Times are changing, though, and many organizations such as NOAA (US National Oceanic and Atmospheric Administration) and the USGS are making NDVI (Normalized Difference Vegetation Index) and land cover satellite data freely available via the Internet. Images are usually available at a coarse (1-4km) resolution and have been well used in many studies. One such long-term system, the Advanced Very High Resolution Radiometer (AVHRR) that operates onboard the NOAA satellites, has data archives covering Africa for the past 20 years. More recently, higher (e.g. 250-500m) resolution data collected by the new MODIS (Moderate-resolution Imaging Spectroradiometer) sensor onboard the US Earth Observation System (EOS) have also become freely available.

Whatever the image, it must be correctly classified so features can be reliably identified in terms of objects or type of cover on the ground. Classification of images is possible because the spectral response of vegetation types and objects (the amount of electromagnetic radiation reflected as a function of wavelength) varies. However, some (e.g. bare soil and roads) have the same spectral response and are difficult to distinguish. In our case, it may be very difficult to differentiate between stands of a native aquatic and water hyacinth if they have similar spectral responses. The process used to overcome this involves setting up training sites (areas representing each plant type) to classify the image, and then verifying accuracy by 'ground truthing': going to the field and checking the classified image against what is really on the ground (or in the water!).

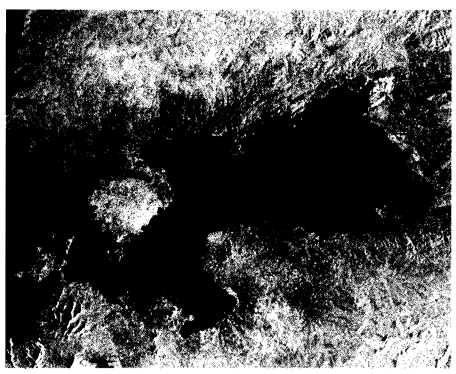
In 1995, a project in Uganda used Landsat images to investigate weed coverage of Lake Kyoga but found that images at a resolution of 30m x 30m were insufficient. However, using images from the French-designed commercial system SPOT (Système Pour l'Observation de la Terre) with a 10-20m spatial resolution, they were able to determine that 1.5% of the lake was covered by water hyacinth and a further 5.4% by papyrus.

At King's College in London (http://www.kcl.ac.uk/kis/schools/hums/geog/mw/lake_page.htm) we have investigated the use of AVHRR images for monitoring water hyacinth on Lake Victoria, where the weed has occurred in very large patches. While this small project has finished, we are hoping to start a new project to investigate the spread of water hyacinth using low spatial resolution imagery. Despite the poor detail, the images are freely available and with many years of data already archived, an historical record of the larger infestations of water hyacinth around the world could potentially be produced. Among the other aspects of the project is the assessment of the different satellite images now available for future monitoring and control programmes.

Currently, Mic Julien at CSIRO Entomology (Australia) and a Dutch firm, Synoptics (http://www.synoptics.nl), are mapping water hyacinth on Lake Victoria. They are using images from the Canadian Space Agency Radarsat system. Radar images are taken at microwave rather than optical wavelengths and have the advantage of being unaffected by clouds. Using radar images covering the whole of Lake Victoria from 1996, 1998 and 2001, they hope to chart the build up and demise of the weed and link this to known changes in weevil numbers. These data can then be cross-checked against actual estimates of coverage from different locales. If successful, the project may be expanded to investigate a dam in Côte d'Ivoire and a retrospective look at the Sepik River System in Papua New Guinea.

In their efforts to review historical and current levels of water hyacinth cover on Lake Victoria and parts of the Upper Kagera river basin, a US environmental company, Clean Lakes, Inc. (http://www.cleanlake.com/), under a Cooperative Research and Development Agreement with USGS are gathering satellite imagery from various spaceborne sensors. A Radarsat image acquired for the Winam Gulf, Kenya reveals a large quantity of floating aquatic vegetation thought to be made up of primarily water hyacinth (see below).

Work has also been published by the US Department of Agriculture (USDA), which used airborne video to map weed distributions in Texas. By knowing the light reflectance characteristics of the different weeds and linking the images obtained to positional data, a map of aquatic weed coverage can be obtained for input into an updateable geographical information system (GIS).



This image, which shows water hyacinth (flat grey) in Winam Gulf, Kenya on 6 Nov 1998, was produced from Radarsat data by the US Geological Survey/EROS Data Center in conjunction with Clean Lakes, Inc. Funding for this work was provided by the US Agency for International Development.

