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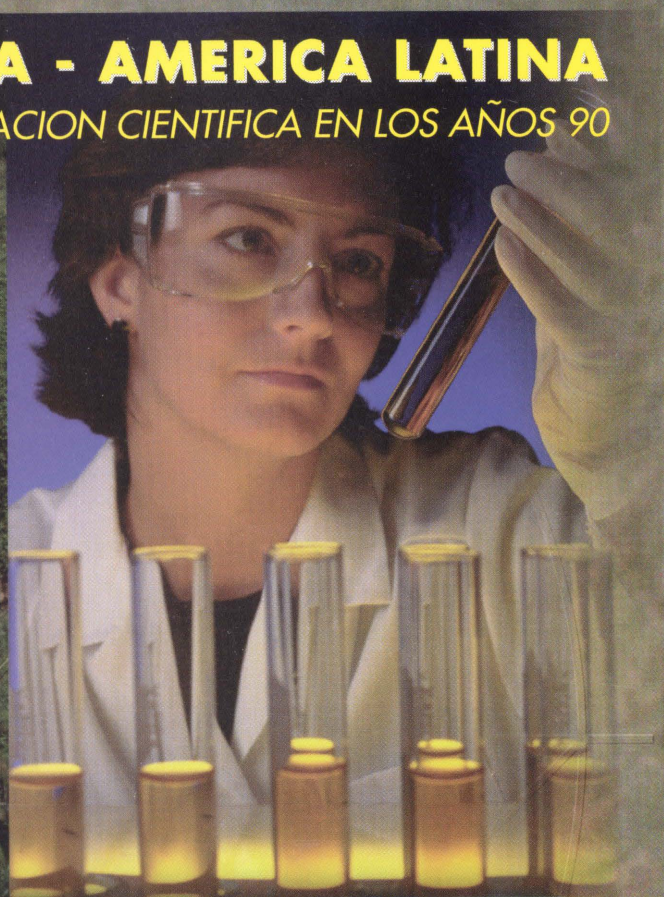

EUROPEAN UNION - LATIN AMERICA

SCIENTIFIC COOPERATION IN THE 90's

Vol III: International Scientific and Technological Cooperation with Developing Countries (INCO-DC)

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EUROPEAN UNION - LATIN AMERICA

SCIENTIFIC COOPERATION IN THE 90' s

UNION EUROPEA - AMERICA LATINA

COOPERACION CIENTIFICA EN LOS AÑOS 90

Vol III: International Scientific and Technological Cooperation with Developing Countries (INCO-DC)

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Preface

European Union - Latin America Scientific Cooperation in the 90's

It gives me great satisfaction to present this overview of the results of almost a decade of continuous support from the European Community to cooperation between our scientists and their Latin American counterparts. In addition, this publication provides researchers with a valuable source of information on the projects supported, their scope, objectives, and results, and gives full details of the teams involved and how to contact them.

The reader will find in the pages that follow the practical results of the Community's policy on scientific cooperation with the Latin American region. As in the case of other developing regions, Community policy has sought to harmonise a contribution to the region's socio-economic progress with our own scientific interests.

Implementation of this policy has allowed Community scientists to gain access to localities displaying particular environmental, agricultural, ecological and public health characteristics, and to undertake their research in these areas. As a counterbalance, we believe that Latin American researchers have derived great benefit from interaction with their European peers. Given their own scientific quality, this sharing of experience places local teams in a privileged position from which to contribute to finding science-based solutions to problems faced by their communities.

It is precisely with the aim of tackling these problems effectively that, after extensive dialogue with the scientific authorities and communities of the region, the Commission selected areas on which to target cooperation. Agriculture and agroindustry, health and environmental issues were considered the most important priorities, as the reader will be able to see in the body of this publication. However, in order to capitalize on the human potential available, research in other relevant fields such as earth sciences, materials and different branches of engineering was also supported when resources permitted.

We firmly believe that our cooperation has led to the creation of a permanent network of scientific interaction, embracing a vast number of Latin American and European scientists, and which is even broader and more far-reaching than the sum of the results of the projects presented here.

The importance of Latin America for the European Community has recently been brought to the forefront by the Summit of Heads of State of Latin America and the Caribbean, and the European Union, which took place last June in Rio de Janeiro. The dialogue that has taken place over the years in different fora has been reinforced by the Heads of State of the two regions with their decision to establish a Working Group of Representatives. This institutionalised Working Group should provide a renewed impetus to our cooperation: whether this will be achieved through the enlargement of the specific programme for cooperation, by further facilitating access to the specific thematic programmes of the framework programmes, by the conclusion of cooperation agreements, or by the combination of some of these options, is still an open question.

The Working Group of Representatives will be the forum for reflection and advice on the most appropriate way to develop the full potential of our cooperation in the future. The Rio Summit underscored the will of both regions to deepen that cooperation, and the European Commission will apply its best efforts and full capacity to the successful achievement of that aim.

Brussels, October 1999

J. Gabolde
Director

Introduction

During the 1990s, the European Community pursued scientific cooperation with Latin America through a series of different programmes.

For the period 1990-1994 two complementary schemes were in operation. First, the Life Sciences and Technologies for Developing Countries (STDIII) programme, which formed part of the EC's Third Framework Programme for Research and Technological Development aimed at mobilizing EC and Developing Country scientists to work on pressing problems of all developing countries, including Latin American countries, in the areas of human health and agriculture. Second, the International Scientific Cooperation (ISC) scheme, which aimed at developing long-lasting working relationships between EC and Latin American scientists, covered a wider range of subjects and set priorities by mutual agreement with the national authorities of individual countries. Through these two schemes a wide-ranging development effort was complemented by a country-specific initiative. The ISC scheme also granted fellowships for Latin American scientists to do research in European laboratories and develop contacts with the European scientific community.

In 1994, a new scheme combining these ideas was introduced. This was the INCO-DC programme (Scientific and Technological Cooperation with Developing Countries), which formed part of the EC's Fourth RTD Framework Programme and which ran until 1998. It focussed specifically on three sectors of widespread importance (sustainable management of renewable natural resources, sustainable improvement of agricultural and agroindustrial production, and health) and used a regional basis, in this case the region being Latin America, on which to set research priorities and build projects.

The newest programme, which started in 1999 and runs for a further four years, is the Research for Development (INCO-DEV) component of the Fifth RTD Framework Programme. This programme targets research of a problem-orientated nature, maintains the regional approach and subject-matter coverage of the earlier INCO-DC programme but adds to it a section on policy research for sustainable development.

This volume contains summaries of joint research projects involving partners in Latin America. It covers all STDIII and INCO-DC projects, and ISC projects which started in the 1992-1994 period. A table summarizing the number of activities carried out and EC financial contribution is given below.

Jaak Sinnaeve
Head of Unit XII-E-4
Research for Development

EC-Latin America S + T cooperation activities			
	Number of activities	Number of institutional partners	EC financial contribution (million ECU)
Joint Research projects			
STD III (1990-1994)	96	388*	31.76
ISC (1990-1994)	363	933	57.88
INCO-DC (1994-1998)	121	818*	58.50
Fellowships (1990-1994)	319	638	10.44
TOTAL	**899**	**2777**	**158.58**

** Includes some partners from non-Latin American developing countries*

Table of Contents

INCO-DC

Health

Contract number: IC18*CT950016

Period: January 1996 to June 1997

SELECTION OF *P. FALCIPARUM* GENES FOR MPES VACCINE DEVELOPMENT

Co-ordinator: Institut Pasteur, Paris, France (Pierre Druilhe)

Objectives :

- Further investigate the vaccine potential of SALSA, STARP, LSA1 and LSA3.
- Improve our understanding of the mechanisms mediating protection against *P. falciparum* MPES in humans.
- Investigate the potential of 10 novel MPES genes so as to choose which deserve further characterization and immunogenicity studies.

Activities

- Characterization of the immune responses in humans exposed in the field, in mice of various haplotypes, in Aotus monkeys and in chimpanzees, to the four lead molecules SALSA, STARP, LSA1 and LSA3, in terms of B, T-helper, and CTL responses. Novel techniques were developed to further this analysis, e.g. a Class-I restricted Elispot technique.
- Development of a novel assay aimed at assessing the homing in the liver of the responding cells, by comparison with peripheral blood, spleen or lymph-nodes cells.
- Development of a very large range of immunization methods, e.g. with the lead molecule LSA3: 12 pro-caryotic recombinants were prepared, cloned in various vectors, l-gt 11, pGEX, 4 different pTcr-His vectors, 4 different naked-DNA vectors, pHIL for expression in the yeast Pichia, 3 different attenuated vaccinia virus and a large series of synthetic peptides, numerous nanomers, many semi-large peptides (24 to 27 AA), four lipopeptides including a formulation prepared in sub-GMP conditions, and more recently, 5 very large synthetic peptides (120 to 180 AA). These formulations were used with an extremely large range of adjuvants, e.g. : Montanide ISA 51, QS 21, Titermax, AFI, AFC, Alum, MPLA, lipopeptide incorporated with MPLA in liposomes, microparticles of various sizes and biodegradable microparticles, SmithKline-licensed new adjuvants, etc.
- 8 of the 10 novel MPES genes planned to be studied have been subcloned in His-taged and DNA vectors, and partially sequenced.
- A molecule homologous to the *P. falciparum* LSA3 gene has been identified in the rodent parasite *P. yoelii*, with which it shares at least 3 B and 2 Th epitopes.
- *In vitro* investigations with *P. falciparum* and *P. yoelii* were performed with primary culture of hepatocytes. A new hepatoma line was isolated, and its susceptibility to invasion and growth by human and rodent plasmodia was investigated.
- In replacement of the previous Thamnonys colony developed, which was destroyed by a viral infection, a new colony of the original host of *P. berghei*, the Grammomys, has been established and is reproducing at fast speed (ca. 400 animals available today). Many immunological investigations and protection studies were carried on in parallel in this natural host, as compared to laboratory rodents.

Selected publications

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Druilhe P. and Perignon J.L. 1996. Vaccins antipaludiques: une aussi longue attente. Infectiologie et Immunologie. **3(2):** 43-53.

Bossus M., BenMohamed L., Londono A., Barbier B., Tartar A., Druilhe P. and Gras-MasseE H. 1996. Improved detection of human antibodies to a *Plasmodium* antigen using a peptide modified with Aib residues.. J. Pept. Sci. **2:** 1-7.

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Contract number: IC18*CT950017

ISOLATION, CHARACTERISATION AND IMMUNOLOGICAL EVALUATION OF RECOMBINANT VACCINES FOR FILARIAL PARASITES

Period: January 1996 to December 1998

Co-ordinator: Salford University, Salford, United Kingdom (Janette E. Bradley)

Objectives

- Development of candidate vaccines against parasitic filarial infections, in particular *Onchocerca volvulus*;
- Identification, isolation and expression of antigens specific to the larval stages of these parasites;
- Analyse of the immune responses of humans exposed to *O. volvulus* and animals infected with *Acanthoche lonema viteae* to these antigens;
- Assess the protective capacity of these antigens in a rodent filarial model system.

Activities

- Production of quantities of L3 and L4 larval stages of the parasites *Onchocerca volvulus* and *Acanthocheilonema viteae*;
- Identification and characterisation of antigens that are specific to the larval stages of the parasite by protein analysis and differential display PCR;
- Cloning and Expression of stage specific antigens from cDNA libraries of each larval stage;
- Characterisation of the cellular and humoral responses to the larval antigens of putatively immune humans exposed to onchocerciasis transmisson;
- Evaluation of the protective capacity of the recombinant larval antigens in a rodent filarial model system.

Expected outcome

⇒ The identification of novel candidate vaccine antigens for onchocerciasis;
⇒ The identification of the type of immune responses that are protectiveagainst filarial infections in humans and animal models.

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Contract number: IC18*CT950020

A CONCERTED EUROPEAN APPROACH TOWARDS THE DEVELOPMENT OF MALARIA VACCINES

Period: January 1996 to June 1998

Co-ordinator: Statens Serum Institut, Copenhagen, Denmark (Søren Jepsen)

Objectives

- Promote a coherent approach of the development of malaria vaccines. This requires focused interactions between vaccine industrialists and scientists in Europe and developing countries, concerned with malaria antigens and with the wider fields that underpin malaria vaccine development.
- Identify and exploit existing structures and resources to support malaria vaccine development and to create forums for the regular exchange of information on planned work, progress and results germane to malaria vaccines.
- Provide a channel for expert advice on malaria vaccine research and development to the European Commission, as well as to other national and international authorities.
- Develop partnerships amongst academia, the Public Sector Vaccine Institutes and the European Vaccine Enterprises and to promote interaction amongst those engaged in malaria vaccine research and development in Europe and elsewhere.

