, Evans and Reeder (2001) argue for a 'longer term' approach to the use of microbial agents, in which isolates are sought at the weed's centre of origin, for release as classical biological agents. However, the introduction of exotic pathogens is in its infancy and there is still a reluctance to the release of pathogens as biological control agents in many countries. As part of the inception phase of the IMPECCA Programme, the scientific collaborators have identified a short-list of fungal species that show most promise in their potential as mycoherbicides for water hyacinth control in Africa. In order of preference, they are:

1. *Alternaria eichhorniae*;
2. *Acremonium zonatum*;
3. *Cercospora piapori*/*C. rodmanii*;
4. *Rhizoctonia solani* and *Alternaria alternata*;
5. *Myrothecium roridum*.

Program of Activities and Progress to Date

Table 1 gives a possible sequence of activities for mycopesticide development, as identified by IMPECCA Programme scientists. The following major activities are in progress or are planned:

- Collection and preservation of pathogen isolates. Over 70 isolates have been collated by CABI Bioscience, and the most promising specimens will be accessioned at CABI Bioscience, UK. Isolate collection is being carried out in strict observance of the International Convention on Biodiversity, and a document has been prepared explaining IMPECCA Programme policy.
- Identification and characterisation of specimens:
 - morphological, molecular and chemotaxonomic identification of fungal isolates,
 - molecular identification of water hyacinth biotypes.

Preliminary analyses of water hyacinth isolates from Africa, India and South America indicate a surprising degree of genetic homogeneity (Alex Reid, pers. comm.). However, we foresee the need

Table 1. Major elements of mycoherbicide development

Issues	Process (may be repeated with new isolate)	Sequence
<i>Survey for indigenous pathogens</i>		1
<i>Pathogen isolation and characterisation</i>	Isolation	2
	Agreement on standard isolates	2
	Preservation of isolates	2
	Identification	3
	Assessment of pathogenicity	4
	Characterisation	5
<i>Working methods</i>	Formation of country networks	1
	Development of models as research tools	1
	Intellectual property issues	1
	Policy on publication	2
<i>Biology</i>	Key protocols: screening techniques/objectives	1
	Water hyacinth biology	1
	Water hyacinth–pathogen interactions/nutrient levels	9
	Persistence, horizontal transmission	10
<i>Quarantine issues</i>		2
<i>Host range determination</i>	For environmental safety	6
	Efficacy	6
<i>Mass production</i>	Laboratory (Petri dishes / bottles)	2
	Pilot plant	7
	Commercial	13
<i>Toxicology</i>	Mammalian safety tests (isolate assessment)	8
	Mammalian safety tests (registration)	12
	Ecotoxicology (includes host range data)	6–12
	Post control impact (succession of water hyacinth with other species) and changes in oxygen demand	10
<i>Delivery systems</i>	Storage	6
	Formulation	7
	Application	8
<i>‘Lab to field’ studies</i>	‘Pre field trials’ for efficacy	9
	Assessment of synergism (e.g. with <i>Neochetina</i> or chemicals/stressors)	9–11
<i>Field testing</i>	Small-scale field trials	10
	Large-scale field trials (operational effectiveness)	12
<i>Strategy for use</i>		10
<i>Socioeconomic assessment</i>		10
<i>Product identification</i>	Ideally one product (maximum 2) – registration is expensive!	11
<i>Registration dossier</i>		12

to characterise approximately 100 samples before any firm conclusions can be drawn.

- Pathogen screening and selection for further mycoherbicide development on the basis of a combination of key criteria including;
 - virulence,
 - host range testing,
 - preliminary mammalian toxicity studies, and
 - an assessment of production and storage characteristics.

A series of standard operating procedures for isolate collection, and assessment of virulence and host range testing, have been agreed or are in preparation. Preliminary observations confirm those of Shabana et al. (1995) that isolates of *Alternaria eichhorniae* appear to be the most promising. These and other useful techniques are being compiled in the form of IMPECCA Technical Guides.