The use of satellite imagery can provide information on the historical scale of an aquatic weed problem and the success of control measures. In theory, remote sensing can also be incorporated in monitoring programmes, supplying reliable, detailed information for water managers and scientists. The new availability of very high spatial resolution imagery, coupled with use of cloud-penetrating radar and low cost or free access lower spatial resolution historical data, means that the use of this technology in the management of aquatic weeds is set to increase.

If you want to check out some other images that have been used to map water hyacinth, try the following from NASA:

http://edcsnw3.cr.usgs.gov/ip/hyacinth/winam1.html

http://edcsnw3.cr.usgs.gov/ip/hyacinth/hyacinth.html

http://www.wisard.org/wisard/shared/asp/projectsummary.asp?Kennummer=2657

http://www.ars.usda.gov/is/pr/1999/990524.htm

By: John Wilson (Imperial College) and Martin Wooster (King's College), University of London, UK

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Microsporidia & *Neochetina*

Microsporidia comprise the most common group of protozoan pathogens found in insects. About 70 genera have been described from most insect orders, particularly Diptera and Coleoptera[1]. The importance of eliminating microsporidial diseases from biological control agents prior to their release has been a subject of controversy[2]. Though typically sublethal, effects of microsporidia can become lethal when the host is stressed. They adversely affect many aspects of insect biology including pupal weight, adult longevity, developmental rates, adult fecundity, mating success, diapause, etc. We consider elimination of microsporidia essential prior to the release of a new biological control agent. On the other hand, it has been argued that decontamination of initial stock is futile as agents often acquire diseases after release anyway (see [3]). Furthermore, decontamination procedures involving the selection of a few 'healthy' individuals from an already limited original stock could unwittingly induce inbreeding depression, thereby reducing the ability of the released population to establish or to control the plants.

Although the weevils *Neochetina eichhorniae* and *N. bruchi*, introduced worldwide for the biological control of water hyacinth, are responsible for one of the most successful weed biological control projects in the world, their effectiveness has been

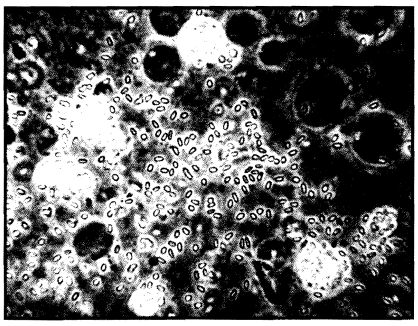
What to Look For

It is always best to examine live insects.

Macerate each one in a drop of distilled water. Remove the pieces of cuticle and place a portion of the remaining fluid on a clean microscope slide. Place a cover slip on the sample and examine the slide using phase-contrast microscopy (± 40x).

The spores will appear to glow due to refraction from the phase-contrast illumination (see photo).

To determine the site of infection, specific tissues can be removed and examined individually.



Microsporidia seen with phase-contrast using a compound microscope.

Shipping Neochetina

Do not include any plant material: the weevils are fine for a few days without food.

Ideally, hold them without food for 1-2 days prior to shipment to ensure their guts are voided. This minimizes the chances of spore-containing frass contaminating the entire stock.

Loosely stuff the shipping container with wooden packing material (e.g. Excelsior) that has been soaked in water. This provides adequate space and humidity.

Then place the weevils in the container.

Rearing Clean Cultures

Sanitation measures used in bacteriology labs provide good guidelines for avoiding contamination problems arising from the presence of microsporidian spores. All counter tops, glassware, dissection instruments, etc. must be routinely sterilized by heat (>50°C) or wiped down with a 2.5-5% commercial bleach (sodium hypochlorite) or other antimicrobial solution.

The primary strategy for eliminating microsporidia depends on the selection of healthy progeny from field-collected parental stock.

1. Collect adults from the original source (virgin adults reared from pupae are ideal, but collection of adults is usually more practical) and place individual pairs on whole plants.

2. Allow sufficient time for them to mate and lay eggs.

3. Examine the parents for the presence of microsporidial spores (see 'What to look for' box).

4. If spores occur in smears from either parent, destroy plant material containing their progeny. Keep only material from disease-free adults. (Microsporidia can be transmitted transovarially, and from male to female, so surface sterilization of eggs is not effective.)

5. Sample and examine progeny (10-15%) of parental stock to ensure that the sanitization procedure produced the desired result.

6. If microsporidia are detected in the progeny, repeat the entire procedure with each subsequent generation until spores are no longer found.