Activities

- The primary core activity of this concerted action will be a series of expert meetings addressing different aspects of malaria vaccine development. These meetings will bring together groups of the INCO-DC (and former STD3) contract holders, other experts in the fields which underpin the science base of a malaria vaccine, and representatives of vaccine manufacturers. The industries and public sector institutes invited to specific meetings include: SmithKline Beecham (Belgium), Pasteur Merieux (France), Chi-ron/ BIOCINE (Italy), Hoffman LaRoche (Switzerland), Swiss Vaccine and Serum Institute (Switzerland), Statens Seruminstitut (Denmark) and RIVM (The Netherlands). Liaison with the European Vaccine Manufactures (EVM) is ensured;
- Assistance tools will be updated and made operational: The Malaria Antigen Database (with WHO/TDR, USAID and NIAID), The compendium of *in vitro* and smaller animal models (with COST/STD and EVM), the PVEN document (with PVEN) and the "Atlas" and guidelines for field trials (with AMVTN).

Expected outcome

Vaccines are the most cost-effective approach to control of transmissible diseases. The benefit of this CA is in its role in expediting and rationalising progress towards malaria vaccine development and production. Continual discussions with representatives from the malaria endemic countries will benefit the process of malaria vaccine development. Developing countries will benefit from the proposed action, and also from the networks (PVEN and

AMVTN) which will enhance the influence of the Developing Country partners on the process of vaccine development.

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Contract number: IC18*CT950021

PRE-CLINICAL STUDY OF THE IMMUNOGENICITY OF MSP3 AND GLURP. TWO *P.FALCIPARUM* ANTIGENS TARGETED BY PROTECTIVE ANTIBODIES

Period: January 1996 to December 1998

Co-ordinator: Statens Serum Institut, Copenhagen, Denmark (Søren Jepsen)

Objectives

- Optimize the immunogenicity of MSP3 and GLURP by using several antigen-presentation systems in
  - mice,
  - saimiri,
  - aotus,
  - chimpanzee.
- Characterize the antibodies (fine epitope specificity, isotype) induced by the various protocols, and analyze their biological effect in defence mechanisms,
  - *in vitro*, invasion and ADCI,
  - *in vivo* by passive transfer in the humanized SCID mouse and in
  - primate. compare the results with *P. falciparum* challenge experiments in the primates immunized with MSP3 and GLURP. Complete the characterization of the B and T cell epitopes from MSP3 and GLURP by epidemiological field studies.

Activities

- Immunizing mice with one lipopeptide, and one recombinant from MSP3 and one peptide and one recombinant derived from GLURP with the different adjuvants. The titer, and isotype as well as the ADCI effect of the antibodies obtained will be determined,
- Aotus will be BCG primed, immunized with PPD-coupled GLURP and MSP3 and subsequently challenged with *P. falciparum*;
- ADCI experiments will be conducted in SCID mice harbouring live *P. falciparum* and human monocytes by passively transferring total IgG from hyperimmune individuals to confirm the model. Anti-R0, anti-R2, and anti-MSP3 and other antibodies will subsequently be analyzed in the SCID model;
- Epidemiological studies with MSP3b and R0, and R2 in Dielmo;
- Mice will be immunized with new peptides and recombinant proteins derived from MSP3 and GLURP with the best performing adjuvant. The titer, and isotype and ADCI effect of the antibodies induced by new constructs will be determined;
- Immunizing Aotus and Saimiri with the GLURP and MSP3 vaccine formulation that induced antibodies which prove to be efficient in ADCI. The humoral and cellular immune responses of the monkeys will be analyzed;
- The antibody reactivity to epitopes identified in MSP3 clone 256 and 256B and peptides derived from GLURP will be analyzed in the population of Dielmo;
- Immunizing Aotus and Saimiri with antigens provided that they are superior to the initial constructs. The immunogenicity of the best performing MSP3 and GLURP antigen formulation will be determined in Chimpanzees.

Expected outcome

The optimal conditions for inducing antibodies against MSP3 and GLURP will be determined and functional protection assays established. Improved understand of the critical epitopes involved in the production of protective antibodies and cross-reactivity to MSP3 and GLURP.

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Contract number IC18*CT950022

Period: January 1996 to December 1998

THE APPLICATION OF TRANSFECTION TECHNOLOGY TO MALARIA VACCINE DEVELOPMENT

Co-ordinator: Rijksuniversiteit Leiden (RUL), Leiden, The Netherlands (A.P. Waters)

Objectives

To develop DNA expression vector systems that will facilitate the introduction of genes encoding proteins with vaccine potential into asexual bloodstages of the rodent malaria parasite, *Plasmodium berghei.* To study the following model genes: Apical membrane antigen (AMA) - 1, erythrocytic vaccine candidate Pbs21 ookinete surface protein, transmission blocking candidate Circumsporozoite (CS) protein, pre-erythrocytic vaccine candidate. To clone and modify these genes in such a way that they can be expressed in recombinant P. *berghei* parasites with a view to analyzing the regulation of their expression and manipulating the immune response to these proteins. To isolate DNA elements (promoters) involved in the control of the stage specific expression of the three P. berghei genes to permit the appropriate expression of recombinant genes upon re-introduction into the parasite. To attempt to knock out these genes in the P. *berghei* parasite genome and demonstrate their immediate biological function and essential nature. To re-introduce modified copies or analogues of the genes into the knockout mutants to 1) restore 2) modulate 3) demonstrate the conserved nature of the function of the encoded Drotein.

Activities

* Appropriate vectors for the expression of genes introduced by genetic transformation into the rodent malaria, P. *berghei,* will be developed based upon the available DHFR/TS selectable marker which donates resistance to the antimalarial drug, pyrimethamine.
* Attempts will be made to develop new selectable markers and vectors for the disruption and modulation of genes.
* The relatively new system for the transfection of malaria parasites will be disseminated throughout the partner groups.
* The technology for the dissection of promoter structure will be developed and dissemin~t~c!

Expected outcome

The study should provide a case example of the utility of transfection technolOgY' as applied to malaria parasites, to investigate the function as well as the immunological and biochemical properties of conserved proteins of malaria parasites that are considered to be candidate components of vaccine formulations. An insight will be gained into the functional structure of stage specific promoters of gene transcription. This can be expected to inClude an identification of those elements which dictate stage and sex specificity and those which direct basal transcription.

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Contract number: IC18*CT960027

Period: October 1996 to September 1998

EARLY EVENTS IN ROTAVIRUS INFECTION: ROLE OF VIRAL PROTEINS ON PARTICLE INTERNALIZATION AND MEMBRANE PERMEABILITY.

Co-ordinator: Institut National de la Recherche Agronomique, Jouy-en-Josas, France (Jean Cohen)

Objectives :

- Identify the domain of VP4 interacting with sialic acids.
- Characterize the mechanism of membrane destabilization and pore formation.
- Determine the viral protein(s) and/or domain(s) responsible for membrane destabilization.
- Study the role of chaperones in folding and oligomerization of ET-associated proteins (VP7, NSP4).
- Elucidate the role of various genes implicated in early events of rotavirus infection.

Expected outcome and results

⇒ Insight in the mechanisms that allows penetration of large nucleoprotein complexes into the cell.
⇒ New strategies for prevention of gastro-intestinal diseases.
⇒ Analysis of the sialic acid binding domain of rotavirus.
⇒ Virus-induced co-entry of the toxin alpha-sarcin.
⇒ Processing of the outer glycoprotein VP7.
⇒ Solubilization of membrane vesicles by rotavirus outer proteins.

Selected publications

Charpilienne A., Abad M.J., Michelangeli F., Alvarado F., Vasseur M., Cohen J. and Ruiz M.C. 1997. Solubilized and cleaved VP7, the outer glycoprotein of rotavirus, induces permeabilization of cell membrane vesicles. J. Gen. Virol. **78**: 1367-1371.

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Contract number: IC18*CT960028

Period: February 1998 to July 1998

A NETWORK APPROACH TO RESEARCH ON LEISHMANIASIS IN CENTRAL AMERICA, WITH EMPHASIS ON DRUG SENSITIVITY IN THE FIELD

Co-ordinator: Keele University, Staffordshire, United Kingdom
(R. Ward/R. Maingon/D. Nimmo)

Objectives

- Obtain information on the nature and mechanisms of drug sensitivity in the field, using a controlled prospective population-based study
- Improve diagnosis and parasite/vector identification in Central America, using traditional and new molecular methods.
- Improve human resources with expertise on leishmaniasis, using a network approach.

Activities

* The project focused upon an examination of drug sensitivity to glucantime by the *Leishmania* species circulating in Guatemala and other American countries.
* Genetic analysis of P-glycoprotein genes in Granada (Spain) was completed by work at Keele (United Kingdom) using broader molecular approaches such as differential display.
* In Nicaragua and Panama, PCR methods were applied to examine for post-treatment parasite persistence.
* Studies in Honduras and El Salvador focused upon isolation of new strains of *L. chagasi* from typical and atypical clinical cases along with preliminary studies on transmission dynamics in the San Juan Bautista and Choluteca areas.

Results

⇒ In Honduras, entomological field trips to San Juan Bautista led to isolation of a single strain of *L. chagasi* along with 12 new strains from non-ulcerated cutaneous human cases. A further six visceral isolates and eight cutaneous isolates have been made from other localities. These have been typed in Nicaragua. Three meetings between the Honduran/Nicaraguan and Guatemalan participants have taken place locally. Local health personnel training continues, and over 245 cutaneous cases in Honduras have been successfully treated. In Spain, it has been shown that resistant *Leishmania* has reduced membrane permeability. Studies continue on the involvement of thiol metabolism in resistance. In resistant strains, the γ-glutamyl cysteine synthetase and ornithine decarboxylase genes are over-expressed. In collaboration with Keele, RAPD studies on wild type and resistant *tropica* have been carried out, and fragments of over-expressed genes have been isolated, subcloned, and sequenced. No significant homology with known gene-coding sequences has been found.

⇒ Differential display (DD) has been applied to a variety of *Leishmania* including resistant strains, and has detected several bands of interest. Sequencing work took place in Granada. Glucantime-resistant clones of *L. mexicana* and *L. braziliensis* have been prepared, and RNA extraction and DD analysis have begun.

Selected publications

Arana FF, Pérez-Victoria J.M., Repetto J.M., Morello Y., Castanys S., and Gamarro F. In press. Involvement of thiol metabolism in the resistance to glucantime in *Leishmania tropica*. Biochemical Pharmacology.

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Contract number: IC18*CT960032

SOUTH AMERICAN BITES AND STINGS PROGRAMME

Period: August 1996 to January 2000

Co-ordinator: Liverpool School of Tropical Medicine, Liverpool, United Kingdom
(Robert D.G Theakston)

Objectives

- Investigate and improve therapy of bites and stings in different areas of Brazil and Ecuador by clinical testing of antivenoms;
- Investigate the extent of the problem of bites and stings in the above areas;
- Investigate the efficacy of plant extracts as possible alternatives to conventional antivenoms;
- Develop enzymes immunoassay as a tool for epidemiological studies, rapid immunodiagnosis and for studying the kinetics of envenoming and therapy.

Methodology and Activities

* Preclinical experimental assessment of antivenoms before clinical studies (Liverpool);
* Randomized clinical trials of antivenoms using clinical and laboratory methods (Belem, Uberlandia, Sao Paulo-Brazil, Shell Pastaza-Ecuador, Oxford-UK);
* Laboratory tests on the pathological effects of venoms;
* Isolation, purification and study of venom components;
* Taxonomic evaluation of medically-important snake species and associated study of venoms from these (Sao Paulo-Brazil, Bangor-UK);
* Epidemiological survey studies in Amazonian Ecuador and Brazil to establish the true extent of bites and stings in these areas (Sao Paulo and Belem-Brazil, Pastaza, Shell-Ecuador);
* Enzyme-linked immunosorbent assay is being used as a tool for examining the kinetics of envenoming and therapy (Liverpool, Paris);
* Isolates from various plants are being prepared in Hannover and tested for antivenom activity in Liverpool;
* Affinity purification of specific venom antigens is being used to increase the specificity and decrease the time taken for immunodiagnosis (Liverpool, Paris).