- Development of a suitable delivery system (Fig. 1) that includes:
 - preparation of prototype formulations,
 - storage stability tests, and
 - selection of appropriate application equipment.

The use of oil formulations has shown great promise for enhancing the efficacy of fungal agents that show potential as insecticides, fungicides and herbicides. Perhaps most importantly, the need for high humidity is overcome: Amsellem et al. (1990) showed that invert emulsions eliminated the need

for a minimum inoculum threshold with *Alternaria cassiae* and *A. crassa*. Such formulations (where oil constitutes the continuous phase) are rather ‘user unfriendly’ being unstable and very viscous, so the use of less viscous vegetable oil suspension emulsions has been investigated by Auld (1993) and Shabana (1997). Bateman et al. (2000) discuss important criteria in the selection of application equipment for biopesticides, and argue that the equipment normally used for conventional pesticides is often most appropriate. Rotary atomisers fitted to aircraft for low volume spraying of chemical herbicides are a common method for water hyacinth control (Julien et al. 1999) and especially suitable for the application of oil-based formulations.

- Field testing and ecological evaluation. A preliminary model has been developed that describes water hyacinth phenology; this will later incorporate (and attempt to interpret) data on the impact of natural enemies and their interactions (Neils Holst, pers. comm.)
- Programme management, liaison with collaborating partners, donors and other projects; agreements, patents, subcontracts, promotion and publicity.

The IMPECCA Programme collaborates with the IOBC by publishing The Water Hyacinth Newsletter (editors: Rebecca Murphy and Martin Hill). News and progress will also be available on our World Wide Web page: <<http://www.impecca.net>>.

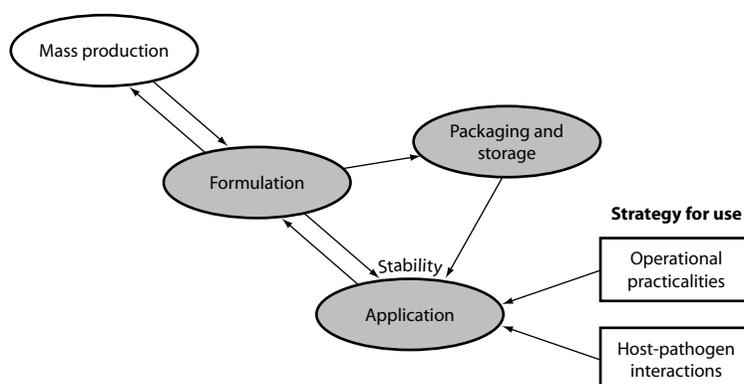


Figure 1. Delivery systems: successful biopesticide development is most likely to be brought about by considering all the various technical aspects of product development at an early stage. There are often important linkages between the mass production, storage and packaging processes. Formulation and application are likewise interdependent and governed by appropriate strategies for use of a microbial agent in the field.

Collaborators

The main investigators of the IMPECCA Programme include:

1. CABI Kenya: Ignace Godonou, Christiaan Kooyman
2. CABI UK: Roy Bateman, Carol Elison, Harry Evans, Jane Gunn, Jeremy Harris, Nina Jenkins, Belinda Luke, Rob Reeder, Alex Reid, Sue Paddon, Emma Thompson
3. Danish Institute of Agricultural Science: Niels Holst (supervising PhD students: Sander Bruun and John Wilson)
4. Department of Research and Specialist Services, Zimbabwe: Bellah Mpofu, Lawrence Jasi
5. International Institute for Tropical Agriculture, Benin: Jürgen Langewald, Fen Beed
6. Plant Protection Research Institute, South Africa: Alana den Breeÿen, Cheryl Lennox
7. University of Mansoura, Egypt: Mahmoud Zahran, Mahomed El-Demerdash, Fathy Mansour, Yasser Shabana (from August 2001), Abdel-Hamid Khedr, Gamal Abdel-Fattah, Ibrahim Mashaly, S. El-Moursy

At the time of writing, the program has also established linkages with representatives from Kenya, Malawi, Rwanda, Tanzania and Uganda.

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