7. Use only disease-free individuals to develop the general colony.

Louis Pasteur pioneered this technique for raising microsporidia-free silkworms, so saving the French silk industry.

limited in some places. This lack of effectiveness may be partially attributable to a microsporidiosis that infects Florida populations, which have provided a source of weevils for many countries. Colonies of both species were purportedly 'spot-checked' for diseases prior to their release in the early 1970s, so we are unsure if the microsporidia were introduced with the weevils or acquired after release. We also don't know if infected weevils were released in other countries.

We have been evaluating the impact of microsporidia on both *Neochetina* species at the US Department of Agriculture - Agricultural Research Service (USDA/ARS) Invasive Plant Research Laboratory in Fort Lauderdale, Florida, USA in collaboration with Dr James Becnel at the USDA/ARS Center for Medical, Agricultural, and Veterinary Entomology (CMAVE) in Gainesville, Florida. The major thrust of this work has involved identifying the species of microsporidia that infect *Neochetina* spp. and determining their impacts on various aspects of water hyacinth weevil biology.

Population studies have now shown that *N. eichhorniae* generally have higher levels of infection than *N. bruchi* on both a yearly (average of 9.3% vs. 4.4%) and seasonal basis (Winter: 10.4% vs. 3.6%; Spring: 9.2% vs. 4%; Summer: 9.6% vs. 6.4%, and Fall: 8% vs. 3.6%). Morphological and molecular studies are showing that the microsporidia species infecting the weevils are quite primitive. While *N. bruchi* harbours a single species, *N. eichhorniae* is possibly infected by two different species (molecular analyses soon will clarify this). Furthermore, infections in *N. eichhorniae* are typically systemic, with the mid-gut, Malphigian tubules and fat body heavily infected, whereas *N. bruchi* usually shows a very light infection with the spores located only in the mid-gut. Spore measurements show slight, although not statistically significant, differences between weevils species with spore widths being equal (average of 1.94µ) but spores from *N. bruchi* being slightly longer (average 3.7µ vs. 3.5µ).

Microsporidia can best be diagnosed using optical phase microscopy to examine fresh tissue smears for the presence of spores [See 'What to Look For' box]. Other workers have suggested that, in order to avoid killing the weevils, microsporidia can be detected in frass samples, so we also examined this possibility. Examination of frass does provide a reliable method for detection of disease in *N. bruchi* (93% accuracy) but in *N. eichhorniae* it fails to detect the pathogen 78% of the time. The explanation for this lies in the fact that in *N. bruchi* the infection is restricted to the gut where the spores are easily excreted. But in *N. eichhorniae* excretion through the gut is unlikely when the infection is localized in the fat body or Malphigian tubules, so spores don't always appear in the frass.

Microsporidiosis is usually chronic and without manifestation of external symptoms exhibited by their hosts. Sublethal effects are commonly evaluated by measuring the impact on host fitness: life span, fertility, and feeding. Preliminary studies suggest that infection reduces fertility (by 46.2% in *N. eichhorniae* and 35.3% in *N. bruchi*) and longevity (by 41.8% in *N. eichhorniae* and 41.1% in *N. bruchi*) in water hyacinth weevils, but feeding rates do not seem to be altered.

Microsporidia clearly can limit the effectiveness of these biological agents, so the introduction and release of microsporidia-free colonies is of paramount importance. Despite it being a very laborious procedure, the Pasteur method (or single breeding method), which produces disease-free breeding lines, is the only way to successfully control transovarially transmitted microsporidia in insect cultures [see 'Rearing Clean Cultures' box]. Precautions must also be taken during

shipment. Often, large numbers of weevils are placed in small containers along with plant material. As a result, the weevils concentrate in a small area to feed on leaves that become covered with frass. This results in massive cross-contamination of the consignment and results in extremely high infection rates. [See 'Shipping *Neochetina*' box, which describes our recommended packing and shipping method.]

[1]Becnel, J.J.; Andreadis, T.G. (1999) Microsporidia in Insects. *In*: Wittner, M. *(ed)* The Microsporidia and microsporidiosis. American Society for Microbiology, pp. 447-502.

[2]Kluge, R.L.; Caldwell, P.W. (1992) Microsporidian diseases and biological weed control agents: to release or not to release? *Biocontrol News and Information* **13**, 43N-47N.

[3]Dunn, P.H.; Andres, L.A. (1981) Entomopathogens associated with insects used for biological control of weeds. *In*: Del Fosse, E.S. *(ed)* Proceedings of the Fifth International Symposium on the Biological Control of Weeds, Brisbane, 1980, pp. 241-246.