Expected outcome

⇒ The optimum antivenoms and antivenom doses will be determined following preclinical tests and possibly clinical trials for use in central and Amazonian Brazil and Amazonian Ecuador;

⇒ Epidemiological studies will result in clarification of the extent of the health problems caused by bites and stings in Brazil and Ecuador;

⇒ The purification and testing of venom components will help in the possible development of novel drugs.

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Contract number: IC18*CT960033

Period: October 1996 to March 2000

MAGNESIUM SULPHATE FOR TREATMENT OF PRE-ECLAMPSIA: A TRIAL TO EVALUATE THE EFFECTS ON WOMEN AND THEIR BABIES (THE MAGPIE TRIAL)

Co-ordinator: Institute of Health Sciences, Oxford, United Kingdom
(Lelia Duley)

Objectives

- Estimate the overall effectiveness and safety for women and their babies, of magnesium sulphate when administered within the existing health services, for women with pre-eclampsia.
- Contribute to *The Cochrane Library* by preparing and maintaining systematic reviews of the care of women with pre-eclampsia, and by ensuring that implications for practice within developing countries are discussed for these reviews.
- Enhance and strengthen existing collaborative networks within developing countries, and increase the capacity to conduct high-quality primary and secondary research, and implement appropriate evidence into practice.

Expected outcome

⇒ The Magpie Trial will provide reliable evidence of direct policy relevance about the effectiveness and safety of magnesium sulphate when used for pre-eclampsia.

⇒ If appropriate, these results may enable an economic evaluation and long term follow up of the children.

⇒ Strengthening of the capacity to design and conduct multicentre trials within developing countries.

⇒

Selected publications

Duley L, Gülmezoglu AM, Henderson-Smart D. 1998. Anticonvulsants for women with pre-eclampsia (Cochrane Review). In: *The Cochrane Library*. Issue 1,. Oxford: Update Software.

Atallah A. 1997. Commentaries on anticonvulsant use for pre-eclampsia and eclampsia. In: *The Reproductive Health Library*. No 1. Oxford: Update Software.

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Contract number: IC18*CT960042

Period: December 1996 to November 1998

POPULATION GENETICS AND CONTROL OF *TRIATOMA BRASILIENSIS* IN NORTHEAST BRAZIL

Co-ordinator: London School of Hygiene and Tropical Medicine, London, United Kingdom
(C.J. Schofield)

Objectives

This study, initiated at the request of the Ministry of Health of Brazil, seeks to improve knowledge of the biology and population genetics of *Triatoma brasiliensis* in support of surveillance and control activities in northeastern Brazil where *T.brasiliensis* is the main vector of Chagas disease. The aim is to assess population growth rates and dispersal parameters in relation to recolonisation of treated communities and to provide markers useful for identifying the origin of new infestations.

Activities

Following preliminary work to characterise the general distribution and habitats of *T.brasiliensis* and to establish baseline-population genetic parameters for this species by using morphometric and biochemical techniques, a field trial was set up in the state of Ceara, whereby some 300 houses and their peridomestic dependencies were treated with deltamethrin to eliminate the bug populations. Over the following year, reinfestations have been carefully monitored and the reinfestant populations have now been collected for genetic comparison with the original domestic, peridomestic and sylvatic populations.

Results

Analysis to date has provided morphometric and biochemical characterisation of *T.brasiliensis* and of related species found in the same regions. Ecological associations have also been characterised, along with a careful evaluation of operational procedures used by the vector-control services. The trial results, following improved operational procedures already show reduced rates of reinfestation compared to previous interventions. Domestic reinfestation has been below 5 % (from a pre-intervention infestation rate of around 20 %, although infestation of peridomestic habitats has remained at about two-thirds of the pre-intervention levels. Direct studies on population dispersal mechanisms suggest that dispersal is primarily in association with vertebrates rather than by flight of the adult bugs, and a possible new enzyme marker for flight capacity is now subject to further study. Initial interpretation of the trial data suggest that the current control interventions are satisfactory in the domestic habitats but less so in peridomestic habitats, and that reinfestation is due primarily to control failures in peridomestic habitats rather than to invasion by syilvatic populations - although confirmation of this interpretation awaits final biochemical comparisons between the populations.

Follow up

This project is nearing completion, and preliminary recommendations for revised control and surveillance procedures are being drawn up for presentation to the Ministry of Health. The results and recommendations will be discussed and finalised during a technical workshop with Ministry of Health personnel, scheduled for October 1998.

Selected publications

Borges E.C., Barbosa S.E., Faria Filho O.F., Carneiro F.F., Schofield C.J., Diotaiuti L., 1997. Estudos morfometricos de populaçoes de Triatoma brasiliensis (Neiva, 1911). (asbstract) XXXIII Congreso da Sociedade Basileira de Medicina Tropical, Belo Horizonte, p.157.

Laranja L.S., Soares R.P.P., Diotaiuti L., 1997. Biology of Triatoma brasiliensis Neiva, 1911 under laboratory conditions - life cycle and feeding aspects. (abstract) XXIV Annual meeting on basic research in Chagas disease, Memorias do Instituto Oswaldo Cruz 92 (suppl.1) p.280.

Margonari C., Pires H.H.R., Faria Filho O.F., Carneiro F.F., Diotaiuti L., 1997. Comparaçao da morfología da genitalia masculina de duas populaçoes de Triatoma brasiliensis (abstract) XXXIII Congreso da Sociedade Basileira de Medicina Tropical, Belo Horizonte, p. 156.

Soares R.P.P. Dujardin J.P., Schofield C.J., Diotaiuti L., 1997. Capacidade de vo das pricipais especies vetoras da doença de Chagas no Brasil (Hemiptera, Reduviidae, Triatominae). XXVII Congreso Brasileira de Entomología, Salvador, p. 289.

Soaeres R.P.P., Romanha A.J., Santoro M.M., Dujardin J.P., Schofield C.J., Diotaiuti L., 1997. x-glycerophosphate dehydrogenase activity correlates with flight muscle activity in triatomine bugs. (abstract) XXIV Annual meeting on basic research in Chagas disease) Memorias do Instituto Oswaldo Cruz 92 (suppl. 1) p. 295.

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REGULATION OF DEVELOPMENT IN MALARIA PARASITES

Period: January 1997 to December 1999

Co-ordinator: University of Leiden, Leiden, The Netherlands (Christofel J. Janse)

Objectives

- Investigation of the regulation of parasite development through studies on 1) gene promotor structure, strength and stage specificity and on 2) function of the proteins encoded by the genes under the control of these promoters;
- The genes studied are: *P. falciparum*, Glycophorin binding protein (GBP) 130, Na+/H+ transport protein; from *P. berghei*, PBS21, pbB7, rRNA units (A-D), 150 family, crk2, EF-1a; from *P. vivax*, crk2, EF-1a, rRNA;
- Development of plasmid systems based upon the *Tet* repressor which will allow the inducible expression of cloned genes in different species of *Plasmodium*;
- Where feasible, develop resources to facilitate the isolation and characterisation of genes encoding proteins with a specific role in development during gametocytogenesis and throughout the mosquito phase of the life cycle in *P. vivax* and *P. berghei.*

Activities

- Where appropriate, clone and complete the full characterisation of the named genes, their sequence, expression and comparative structures;
- Study the structure and function of promoters of transcription of RNA polymerases I and II in both *P. berghei* and *P. falciparum* using stable and transient transfection technologies;
- Investigate the species specificity of promoter structure through an investigation of the ability of defined promoter regions of genes isolated from *P. vivax* to accurately control transcription in *P. berghei*;
- Develop plasmid systems based upon the *Tet* repressor which will allow the inducible expression of cloned genes in different species of *Plasmodium*;
- Initiate a study of structure/function relationships of specific parasite structures through gene mutagenesis, replacement, over-expression and/or knock out to establish the role of the protein;
- Study the control of gene expression in *P. berghei* at the post-transcriptional level in female gametocytes using the Pbs21 (female gametocyte specific), 150 gene family (variant 3' UTR) and pbB7 (nuclear protein gene) as paradigms;
- Where feasible develop resources to facilitate the isolation and characterisation of genes encoding proteins with a specific role in development during gamecytogenesis and throughout the mosquito phase of the life cycle in *P. vivax* and *P. berghei*;
- Continue to expand the investigation of the relationship between genome organisation and sexual development.

Expected outcome

⇒ The programme should provide a functional analysis of the genes under study and thus provide insights into their role during the complex development cycle of *Plasmodium*;

⇒ An insight will be gained into the functional structure of stage specific promoters of gene transcription. This can be expected to include an identification of those elements which dictate stage- and sex-specificity and those which direct basal transcription;

⇒ The study will provide reagents and materials, which are essential for further studies on the sexual development of *Plasmodium*.

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Contract number: IC18*CT960056

PHOSPHOLIPID METABOLISM, A NOVEL TARGET FOR ANTIMALARIAL DRUGS: DEVELOPMENT OF THE PHARMACOLOGICAL MODEL

Period: October 1996 to October 1999

Co-ordinator: Université Montpellier II, Montpellier, France (Henri Vial)

Objectives

The main objective of the project is the development of new antimalarial drugs that interact with the malarial parasite phospholipid metabolism and could provide a solution to *P. falciparum* polychemoresistant malaria. Although there is no indication of potential resistance to date, we believe that we must actively initiate studies concerning mechanisms that could be involved in potential acquisition of resistance to the effectors. This molecular approach is worth immediate investigation since precise mechanisms can be expected from the suspected drug site. Results could lead to the knowledge of a whole set of metabolic pathways vital for parasite growth.

- Synthesize new series of original and potentially alternative compounds aimed at improving tolerance and oral absorption. Identification, isolation and characterization of the pharmacological target.
- Carry out thorough antimalarial activity studies including chemosensitivity, therapeutic index after *in vivo* oral formulations in *P. falciparum*-infected monkeys, and against others stages (non erythrocytic) or species (e.g. *P. vivax*). To describe the pharmacokinetic properties and toxic evaluation of lead compounds will also be studied. Experimental induction of resistances, characterisation of effector-resistant *P. falciparum* malaria and alternative to resistance. In case of resistance, combinations with other current approaches would be studied.

Activities

- Chemical synthesis of compounds aimed at improving tolerance and oral absorption. This includes compounds with new cationic heads that could be used if the current lead compounds may prove to have unacceptable drawbacks, and also the synthesis of prodrugs with the aim of improving oral absorption and, eventually to promote the development a new generation of effectors;
- Identification and characterisation of the pharmacological target. Affinity chromatography using a column of immobilized effectors is proposed. If necessary, chemists will also synthesise photoreactive lead compound derivatives. Pharmacological target cloning should allow its complete characterisation, determination of the active site and help in the design of new effectors;
- It is of utmost interest to study the mechanisms of regulation of PL biosynthesis pathways in Plasmodium. More than just a problem of metabolic regulation, this program concerns mechanisms which could be involved in resistance that the parasite could develop when the supply of choline is blocked due to pharmacological interference. Biochemical and genetical approaches will be used as a powerful tool for the elucidation of metabolic regulations as well as the biological significance of the different metabolic pathways in Plasmodium. We will be particularly concerned by the metabolites and activities of CDP-

choline pathway which synthesizes de novo PC. Additionally, we will focus on PS Decarboxylase activity which also provides Plasmodium for an important part of PC;

- Antimalarial activity, pharmacokinetics and toxic evaluation of lead compounds. The first priority tasks will be (1) in vitro and in vivo evaluation of antimalarial activity against *P. falciparum* blood stages. (2) 4 to 5 lead compounds will be tested in the *P. falciparum* / SCID mouse model , (3) therapeutic index of various formulations (intramuscular and oral modes) of 2-3 compounds in Aotus monkeys infected with P. falciparum. Blood samples will also be collected to perform bio-assays of the seric compounds;
- Activity of 2-3 lead compounds against *P. falciparum* isolates with various degrees of resistance will be determined;
- Evaluate antimalarial activity against *P. vivax/P. cynomolgi* blood stages. (2-4 lead compounds). According to results, to evaluate against *P. cynomolgi* in rhesus monkey and against *P. vivax* in Saimiri monkey for comparison with *P. falciparum*/Aotus results;
- Test the activity of the lead compounds against the non-erythrocytic stages of Plasmodium;
- Determine pharmacokinetics properties of lead compounds (ex vivo tests), and to determine toxicity of lead compounds;
- Resistance mechanism and alternative to resistance. This includes the in vitro induction of resistance against choline analogs, the characterisation of effector-resistant *P. falciparum* malaria (pharmacological target and lipid metabolism), and examination of genes associated with resistance to standard antimalarial drugs. Alternatives to resistance would include combined action of PL metabolism inhibitors with known antimalarial drugs.

Expected outcome

The work content is totally devoted to the establishment of a new pharmacological model. Although the outcome of fundamental and experimental research can never be known in advance, the numerous complementary experimental approaches that are planned within the different partner laboratories, should allow to reach the proposed objectives. It is anticipated that some of our research effort will lead to potential industrial or pharmacological outcomes.
Concerning malaria, by now, the most urgent need concerns a first-line oral substitute to chloroquine. That is one of the reasons we want to achieve an oral formulation of our compounds, rather than risking the development of a non-oral administerable compound.
The target could be a common one between different parasites. However, until now, significant inhibition at concentrations lower that 1 mg/l has not been observed for any other parasites except *Babesia*. On the other hand, the susceptibility of the protozoan parasite *Babesia*, that also invades erythrocytes but is not sensitive to haemoglobin degradation-related lysomotropic agents, is interesting as it confirms the absence of cross-resistance of the PL metabolism pharmacological effectors with the current lysomotropic antimalarial agents.

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Contract number: IC18*CT960058

DEVELOPMENTS REACHED BY THE HEALTH SYSTEM IN EL SALVADOR AND NICARAGUA IN THE POST-WAR PERIOD (1990-1995), FOCUSING ON THE EFFORTS OF CIVIL SOCIETY

Period: February 1997 to January 2000

Co-ordinator: Universidad Nacional Autónoma de Nicaragua, Managua, Nicaragua
(Gladys Ricarte Gutierrez)

In Nicaragua and El Salvador the period 1990-1995 can be characterised as a post-war period in which structural adjustment programs were implemented. State reform and health sector reform were central elements of these programmes, but their impact on the health status of the population and the health service organisation has not been analysed. Furthermore the health sector reform of the state was complemented by a series of local initiatives in civil society, to respond to the growing needs of the population.

Objectives

The general objective of this research project is to analyse the stage of development reached by the health systems in El Salvador and Nicaragua in the post-war period (1990-1995), focusing on the efforts of civilan society.

Activities

- An inventory of these local health initiatives will be made and systematised;
- These initiatives will be placed in an overall analysis of the specificity of health sector development in these two countries;
- The possibilities of a multiplication of the best experiences will be examined on the basis of a detailed evaluation of these experiences.

This evaluation of local experiences in health sector development by different actors of civil society will focus on their effectiveness, their sustainability, the degree of participation by the population, and their capacity to enhance the autonomy and equity in health services delivery. Special emphasis will be put on the relationship between the public service and the non-profit private sector, their complementarity and conflicts during this complex period of post-war reorientation and structural adjustment.

Expected outcome

This research project is expected to develop guidelines to improve the impact of local initiatives in the health sector. An exchange of successful experiences will be organised within civil society and between civil society and the public sector. At the health policy level, elements for the incorporation of local initiatives in the national health policy will be identified.

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Contract number: IC18*CT960060

A MOUSE MODEL FOR LATENT TUBERCULOSIS AND PREVENTION OF REACTIVATION OF THE DISEASE

Period: December 1996 to November 1999

Co-ordinator: University of Bergen, Bergen, Norway (Gunnar Bjune)

Objectives

- Identify the sites where dormant bacilli survive, whether their metabolism is different from that of actively growing bacilli and whether they express different antigens;
- Study the nature of the immune response that maintains the latent state and differences between the immune response in men and mice and in latent and progressive tuberculosis;
- Define the immunological and endocrine factors which induce reactivation.

Activities

* Establish a mouse model for asymptomatic lifelong infection with stable bacillary counts to study histopathology and number of bacilli throughout lungs, spleen, liver and bone narrow;
* Study antigen expression in actively dividing bacilli and in dormant bacilli through immune response to specific antigens, purification and characterisation and identification of gene activation;
* Study T-cell subsets and cytokines in various stages of tuberculosis infection in mice and men;
* Follow the antigen specificity of T-cell responses and antibodies throughout infection in men and mice;
* Study the importance of hormones, growth factors and non peptide biological active components from Mycobacterium tuberculosis in latency and reactivation of the disease.

Expected outcome

⇒ Relevant mouse model for latent tuberculosis and reactivation of the disease in man. Knowledge of what antigens and biological mechanisms which induce keep-up and terminate latency and which are involved in reactivation of the disease;

⇒ Strengthen research capability and training in two DC laboratories in tuberculosis high endemic countries;

⇒ Create a co-operative basis for tuberculosis research and new vaccine development.

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Contract number: IC18*CT960061

Period: January 1997 to December 1999

DETECTION AND CHARACTERIZATION OF PATHOGENIC *ENTAMOEBA HISTOLYTICA*

Co-ordinator: Centro de Investigaciones en Microbiología y Parasitología Tropical, Santa Fé de Bogotá, Bogotá, Colombia (Felipe Guhl)

Objectives

- Distinguish and assess the frequency of *Entamoeba dispar* (invasive) and *Entamoeba dispar* (non invasive) infections in endemic areas in Latin America and in travellers returning from the tropics to non-endemic areas in Europe
- Evaluate the assay performance of PCR-SHELA as a tool for the specific and rapid differential diagnosis of *E. histolytica* from *E. dispar* infections and for the diagnosis by exclusion of bacterial dysenteric syndromes, inflammatory bowel disease and carcinoma.
- Study the relationship between genetic markers in clinical isolates of *E. histolytica* (strain variability) and the degree of virulence according to the features of invasive disease and response to treatment.
- Establish the prevalence of asymptomatic carriers of *E. histolytica* in endemic and non endemic areas, and the risk of those carriers and their contacts to develop invasive disease.
- Establish the prevalence of E. Histolytica and *E. dispar* mixed infections in the populations studied.

Results

⇒ DNA obtained from 436 samples (including 106 faeces containing no cysts or cysts of other amoebas than *E. histolytica* or *E. dispar*) and cultures, was tested by PCR-SHELA. Overall positive results on PCR-SHELA revealed (12.7%) *E. histolytica*-positive samples and 87.3% *E. dispar* carriers

⇒ The evaluation of PCR-SHELA directly in faeces against microscopy was carried out by the collaborators at the laboratory of Parasitology at Leiden. The same samples were used to evaluate the CELISA (CELLABS, 1996).

⇒ The same team compared PCR-SHELA results of DNA extracted from faeces and the corresponding culture of 31 *E. histolytica/E. dispar* cyst positive samples, against other PCR systems. PCR-SHELA correctly identified 9 *E. histolytica* and 21 *E. dispar* strains, in full agreement with the hexokinase results. Hereunder are the percentages of infection among microscopically positive samples, according to countries of origin:

Country	E. histolytica	E. dispar
Netherlands	12	88
Spain	15	85
Colombia [1]	22	78
Mexico	50	50

[1] Isolates from Colombia, Venezuela and Peru, with zymodemes I, II, IX, X, XV, XVI, and XX

Whether those results reflect a particular difference in the transmission dynamics in Mexico remains to be assessed during the follow-up program and using all the PCR systems contemplated in the project (RAPD, SSG, r SSU DNA, and RFLP analyses).

Publications

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ANALYSIS OF VAR GENES FROM *P. VIVAX* AND *P. FALCIPARUM*

Period: January 1997 to June 1999

Co-ordinator: Zentrum für Infektionsforschung, Universität Würzburg, Würzburg, Germany
(M. Lanzer)

Objectives

- Determine the genomic organization of *var* genes in *P. falciparum.*
- Examine the mechanism of differential *var* gene expression in *P. falciparum.*
- Examine the adhesive phenotypes of specific *var* gene variants.
- Verify the existence of *var* gene homologous genes in *P. vivax.*

Activities

- Production of a *P. vivax* YAC library.
- Assortment of *P. vivax* YAC clones into chromosomal contig maps.
- Exploration of *P. vivax* var genes homologues in *P. vivax.*
- Production of a 3D7 clone tree, and cloning of expressed *var* genes.
- Mapping a rosetting locus in *P. falciparum.*
- Genomic analysis of *var* genes.
- Mapping the chromosomal location of silent and expressed *var* gene variants.
- Analysis of *var* genes in field samples (*P. vivax* and *P. falciparum*).

Expected outcome

⇒ The study will provide a better understanding of the mechanisms responsible for differential *var* gene expression and the pathological consequences *var* genes cause.

⇒ The study will provide tools, such as YAC libraries and chromosomal contig maps, that will be useful in other areas of malaria research.

Results

- A *P. vivax* YAC library as well as a representative cDNA library have been generated and the data published.
- First evidence of *var* gene homologous in *P. vivax* have been found.
- The chromosomal position of all *var* gene variants present in the genomes of the *P. falciparum* clones Dd2 and 3D7 have been mapped and tagged:
  - It was found that *var* genes are expressed *in situ*, irrespective of their chromosomal location and independent of conserved expression sites.
  - A 3D7 clone tree has been produced.

- It was found that particular *var* gene variants mediate defined adhesive phenotypes.
- The collection of specific *var* gene tags from geographically dispersed *P. falciparum* field isolates has begun.

Publications

Camargo A.A., Fischer K. and Lanzer M. 1997. Construction and rapid screening of a representative YAC library from the *P. falciparum* strain Dd2. Parasitol. Res. **83**, 87-89.

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Period: January 1997 to June 1999

PLASMODIAL CHROMATIN: STRUCTURE AND FUNCTION

Co-ordinator: Universität Würzburg, Würzburg, Germany (M. Lanzer)

Objectives

- Identify a *P. falciparum* centromere sequence
- Analyze spatial and temporal chromatin changes and their effect on transcriptional activity in *P. falciparum*
- Develop an *in vitro* plasmodial telomerase assay
- Identify and clone the *P. falciparum* telomerase including its RNA subunit
- Characterize subtelomeric domains in *P. vivax*
- Identify and clone non-histone nuclear proteins
- Identify origins of replication

Expected outcome

⇒ Characterization of those structural aspects of plasmodial chromatin that are relevant to the multiple functions in which chromatin is involved, such as transcription, replication, segregation, chromosomal stability vs. (possibly programmed) rearrangements.

⇒ Identification ofnew targets for rational drug design.

Results

⇒ An *in vitro* plasmodial telomerase assay has been successfully established. It was shown that this enzyme adds *de novo* telomere repeat sequences onto single stranded DNA molecules provided these primers contain a G-rich cassette upstream of the 3' terminus.

⇒ We have found that the *P. falciparum* telomerase is responsible for healing broken chromosome ends, thereby stabilizing the truncated chromosome arm. Oligonucleotides resembling known chromosome breakage site are used as substrates by the *P. falciparum* telomerase.

⇒ It was found that nucleotide analogues, such as ddGTP, are effective inhibitors of *P. falciparum* telomerase activity *in vitro*. This finding identifies the *P. falciparum* telomerase as a new drug target.

⇒ As a prerequisite to the identification of plasmodial origins of replication and centromere sequences, transfection of malarial parasites has been successfully established in all the partner laboratories

⇒ We have found that stage specific promoters loose their developmental restriction when episomally located, a phenomenon correlated with improper chromatin assembly onto the plasmid.

⇒ A factor has been identified and cloned that may play a role in chromatin assembly in *P. berghei.*

⇒ Initial data suggest that the chromatin density and possibly structure varies during the parasite's life cycle. Initial data show that chromosomes are looser packed in gametocytes

than in asexual intraerythrocytic stages. This may be a reflection of the high transcription activity of gametocytes.