By: M. Teresa Rebelo & Ted D. Center

M. Teresa Rebelo, a graduate student, is working towards her PhD from the Faculdade de Ciencias de Lisboa, Departamento de Zoologia e Antropologia, Centro de Biologia Ambiental in Lisbon, Portugal. She received a grant from Luso-American Foundation for Development to pursue studies at the USDA, ARS Invasive Plant Research Laboratory in Fort Lauderdale, Florida working with Drs Ted D. Center and G.S. Wheeler.

IMPECCA Spotlight

Continued from back page ...

Zimbabwe and South Africa. Future surveys will also include Sudan, Mozambique, Malawi and Zambia and others if circumstances allow. Following the successful scientists' meeting and training workshop held in Kenya, a network of collaborating national scientists is being developed further in western, eastern and southern Africa to assist in the collection and screening of pathogen isolates.

Pathogens collected in Africa have been through a preliminary screening process to identify the most promising pathogens. Of these, CABI Bioscience, UK now holds 211 fungal isolates in centralized storage. Searches and pathogenicity tests carried out on the fungal isolates collected to date appear to confirm that, of the fungal species found in Africa, isolates of *Alternaria eichhorniae* show most promise for mycoherbicide development. Surveys continue not only to search for better pathogens of water hyacinth, but also to provide data on the distribution of the pathogens already collected, which will be essential in the registration of a mycoherbicide product. Surveys are also enabling the identification of sites that may be suitable for future field testing of a mycoherbicide.

Floating mats of water hyacinth at the village of Gattawani, Niger cause great difficulties for the local fishermen and act as a reservoir of plants to cause infestations downstream. This photo was taken during recent joint surveys by the International Institute of Tropical Agriculture (IITA) and the Ministry of the Environment along the whole length of the Niger River for water hyacinth pathogens. Fungi collected included Alternaria eichhorniae, Myrothecium roridum, Cercospora piaropi *and* Rhizoctonia solani. *Fen Beed.*

Preliminary mass production techniques have been developed for an isolate of *A. eichhorniae* and sufficient quantities can now be produced for use in small-scale trials. A number of experimental formulations of fungal propagules in oil and alginate have been developed, and a relatively high level of conidial survival can apparently be maintained for more than 3 months by storage in certain vegetable oils.

Development and testing of the mycoherbicide formulations has continued and pre-field trials using small paddling pools of water hyacinth will be initiated in the UK and across Africa in October.

In addition, the IMPECCA Programme is making a significant contribution to wider issues in the integrated management of the water hyacinth problem. Sponsorship of *Water Hyacinth News*, technical workshops and the IMPECCA website are helping to disseminate information and develop regional and international linkages in Africa that might not otherwise have been made. Activities undertaken on molecular characterization of water hyacinth isolates, and the development of computer models that characterize and simulate biological and phenological phenomena related to water hyacinth should prove to be of considerable importance to scientific and weed control initiatives other than solely mycoherbicide development.

Farewell and Welcome!

At the end of September, IMPECCA bade a fond farewell and good luck to Jeremy Harris, who leaves CABI Bioscience to train as an accountant. There is a happy ending to this story however, as we welcome two new members to the IMPECCA team.

Dr Dave Moore, also of CABI Bioscience, takes on the role of IMPECCA Programme Manager. Dave has extensive experience of managing biopesticide projects in Africa, South America and Europe, and was a major contributor to the international collaboration, LUBILOSA, which successfully developed a mycoinsecticide for the control of locusts in Africa. Dave is looking forward to joining the IMPECCA team to lead another similar international collaboration, this time in the development of a mycoherbicide.

On a technical level, Dave has expertise in storage and persistence, in relation to ultra-violet radiation, of fungal spores used in biopesticides, although his publications reflect a wide range of experience in the development of biopesticides.

Another new addition, Dr Yasser Shabana, joins the IMPECCA team in Egypt after completing a 2-year research fellowship at the University of Hohenheim, Germany. A scientist of international repute, Yasser has had a particular research interest in the use of *Alternaria eichhorniae* as a biocontrol agent of water hyacinth and the development of formulations for its use as a mycoherbicide for the past 18 years. Yasser has already shared his considerable experience with the IMPECCA team, and we now look forward to the opportunity to work more closely with him.

Dr Shabana (3rd right) and some members of the CABI Bioscience IMPECCA team during his visit to the UK in May.

Coming Soon: New Website Updates

Keep an eye out for new additions to the IMPECCA website, which will include:

- a non-specialist guide to the key features of mycoherbicide development with cross-references to the Technical Guide
- colour illustrations of disease symptoms and of the different flower morphs of water hyacinth
- identification keys
- useful website links
- a database of related scientific publications