⇒ Subtelomeric regions from *P. vivax* chromosome have been cloned as artificial chromosomes in yeast. A comparative restriction analysis suggests a structure of *P. vivax* chromosome ends different from that of *P. falciparum.*

Publications

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Contract number: IC18*CT960074

Period: November 1996 to October 1999

DEVELOPMENT OF NOVEL DRUGS AGAINST MALARIA

Co-ordinator: Rigshospitalet, Copenhagen, Denmark (A. Kharazami)

Objectives

The objective of this research is to identify the most effective oxygenated chalcone as a potential candidate for a drug against malaria. The project is divided into the following sections:

- **Medicinal chemistry and quantitative structure-activity (QSAR) studies:**
  The methods for the synthesis of chalcones have been developed and a large number of compounds have been synthesized. The QSAR model has been developed. The model points to certain structures which can improve the potency of some of the compounds against malaria parasites.
- ***In vitro* activity studies using different strains of *Plasmodium falciparum*.**
  A large number of chalcones exhibited potent *in vitro* activity at a range of 5-10 μg/ml against both chloroquine sensitive and chloroquine resistant strains of human malaria parasite *P. falciparum.*
- **Pharmaceutical preformulation studies.**
  These activities have included physico-chemical characterization, preliminary formulation, pharmaceutical formulations for *in vivo* studies and development of HPLC analysis methods for selected chalcones. A number of preliminary formulations have been developed. These include oil solution, micronised suspension, cubic phase dispersion and self emulsifying oil formulation. These formulations are being tested for oral bio-availibility and efficacy studies.
- ***In vivo* activity studies in murine (*P. berghei*) and monkey (*P. falciparum*) models.**
  — ***Murine studies***. Several chalcones protected mice from the lethal infection of P. berghei. This protection was achieved by all the three routes of oral, peritoneal and subcutaneous administration.
  — ***Monkey studies.*** The studies in Aotus monkeys have been carried out in Cali, Colombia. Preliminary studies have been carried out on 3 compounds in the monkeys infected with *P. falciparum.* The tested compounds were able to reduce parasitemia in the treated monkeys but none of them were able to protect the animals from the infection. Studies on other compounds have been planned.
- **Studies on the mechanism of action of chalcones.** The studies on the mechanism of action include the mitochondrial ultrastructur, the parasite respiration and some of the enzymes involved in the electron transport chain of the mitochondria. These studies clearly indicate that chalcones interfere with the energy metabolism of the parasite mitochondria.

Publications

Chen M., Christensen S.B., Zhai L., Rasmussen M., Theander T.G., Frøkjær S., Steffansen B., Davidsen J., and Kharazmi A. 1997. The Novel oxygenated chalcone 2,4 dimethyloxy-4'-butoxychalcone, exhibits potent activity against human malaria parasite *Plasmodium falciparum in vitro* and rodnet parasites *Plasmodium berghei* and *Plasmodium yoelii in vivo*. J. Infect. Dis. **176**:1327-33.

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Zhai L., Chen M., Blom J., Brøgger Christensen S., Theander T.G. and Kharazmi A. 1997. Oxygenated chalcones inhibit *Leishmania* parasites by interfering with energy metabolism of the mitochondria. Submitted.

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TS3*CT940263

Period: November 1996 to August 1998

ANALYSIS AND CHARACTERIZATION OF PHOSPHOFRUCTOKINASE AND PYRUVATE KINASE OF *LEISHMANIA*, POTENTIAL TARGETS FOR NEW DRUGS

Co-ordinator: Christian de Duve Institute of Cellular Pathology (ICP), Brussels, Belgium
(P. Michels)

Objectives

- Study the structure and kinetics of phosphofructokinase (PFK) and pyruvate kinase (PYK) of *Leishmania*, key enzymes in the metabolism of the parasite, and determine differences with the corresponding mammalian enzymes.
- Design and synthesize selective inhibitors of the *Leishmania* enzymes, based on their differences with the mammalian enzymes

Activities

- Cloning and sequence determination of the *Leishmania* PFK and PYK genes.
- Overexpression of the *Leishmania* enzymes in bacteria (*Escherichia coli*) or yeast (*Hansenula polymorpha*).
- Purification of the recombinant enzymes.
- Kinetic analysis of the purified enzymes.
- Structure modelling of the *Leishmania* enzymes, using the X-ray coordinates of the crystal structures of homologous enzymes.
- Structure-function analysis of residues potentially important for inhibitor design by site-directed mutagenesis.
- Crystallization trials of recombinant *Leishmania* PFK and PYK.
- Synthesis of potentially selective inhibitors of *Leishmania* PFK and PYK.

Results so far

⇒ *Leishmania* PFK and PYK genes have been cloned and characterized.
⇒ *Leishmania* PFK and PYK have been overexpressed in *Escherichia coli*, purified and kinetically characterized.
⇒ Well-diffracting crystals of *Leishmania* PYK have been obtained and are being used for resolution of the enzyme's three-dimensional structure.
⇒ Fructose analogues have been synthesized that inhibit *Leishmania* PFK.

Follow-up

- Resolution of the three-dimensional structure of *Leishmania* PFK and PYK.
- Design and synthesis of highly selective and potent inhibitors of the *Leishmania* enzymes.
- Use of inhibitors selective for *Leishmania* PFK and PYK for the development of compounds with antiparasitic activity.

Selected publications

Michels et al. 1997. The glycosomal ATP-dependent phosphofructokinase of *Trypanosoma brucei* must have evolved from an ancestral pyrophosphate-dependent enzyme. Eur. J. Biochem., **250**, 698-704.

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Contract number: IC18*CT960084

Period: October 1996 to September 1999

ANTILEISHMANIAL AND ANTITRYPANOSOMAL ACTIVITIES OF ALKYL-LYSOPHOSPHOLOPIDS

Co-ordinator: London School of Hygiene and Tropical Medicine, London, United Kingdom
(S.L. Croft)

Objectives

- Determine the mechanisms of action of alkyllysophospholipids (ALPs) against *Leishmania* and *Trypanosoma cruzi*, including effects on the host immune response.
- Identify novel biochemical and molecular targets in *Leishmania* and *Trypanosoma*.
- Establish ALP-resistant clones and identify mechanisms of resistance.
- Define inter-species and inter-strain variations in sensitivity to ALPs.
- Define a structure-activity relationship of the antileishmanial and antitrypanosomal activities of ALPs as they represent a new selective model for further antiprotozoal drug development.
- Define rational drug combinations to be used in treatment.

Activities

* The activities of ALPS, alone and in combination with other drugs, will be determined against both extracellular and intracellular forms of different strains/species of *Leishmania* and *Trypanosoma*, by microscopical and biochemical techniques.
* The effects of ALPs on membrane pathways, in particular sterol, lipid, and glycosylphosphatidylinositol (GP) anchor biosynthesis, will be studied.
* Effects on parasite differentiation and signal transduction will be examined, in particular in relation to roles of protein kinases, phospholipases, calcium levels, and adenylate cyclase.
* The uptake and distribution of ALPs by parasites and host cells will be measured by isotopic and chromatographic methods.
* Immunomodulating properties of ALPs will be studied in relation to killing of intracellular stages of *Leishmania* and *Trypanosoma cruzi* in macrophages.
* Resistant clones of *Leishmania* and *Trypanosoma cruzi* will be established through the stepwise exposure of extracellular parasites to increasing concentrations of ALPs.

Expected outcome

⇒ An understanding of the mechanisms of activity of a novel group of antiprotozoal drugs and the identification of novel drug targets.
⇒ Data on drug activities and drug combinations useful for clinical studies on Leishmaniasis and Trypanosomiasis.
⇒ PhD students will be trained in laboratories in Europe and South America, and international links will be cemented.
⇒ Results will be published in international journals, presented at international meetings, and

be the central focus of an EC meeting to which representatives of pharmaceutical companies will be invited.

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Contract number: IC18*CT960086

TROPICAL MEDICINE ON TRIAL: PRODUCING RELIABLE REVIEWS, DESIGNING BETTER INTERVENTION STUDIES, AND USING SYSTEMATIC REVIEWS TO INFORM PRACTICE

Period: October 1996 to September 1999

Co-ordinator: Liverpool School of Tropical Medicine, Liverpool, United Kingdom (Paul Garner)

Objectives

- Produce and update reliable systematic reviews of randomised controlled trials in parasitic and tropical diseases, and other conditions relevant to the tropics;
- Develop relevant research questions and trial protocols in parasitic and tropical diseases;
- Develop and evaluate approaches using systematic reviews to improve clinical and public health practice in various professional specialities and regions.

Activities

* Encourage, support and produce protocols to conduct systematic reviews of randomised controlled trials in conditions relevant to the topics;
* Encourage, support and produce completed systematic reviews from these protocols;
* Encourage, support and ensure publication of these reviews on *The Cochrane Library* and in relevant specialist journals;
* Assist individuals to produce good research questions and trial protocols to answer these questions;
* Set up nodal points for networks stimulating the use of evidence to improve clinical and public health practice.

Expected outcome

⇒ Completed systematic review protocols produced within the network and published on *The Cochrane Library*;
⇒ Completed systematic reviews produced within the network and published on *The Cochrane Library* and in journals;
⇒ Full research trial protocols submitted for funding;
⇒ Units will be established within developing countries with a range of dissemination, research and development activities promoting evidence-based health care.

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Contract number: IC18*CT960115

MEASURING AND MONITORING THE PERFORMANCE OF REFORMING HEALTH SYSTEMS

Period: November 1996 to October 1999

Co-ordinator: Institute for Health Sector Development, London, United Kingdom
(Peter Sandiford)

Objectives

- Identify the various forms of utility that are being provided, or that could be offered by health systems;
- Identify the types of utility and social benefits that are not currently being obtained from health systems;
- Document the stated and unstated objectives of health sector reform programmes;
- Contrast the goals (stated and unstated) of health sector reform programmes with the desires and expectations of tax-payers and users of health services;
- Develop quantifiable indicators of each form of health system-derived utility;
- Develop a technique for weighing the indicators of each source of utility such that their sum measures total health system derived utility or benefit;
- Test the value of the techniques as tools for policy formulation, taking as the concrete example options for rationing and prioritising health services, including the establishment of 'basic packages';
- Develop tools that allow funders, purchasers and users to monitor performance of decentralised health district or regions in terms of their full range of social benefits.

Activities

* Ten focus group discussions, two in each of the countries (Mexico, Guatemala, El Salvador, Nicaragua, Costa Rica) interviewing extremely diverse socio-economic groups of different age and sex composition as to the different forms of utility that they currently obtain from the health system in their country, and other forms of utility that they are not currently obtaining, or would like to gain to a greater extent;
* Quantitative surveys in each of the five developing countries to determine the relative importance given to the different forms of health service-derived utility identified through activity 1;
* Document review and semi-structured interview with key informants to determine the stated and unstated aims of health sector reform programmes and their relative priorities;
* Analytical desk-work contrasting the implications of results from activities 2 and 3 in terms of the congruence or incompatibility of government and donor policy objectives for the health sector with the desires of the population as a whole and certain key subgroups within it (the poor, women, ethnic minorities etc.);
* Series of pre-tests and pilot studies to identify a set of objective and subjective indicators which can be used to obtain quantitative measurements of the extent to which the major forms of health system-derived utility are being produced by the health sector. Analysis to assess the consistency and validity of these various indicators;
* Experimentation with trade-off, willingness to pay, standard gamble and other methods for measuring utility, in order to develop a technique which would enable the indicators

developed in activity 5 to be weighted so that their sum provides a valid composite index of total health system-derived utility;

* Application of the utility measurement and weighing techniques developed in activities 5 and 6 to the definition of a 'basic package' of health services which when provided by the public sector would maximise total health system-derived utility. This will be done by in-depth interviews with the 10 different social groups identified in activity 1. The composition of such a package will be compared and contrasted with other existing or proposed packages defined by policy-makers or technicians seeking to maximise health gain;

* Application of the utility measurement and weighing techniques developed in activities 5 and 6 to a quantitative assessment of the aggregate health system-derived utility for a defined population within each of the five developing countries participating in the study. This would entail a population-based survey using a structured questionnaire.

Expected outcome

⇒ A greater understanding of the full range of benefits that health systems can and do produce, the relative importance given to each, and how much peoples' assessment of what is important for a health system to produce varies between different population subgroups;

⇒ An indication of the areas where health systems in developing countries are failing to produce the benefits expected of them by the population, and whether governments' or donors' objectives in health sector reform programmes accurately reflect the expressed desires of the population;

⇒ Development of techniques for measuring the full range of benefits produced by health systems including methods to enable different forms of utility to be weighed against one another;

⇒ A test of the applicability of these new techniques as means to measure and monitor the health system performance of different countries, health systems or regions within countries in terms of the utility they generate;

⇒ A test of the applicability of these new techniques to the development of policies which will maximise aggregate health system utility.

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Contract number: IC18*960123

Period: October 1996 to September 1998

CLINICAL VARIABILITY OF AMERICAN TEGUMENTARY LEISHMANIASIS IN PERU AND BOLIVIA: RELATIONSHIP WITH POLYMORPHISM OF THE PARASITE WITHIN THE *LEISHMANIA BRAZILIENSIS* COMPLEX OF SPECIES (SYN. SUBGENUS *VIANNIA*)

Co-ordinator: Prince Leopold Institute of Tropical Medicine, Antwerpen, Belgium
(Dominique Le Ray)

Objectives

Identification of parasite characters underlying clinical variability in infection and disease caused by the *Leishmania braziliensis* complex.

Activities

- Collection of comparable epidemiological and ecological data in 3 foci (Peru: Pilcopata, Amazonian foothills and Huanuco, Andean valley; Bolivia: Isiboro Secure)
- Quantification of clinical variability and isolation of parasites from different clinical categories
- Genomic and genetic characterization of parasites (including those isolated during previous project)
- Verification of the correlations between genetic and clinical variability
- Qualification of the predictive value of potential markers by (i) analysis of the genetic structure of populations under study, and (ii) characterization of genomic markers
- Development of *in vitro* and *in vivo* assays for biological comparison of parasites
- long PCR analysis of ribosomal genes.

Preliminary results

- Constraints: presently : low rate of isolation due to decrease in transmission
- Different epidemiological patterns between Huanuco and the 2 other areas: of virulence, age-dependence
- Characterization of 20 additional isolates from previous project: a correlation between size of gp63-bearing chromosome is confirmed in Pilcopata, but is not observed in Isiboro Secure
- Characterization of genomic markers previously found to be correlated with differences in pathology: further to gp63 genes, size variation of chromosomes bearing rDNA or mini-exon genes (between *L.(V.)braziliensis* and *L.(V.)peruviana*) is due to dosage of the respective genes.
- RAPD: identification of primers generating bands specific of clinical categories (on a limited number of stocks)
- Development of a specific RT-PCR assay for analysis of gp63 transcription
- Inoculation of 4 stocks into hamster: *L.(V.)braziliensis* (cutaneous or mucosal) and *L.(V.)peruviana* (large or small cutaneous lesions); in parallel, measures of *in vitro* growth parameters.

* Development of systems for measuring susceptibility of these parasites to hydrogen peroxide.

Follow-up

* Continue the epidemiological survey
* Carry out further genetic analysis of isolates (specially those from patients muco-converted during present and previous projects) with the new battery of methods developed
* Compare gp63 transcription patterns between parasites of the different clinical categories

Selected publications

Inga R., De Doncker S., Gómez J., López M., García R., Le Ray D., Arevalo J. & Dujardin J.C. 1998. Relation between variation in copy number of ribosomal RNA encoding genes and size of harbouring chromosomes in *Leishmania* of subgenus *Viannia*. Mol.Biochem.Parasitol. In press.

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Contract number: IC18*CT960125

Period: September 1996 to August 1999

CONCERTED ACTION IN SUPPORT OF HIGH-QUALITY NON-HUMAN PRIMATE (NHP) BREEDING AND BIOMEDICAL RESEARCH IN NHP SOURCE COUNTRIES

Co-ordinator: Biomedical Primate Research Centre, Rijswijk, The Netherlands
(Alan W. Thomas)

Objectives

- To develop an organisational and communication framework between European and developing country primate research centres within which improvements in the capabilities for research on health problems in developing countries and improvements in animal welfare can most effectively be realized
- To identify the areas for improvement that can most benefit from collaborative efforts and co-ordinate the implementation of such efforts

Results

Activities 1 and 2 implemented. Training on transfer of malaria transfection technology to IPR, Kenya underway. Study of mAb reactivities with cells of primate sytem completed (ref 2) and second phase of study initiated.

Expected outcome

⇒ Clearly identified priorities for collaborative development of research and reference capabilities

⇒ Management and communication structures that allow rapid and free exchange of information between primate centres

⇒ Co-ordination of the improvement of capabilities, ensuring that duplication of effort is substantially reduced and that optimal use of scarce resources is achieved

Selected Publications

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Contract number: IC18*CT970212

EVALUATION OF THE RADIATION-ATTENUATED SCHISTOSOME VACCINE IN PRIMATES AS A MODEL FOR HUMAN VACCINE DEVELOPMENT

Period: January 1998 to June 2000

Co-ordinator: University of York, York, United Kingdom, (Alan Wilson)

Objectives

- Investigate the radiation-attenuated (RA) schistosome vaccine in chimpanzees and simultaneously examine the development of the granulomatous responses to schistosome egg disposition in the liver of control animals;
- Compare the immunological and pathological responses of human patients exposed to schistosome infection in endemic areas of Brazil, with those of the chimpanzees;
- Explore in baboons aspects of the RA vaccine, crucial to its evaluation as a model for a human recombinant vaccine.

Activities

- A core vaccination experiment will be performed involving three test and three control chimpanzees.
- The immune responses to vaccination and challenge will be compared in two distinct physiological compartments: the peripheral blood and the airways of the lung. Serum will be obtained for determination of specific antibody responses. Leucocytes will be recovered from blood for determination of antigen-driven proliferation and cytokine production after 72-96h of *in vitro* culture. Lymphocytes in whole blood will be phenotyped by flow cytometric analysis. Airway leucocytes will be recovered from test and control animals by bronchoalveolar lavage over the vaccination period to determine whether the lungs have been pre-armed with schistosome-reactive cells. The efficacy of vaccination after challenge with normal cercariae will be estimated from faecal egg counts. Mature worm burdens will also be estimated by the measurement of parasite gut-derived circulating antigens (CAA and CCA) in serum and urine. The pathogenic mechanisms operating after the start of egg deposition in challenged animals will also be intensively monitored. The liver will be sampled at regular intervals by needle biopsy. A wedge surgical biopsy will be taken late in the study. The recovered tissue will be subjected to a detailed histopathological and immunocytochemical analysis. Observations will be made on the gross pathology induced by a schistosome infection by estimating the extent of hepatic fibrosis using non-invasive ultrasound scanning of the liver.
- Human responses to schistosome infection will be evaluated in a cross-sectional study of Brazilians patients during the acute and chronic phases of the disease. Characterization will use primarily peripheral blood leucocytes, but also cells from other compartments that may become available. Phenotypic analysis of lymphocytes will be performed for the same range of CD markers as in chimpanzees. The expanded T cell populations will also be phenotype to pinpoint the characteristics of schistosome-reactive T cell subsets. Cytokine levels in all PBL cultures will be evaluated by ELISA. Biopsy samples of livers and spleen, obtained from patients with chronic (hepatosplenic) disease, will be analysed by immunocytochemistry.
- Two experiments will be undertaken in baboons to define further certain parameters of the RA vaccine. The question of whether protection is long-lasting will be addressed in

an experiment requiring 15 test and 15 control baboons. The test animals will be given 3 vaccinations using the same pools of parasites. Protection will then be measured by portal perfusion to determine adult worm burden at one, three and six months by challenging five test and five control animals on each occasion.

* A second experiment is designed to discover whether there is a celling to protection. Three groups of test animals will receive five, three of one vaccinations, respectively, before they and a single group of controls are all challenged with the same pool of normal cercariae; protection will be measured six weeks after challenge. Assays of immune reactivity, including antigen-driven proliferation, secretion of some cytokine proteins (IL-2, 4, 5 and IFNγ), and specific IgG titres, will be performed. Serum samples will be obtained at perfusion, to determine circulating antigen levels.

Expected outcome

Immunological parameters will be monitored in primates from the start of experiments so that the effects of exposure to the RA vaccine should rapidly become apparent. Studies in human patients in Brazil will take place in parallel with the primate vaccination experiments and attempts will be made to relate these to the experimental findings in order to evaluate the utility of the primates as models for human schistosomiasis. These cross-comparisons will also be important in the studies on liver pathogenesis in the chimpanzee.

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Contract number: IC18*CT970213

Period: January 1998 to December 2000

HOST-PARASITE RELATIONSHIP IN CANINE VISCERAL LEISHMANIASIS (*L. INFANTUM/ L. CHAGASI*): DEVELOPMENT AND VALIDATION OF THE DOG MODEL

Co-ordinator: Centro Nacional de Microbiologia, Majadahonda, Spain (J. Alvar)

Objectives

- Describe the natural history of canine leishmaniasis, with emphasis on the pre-patent period, defining the kinetics and characteristics of infection, immune response, and clinical evolution of *Leishmania infantum* infection in the dog.
- Develop reagents that may have prognostic value.
- Establish a reproducible infection protocol for consistent, parasitological, immunological, and clinical patterns comparable with natural infection in endemic areas (New and Old World), and develop an experimental model useful for future immunoprophylactic and therapeutic studies.

Activities

The natural history of canine leishmaniasis in inbred dogs will be investigated, with emphasis on the pre-patent period, by:

- Clinical analysis including analytical biochemistry and blood-cell count. Measurement of antibody isotype responses, in particular IgM, IgG and IgE.
- Measurement of systemic and cutaneous cell-mediated immunity responses. Cytokines will be determined, the specific response to defined antigens will be studied, and T-cell subsets will be established.
- Investigation of the immunostimulatory and effector roles of dendritic cells in a canine model of visceral leishmaniasis.
- Parasite burden and parasite distribution will be established by direct microscopy, by culture in NNN medium, and by PCR.
- Infectivity for sandflies (epidemiological risk) will be assessed at different time points by xenodiagnosis with *Phlebotomus perniciosus* or *Lutzomuia longipalpis,* and asymptomatic and symptomatic dogs from endemic areas (Spain and Colombia).
- Reagents that may have prognostic value, including primers for cytokines, will be developed together with a quantitative PCR of genomic DNA for determining parasite burden. The experimental model will be validated with natural infection in endemic areas (New and Old World) by comparing clinical, immunological, and parasitological data of asymptomatic dogs (defined by parasite burden and biochemical analysis) from endemic areas in Colombia and Spain.

Expected outcome

⇒ Better understanding of the infection, on the basis of correlating the immunobiological, clinical and epidemiological features.

⇒ Practical recommendations will result from this project in terms of risk for humans and control measures, through a better understanding of the natural history of canine leishmaniasis.

⇒ Moreover, the response to defined antigens will establish the immunological basis for further projects related to vaccine development or for drug/immunological synergy.

⇒ Finally, several reagents are expected to be obtained for both diagnosis and cytokine detection, that will be of value for the scientific community.

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Contract number: IC18*CT970220

Period: October 1997 to September 2000

NEW TRYPANOCIDAL COMPOUNDS BASED ON INHIBITORS OF GLYCOLYSIS AND THE SPECIFIC IMPORT OF THESE INHIBITORS INTO THE PARASITE

Co-ordinator: Université Paul Sabatier, Toulouse, France (J. Perie)

Objectives

- Develop novel compounds active against human trypanosomiases and leishmaniasis;
- Take advantage of an essential metabolism in these trypanosomatidae that glycolysis represents;
- Design irreversible and quasi-irreversible inhibitors of glycolytic enzymes based on differences between parasites and mammalian enzymes and to import them into the cell either via passive diffusion or via the glucose THT1 transporter.

Activities

* The first set of irreversible GAPDH inhibitors will be extended to structures directed towards Arg 231 and the compounds will be transformed into the corresponding prodrugs. Cocrystallisation experiments of these compounds with the enzyme GAPDH from *Trypanosoma brucei* will be made for the design of improved structures after corresponding modelling.
* Parallel work will be done on the *T. brucei* aldolase enzyme, starting from active structures already identified. In both cases the residue responsible for the formation of the covalent bond will be identified using mutants for the most likely locations.
* All the compounds will be assayed for their transport by the glucose transporter THT1 and also on Trypanosome cytosolic esterases which are expected to transform prodrugs into drugs within the cell. Compounds of natural origin will also be studied.

Expected outcome

The study will develop compounds capable of specifically blocking glycolysis and therefore limiting the survival of trypanosomes. The work will lead to an improved knowledge of the glycolytic enzymes and transport systems in trypanosomes and leishmania.

Selected publications

Tetaud E., Barrett M.P., Bringaud F. and Baltz T. 1997. Kinetoplastid glucose transport. Biochem Journal. 325: 569-580.

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Calustre S., Azema L., Baron R., Bringaud F., Perie J., and Willson M. 1998. An easy stereospecific synthesis of 1-amino-1-deoxy-2,5-anhydro mannitol. Activity on THT1 protein. Carbohydrate Research (submitted).

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Contract number: IC18*CT970222

Period: January 1998 to December 2000

ENVIRONMENTAL AND OCCUPATIONAL CANCER IN MERCOSUL COUNTRIES

Co-ordinator: International Agency for Cancer Research, Lyon, France (Paolo Bofetta)

Objectives

- To carry out an international multicentric population-based study of occupational factors of laryngeal cancer;
- To organize an international conference on occupational and environmental cancer in developing countries, with emphasis on Latin America.

Activities

* The proposed approach is a multicentric case-control study of laryngeal cancer and exposure to occupational risk factors, taking into account other risk factors including lifestyle, HPV infection and genetic susceptibility. The study will be conducted in Rio de Janeiro, Sao Paulo, Pelotas and Porto Alegre (Brazil) and Buenos Aires (Argentina). It will include approximately 1200 cases of laryngeal cancer and a similar number of controls. A detailed occupational questionnaire will be used to interview cases and controls, and will then be interpreted on an individual basis by a team of local experts, which will assess exposure to a list of known or suspected occupational laryngeal carcinogens in terms of probability, frequency and level. The preliminary list of exposures to be assessed includes asbestos, man-made mineral fibres, wood dust, strong inorganic acid mists, diesel engine exhaust, environmental tobacco smoke, other sources of PAHs, chromium, nickel, arsenic, formaldehyde, infection with HPV, infection with animal viruses. A blood sample will be collected, whenever feasible, from cases and controls. Laryngeal tumour samples will be collected, whenever feasible, from cases.
* The analysis of genetic polymorphism to GST M1 and NAT2 enzymes will be performed on DNA extracted from lymphocytes. Tumour samples of a subgroup of cases selected according to relevant exposures (e.g., exposed and unexposed to occupational carcinogens) will be analysed for mutations in the p53 and *ras* genes using denaturing gradient gel electrophoresis.

Expected outcome and results so far

⇒ An initial meeting of the case-control study group was held in Sao Paolo in April 1998 during which the study protocol was finalised. The study has now started in all centres and case recruitment is ongoing. It is expected that the study will contribute to occupational exposures to laryngeal cancer and will contribute original information on the interaction between genetic factors, known risk factors, such as tobacco and alcohol, and occupational exposures. These data will be useful in the prevention of cancers of the larynx and other organs sharing some of the risk factors, such as the lung, oral cavity, oesophagus and bladder.

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Contract number: IC18*CT970224

Period: October 1997 to September 2000

HEALTH AND HUMAN SETTLEMENTS IN LATIN AMERICA

Co-ordinator: South Bank University, School of Urban Development and Policy, London, United Kingdom (T. Harpham)

Objectives

The aim of this concerted action project was to use the combined, existing knowledge of partners in a more concerted manner to develop proposals to design and evaluate interventions to improve the health of disadvantaged urban populations. Specifically:
- consolidate knowledge;
- build capacity;
- develop proposal; and
- enhance the impact of research on policy.

Activities

The approach was multisectoral, with the aim of putting urban health issues on agendas that fall outside the health sector. Health is defined as physical, mental and social well-being, in line with WHO's definition. The environment is equally broadly defined as incorporating physical and social aspects. The project brought together, for the first time, a wide range of urban health researchers in the North and South in order that they could co-ordinate their efforts over a three year period to maximise the effectivenessand impact of research. It focused on urban environmental health and urban health services issues. Specific activities involved :
- holding two workshops;
- expanding the "Bulletin of urban health and development" (published by MRC, South Africa);
- producing a "State of the art of urban health in Latin America";
- carrying out exchange visits;
- carrying out project leader missions;
- producing three short reports on the activities of partners;
- mounting an urban health course in Latin America;
- producing research proposals; and
- disseminating the activities of the concerted action.

Results

The first workshop was held in Sao Paulo in February 1998. Over a period of five days partners and other participants met to: share research findings; discuss collaborative initiatives, plan dissemination activities; discuss urban health training initiatives; and define the structure of the "State of the art of urban health in Latin America". A summary version of the papers presented by partners at thye workshop will be published in a future edition of the "Bulletin of Urban Health and Development". In addition, since the inception of the project a

project mailing list has been set up and there are currently 120 individuals listed, a database of key urban health literature (as identified by partners) has been created, and a database of funding organisations has been compiled.

Expected outcome

- Increased reference to urban health in strategic international public health policy plans.
- Increased understanding of urban health in Latin America.
- Increased training opportunities in urban health in Latin America.

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Contract number: IC18*CT970225

Period: October 1997 to September 2001

PRODUCTION AND CHARACTERIZATION OF SYNTHETIC INHIBITORS OF PARASITE OF PROTEASES AS DRUG CANDIDATES FOR THE PREDOMINANT PROTOZOAL DISEASES OF SOUTH AMERICA AND OTHER DEVELOPING COUNTRIES

Co-ordinator: Carlsberg Laboratories, Valby, Denmark (M. Meldal)

Objectives

To develop specific inhibitors of parasitic cysteine proteases as drug candidates for the treatment of the predominant protozoal diseases (in particular *Leishmaniasis* and Chagas disease) of South America and other developing countries. This goal will be attained through the implementation of molecular approaches and state-of-the-art combinatorial chemical-library techniques.

Activities

* Solid-phase synthetic methodology will be developed simultaneously with molecular and recombinant technologies for the generation of large quantities of cysteine proteases (CPs) for screening. An iterative process of screening and optimization will lead to the target drug candidates.
* Establishment of a multi-disciplinary approach in LA-EEC that fosters development of the field of drug discovery by using an interdisciplinary approach involving state-of-the-art molecular and biochemical techniques and combinatorial chemistry.

Results

Synthetic Methodology:

⇒ Two inert resins were developed and are being utilized in the solid-phase synthesis of CP inhibitors.

⇒ Substrate libraries were synthesized and screened using papain, the archetypal CP for comparative purposes and for elucidation of enzyme activity on solid phase compared to in-solution.

⇒ A novel synthesizer for manual organic library synthesis with the capacity to provide inert reaction conditions, temperature control and refluxing conditions has been constructed.

Biochemistry, and molecular and recombinant technology:

⇒ An isoform of the *Leishmania mexicana* Type I cysteine proteinases was over-expressed in *E. Coli* and active enzyme successfully purified from both the soluble phase and inclusion bodies.

⇒ Cruzain was successfully expressed in *E. coli* and isolated and purified from the bacterial inclusion bodies.

⇒ Cruzipain 2 was expressed in *S. cerevisiae* and the substrate specificity was determined using synthetic fluorogenic substrates. Results suggest that cruzipain, cruzipain 2 and cruzain are distinct enzymes.

⇒ Cruzipain 2 was successfully over-expressed in epimastigotes of *T. cruzi* and the infectivity of the transfected parasites was then investigated.
⇒ The CP activity of different species of *Leishmania* was investigated. CPs vary widely in expression in the different species.
⇒ CPs were isolated and purified from *Leishmania* Lp52 parasite.
⇒ The substrate specificity of cathepsin B was investigated in order to determine differences from parasitic cysteine proteases.
⇒ An *in-vitro* system to study CP inhibitors from combinatorial libraries has been developed.

Publications

Meldal M., Svendsen I., Juliano L., Juliano M.A., Del Nery E., Scharfstein J. 1998. Inhibition of cruzipain visualized in a fluorescence-quenched solid-phase inhibitor library assay. D-amino acid inhibitors for cruzipain, cathepsin B and cathepsin L. J. Peptide Sci. **4:** 83-91.

Coombs G.H., Mottram J.C., Sanderson S.J. April 1998. Purification of an active, recombinant cysteine proteinase of *Leishmania mexicana trypanosomiasis* and *leishmaniasis* symposium Arcachon, France.

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Contract number: IC18*CT970235

Period: September 1997 to August 2000

SUPPORTING COLLABORATIVE RESEARCH ON PUBLIC-PRIVATE RELATIONSHIPS IN HEALTH CARE : AN INTERNATIONAL NETWORK

Co-ordinator: Centre for Health Policy, Johannesburg, South Africa (Neil Söderlund)

Objectives

Link researchers and policy makers in 14 countries for the purposes of initiating and supporting research on the public-private mix in health care in developing countries.
Specifically, the network concentrated on support for research in two areas :

- regulation and incentive setting for private health sector players,
- the selective involvement of private sector players to achieve public policy goals through contracting arrangements, allowing private practice by public sector doctors, and expanding the role of private practionners in the delivery of public health services.

Activities

- Development of internet connections to facilitate exchange of ideas, circulation of research methods and results and the dissemination of key findings in this field. This was done using text-based automatic mailing systems (known as "mailbases") and an interactive World Wide Web site.
- Structured meetings were held between all participants to share research, provide access to technical experts, and inform dissemination strategies and approaches to improve the impact of work on policy.
- Staff exchanged visits to provide support to facilitate collaborative projects.
- Research products were prepared in the form of publications and conference addresses.
- Methodological reviews were prepared to guide current and future work in this area internationally.

Expected outcome

⇒ Firstly, the network will increase the volume and quality of research into public-private mix issues in developing countries. It will also provide a streamlined route for new entrants to this field to access available literature and human resources, and, by means of a project register, reduce duplication of existing work. Finally, the network should increase the profile of research results around public private mix issues for both national and international policy makers, thus improve health care organisation, financing and provision practice in developing countries.

⇒ So far an electronic communication network has been set up and a steering group meeting held to determine the specific activities that the network has undertaken.

Selected publications

Bennett S., Mc Pake B., Mills A., 1997. Private Health Providers in Developing Countries – Serving the Public Interest. Zed Books, London.

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Contract number IC18*CT970236

Period: November 1997 to April 2001

IDENTIFICATION OF PROTECTIVE IMMUNE RESPONSES TO PATHOGENIC MYCOBACTERIA

Co-ordinator: London School of Hygiene & Tropical Medicine, United Kingdom
(H.M. Dockrell)

Objectives

To evaluate the role of T cell-associated lytic mechanisms in the killing of intracellular mycobacteria, and the contribution of these effector pathways to immunity against tuberculosis and leprosy.

Activities

The function of various T-cell subsets in immunity to tuberculosis will be assessed in patients with tuberculosis, without or with co-infection with HIV, and in normal BCG-vaccinated healthy controls. Further studies will assess expression of the P2Z (P2X7) receptor in patients with tuberculosis or leprosy, and in healthy controls. Specific areas of investigation are as follows:

- To evaluate whether antigen-specific CD4+ and CD8+ T-cell mediated cytotoxicity reduces the survival of intracellular maycobacteria within macrophages, and the relative contribution of various cytolytic effector mechanisms to this T cell-induced macrophage death (LSHTM, London , University of Oxford, and MRC Laboratories, The Gambia).
- To assess the role of the gamma delta T cell subset in cytolysis and cytokine secretion in tuberculosis with/without HIV co-infection (CMDT, Lisbon).
- To investigate the mechanism(s) by which extracellular ATP-induced macrophage death (occurring via the P2Z-receptor mediated pathway) reduces survival of intracellular *M.bovis* BCG (University of Birmingham).
- To investigate the role of genetic heterogeneity of extracellular ATP-induced macrophage death and intracellular mycobacterial killing in conferring resistance to mycobacterial disease, by comparing P2Z (P2X7) receptor expression on monocyte-derived macrophages from patients with tuberculosis (with/without HIV), lepromatous leprosy, and endemic controls (University of Birmingham, CMDT Lisbon, MRC Laboratories, The Gambia and IMSS Mexico City).

Expected Outcome

The study will obtain scientific data allowing the relative importance of these immune responses in protection against mycobacterial disease, and the heterogeneity of the P2Z (P2X7) receptor in susceptibility to mycobacterial disease, to be evaluated.

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Contract number: IC18*CT970249

HEALTH SECTOR REFORM: TOWARDS A MORE GLOBAL APPROACH OF CHILD HEALTH

Period: December 1997 to May 2001

Co-ordinator: Universidad Peruana Cayetano Heredia, Lima, Peru (Luis Benavente)

Objectives

The research proposal aimed to develop and apply an holistic approach through which health services would rationalise health care delivery concerning the health problems children face in a community. In particular:

- Identify health risks children face during their growth and development in general and in the particular study areas (Peru, Bolivia), with the participation of the parents and the community;
- Identify prevailing representations of child development and health of both the health professionals and the community;
- Identify activities which can be implemented in the given contexts of health delivery;
- Define criteria for the selection and the modification of existing activities directed at the safeguard of child health;
- Develop support mechanisms to increase parental participation;
- Increase the competence and the attitude of the health staff;
- Identify obstacles in the implementation of these activities in the health system and for the participation of the parents;
- Measure the improvement in quality of care and coverage, after rationalisation of the various specific activities;
- Evaluate the changes in autonomy and caring practices of the parents with regard to the health and development of their children.

Activities

* Two major phases can be distinguished in the overall research : a descriptive phase and a participatory phase.
  - the descriptive phase will consist first in the identification of the risks children face during their growth and development.
  - the identification of the risks specific for a given geographical area will be done in the study areas, using sociological and anthropological tools to ensure participation of the parents.
* This will result in an operational plan describing the necessary changes in the existing health system and identifying the role of the parents and the community.
* The participatory action research phase is a collaborative research between the health providers, the population and the supporting institutions. It implies the implementation and evaluation of the operational plan. The evaluation will be based on quantitative aspects of health provision and on the quality of the service offered. Rapid sociological-anthropological tools will also be used.

Expected outcome

⇒ The expected outcomes are in relation to the specific objectives formulated, and implies the implementation of the results at the research setting: a holistic approach by the health services towards the health problems of the children. An attempt will be made to translate the methodology and results beyond the local research level.

⇒ The results will be published in regional and international journals, presented at international workshops and integrated in national and international public health courses.

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Contract number: IC18*CT970250

Period: October 1997 to March 2001

EVALUATION OF A STRATEGY TO CONTROL THE EPIDEMIC OF CAESAREAN SECTIONS IN LATIN-AMERICA

Co-ordinator: Université Libre de Bruxelles, Brussels, Belgium (S. Alexander)

Objectives

- To evaluate, using a randomized trial, the effect of an intervention aimed at reducing Caesarean Section (CS) rates in Latin America. The intervention consists of: (i) systematic second opinion before surgery (ii) practitioner education about decreasing CS rates through alternative guidelines for effective and safe management of childbirth.
- Exploratory assessment of rates and needs of CS in Africa and Asia
- Dissemination of results of a study of maternal needs and demands concerning childbirth.

Activities

- The efficacy of the intervention will be assessed in a multicentre, cluster randomized controlled trial with two (three) further nested studies: (i) assessment of women's opinions (ii) care-giver's opinions (this survey is optional) and (iii) organisational and cost survey.
- Practitioners will systematically discuss the indication for caesarean section with a colleague before resorting to surgery (obtain a second opinion) and fill in a special form. Educational seminars on ways of decreasing CS will also be organized for the care-givers in the intervention arm maternity units. The trial will be conducted in six Latin American countries: Argentina, Brazil, Chile, Cuba, Guatemala and Mexico contributing a minimum of 17 pairs of maternity units.
- The main outcome is CS rate; secondary outcomes include measures of maternal and perinatal morbidity. Satisfaction, acceptability, and economic aspects will be assessed in the nested studies. Because of the contamination risk between intervention and non-intervention units, CS levels will be assessed in all participating units prior to the study.
- Prevalence and needs of CS in Africa and Asia will be assessed.
- Translation and dissemination of a related previous study will be performed.

Expected Outcome

⇒ Assess the effectiveness of a package of co-interventions aimed at curbing the excess rate of CS in Latin America. Should this prove effective, replicating the interventions should not be an issue, as they are mainly behavioural, and therefore their extension to routine care should be neither costly nor complicated. Should the interventions not be effective within the framework of this trial, further assessment of the determinants of the failure will be essential and further in-depth work along the Bradby study would be recommended.

⇒ Finally, it must be remembered that the Latin American CS epidemic is but one end of the spectrum. In other regions there is a dearth of necessary caesarean sections. This aspect will be explored by the Ghent partners, and we hope to be able to make sensible

recommendations as to an approved bracket of CS rates, and as to measures to achieve this.

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Contract number: IC18*CT970253

Period: December 1997 to May 2001

THE PATHOGENESIS OF TUBERCULOSIS; GROWTH RATE REGULATION AND RIBOSOME SYNTHESIS

Co-ordinator: Medical Research Council, London, United Kingdom (M.J. Colston)

Objectives

To investigate the ways in which pathogenic mycobacteria are able to regulate their growth rate and survive within an infected host by:

- Analyzing the expression of ribosomal RNA (rRNA) genes when mycobacteria are grown under a variety of conditions, including in host tissue.
- Studying the role of ribosomal protein S10 in the transcription of mycobacterial rRNA operons.
- Identifying additional transcription factors involved in expressing genes involved in ribosome synthesis.

Activities

- The expression of rRNA genes is being investigated by identifying promoters involved and studying their relative levels of expression under different conditions of growth, including growth in infected tissue.
- The role of the ribosomal protein S10 is being investigated by preparing a purified recombinant protein and studying its interaction with RNA sequences and with other proteins of *M. tuberculosis*.
- Additional transcription and antitermination factors will be identified and characterised.

Expected outcome

This study will identify the strategies used by pathogenic mycobacteria to regulate ribosome synthesis and hence to regulate growth rates. By identifying specific components of the transcriptional machinery of *M. tuberculosis*, we expect to identify potential targets for the development of novel anti-tuberculosis drugs.

Selected publications

Gonzalez-y-Merchand J. A., Colston M. J., and Cox R. A. 1997. Strategies used by pathogenic and non-pathogenic mycobacteria to synthesize rRNA. Journal of Bacteriology. **179** (22), 6949-6958.

Gonzalez-y-Merchand J. A., Colston M. J., and Cox R. A. The role of multiple promoters in transcription of rDNA; the effects of growth conditions on precursor rRNA synthesis in mycobacteria. Submitted for publication.

Contract number: IC18*CT970253

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Contract number IC18*CT980338

IMPROVING EFFICIENCY AND QUALITY OF HEALTH NETWORKS IN URBAN AREAS

Period: September 1998 to August 2002

Co-ordinator: Deutsche Gesellschaft für Technische Zusammenarbeit, Eschborn, Germany (Ulrich Knobloch)

Objectives

The project aims at improving urban health initiatives in North and South alike, on the basis of scientific evidence. Specifically this means to:

- Systematically collect and compile comprehensive experience relating to urban health initiatives, and to identify the most valuable approaches using established analytical criteria;
- Conduct a process analysis of the most relevant experiences (lessons learned approach);
- Improve the efficiency, efficacy and relevance of urban health initiatives in the participating cities by increasing the frequency and quality of exchanges between the various projects/initiatives under way (cross-fertilisation process);
- Initiate an advocacy system - on a national and international level - to improve the dissemination and formulation of urban health strategies;
- Dynamize multi- and interdisciplinary actions in urban health systems.

Activities

- The general methodology is a concerted action in order to bring together a critical mass of urban health specialists in the North and in the South. The goal is to enhance collaboration between the different partners in order to improve the health situation of disadvantaged urban populations.
- A preliminary review of relevant literature has revealed that there are already several ways of approaching the development of urban health systems, but none has been reviewed in the manner proposed by this CA. The main techniques include an intensive literature review, a process analysis according to the criteria established, an initial and a wrap-up meeting as well as peer consultancies, a capable dissemination strategy between the partners and a set of toolboxes and guidelines.
- The choice of subjects will depend on each partner's priorities.

Expected outcome

⇒ Increased understanding of specific urban health issues;
⇒ Tools elaborated for integrating health issues into sustainable urban development patterns, as manifested in Agenda 21;
⇒ Active scientific exchange strengthened;
⇒ Commitment and co-operation of local government enhanced (described by case studies);

⇒ Local decision-makers, ministries and donor agencies sensitised in a more rational and systematic way;
⇒ Case studies on research issues and country programs analysed and well documented;
⇒ Additional participants have joined the network;
⇒ Scientific publications about urban health issues and related topics disseminated and made available to a wide public;
⇒ Links to other networks and concerted actions established.

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Contract number: IC18*CT980339

THE IMPLICATIONS OF HEALTH SECTOR REFORM IN ECUADOR, COLOMBIA AND NICARAGUA FOR BASIC HEALTH PROGRAMMES: OPERATIONAL RESEARCH ON THE PROCESS, COST EFFECTIVNESS AND OUTCOME.

Period: September 1998 to August 2001

Co-ordinator: Liverpool School of Tropical Medicine, Liverpool, United Kingdom
(Axel Kroeger)

Objectives

General

Determine - through Health Systems Research - the most cost-effective control of common diseases - using malaria, tuberculosis and immunopreventable diseases as an example - by local health services (reduction of morbidity, mortality and economic loss), in the context of the ongoing transformation of the national control programmes in Nicaragua, Ecuador and Colombia, which includes structural changes (partial or full integration of the malaria and Tb control operations into the district health systems), a diversification of the control interventions, and the improvement of the managerial system.

Specific

1. Develop a comprehensive framework for measuring the impact of health sector reform and of the integration of vertical control programmes into district health systems testing the following research hypotheses:
   - 1.1 *The integration of vertical disease control programmes* into district health systems leads to contradictory results: deterioration of malaria control; continuity Tb control at a poor level and of immunisation programmes at an acceptable level.
   - 1.2 When *intervening* in 2 major problem areas, the situation can be significantly improved. These areas include:
     - 1.2.1 Using a *mix of different interventions (through governmental health services):*
       - a) introduce bednet impregnation for malaria control and passive case finding for Tb control;
       - b) improve quality of diagnostic procedures;
       - c) enhance community participation and improve health seeking behaviour;
       - d) improve field workers' performance.
     - 1.2.2 Improving the *management system* through management training will:
       - a) lead to a more efficient use of resources;
       - b) facilitate the early detection and solution of operational problems;
       - c) improve field workers' performance;
       - d) enhance the integration of the control into the district health system.

2. To improve local and national control of important endemic diseases through the joint development of guidelines for control operations.
3. To strengthen Operational Research at local level as a tool for improving health services.

Activities

- ✶ The study will consist of a pilot phase, three research phases and finally, a short dissemination phase.
- ✶ In the *pilot phase* the research instruments will be tested and field staff trained (including local health staff). The three *research phases* include *Phase I*: baseline studies (cross sectional) in the 4 target groups indicated in Fig. *I*; *Phase II*: longitudinal studies during the intervention stage; and *Phase III*: follow-up studies (cross sectional) the results of which will be compared with the baseline studies.
- ✶ The *analysis and dissemination phase* includes the joint analysis of study results, the incorporation of the results into local health services and the joint development of national guidelines for basic health programmes as indicated in our second specific objective.

Expected outcome

⇒ End of *phase I:* Description of the process and results of the health reforms with respect to basic health services (control of malaria, Tb, immunopreventable diseases) and factors explaining success and failure.

⇒ End of *phase II:* Report on experiences with the interventions and related research activities.

⇒ End of *phase III:* Presentation of the:

a) analysis of favourable and limiting factors for the improved delivery of basic health packages in the context of health reforms
b) possibilities and preconditions of cost-effective interventions
c) development of policy recommendations for Latin American countries
d) analysis of research co-operation among the partners involved

⇒ At the end of *dissemination phase:* Comprehensive scientific report and publications.

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ASSESSING BARRIERS AND OPPORTUNITIES FOR USERS INVOLVEMENT IN HEALTH CARE QUALITY CONTROL: AN EVALUATIVE STUDY IN COLOMBIA AND BRAZIL

Period: November 1998 to October 2000

Co-ordinator: Escuela Andaluza de Salud Pública, Granada, Spain (Mariano Hernan Garcia)

Objectives

General:

- Evaluate the effectiveness of current health sector reform policy for strengthening user involvement in health care quality control in the health system in Colombia and Brazil.

Specific:

- Analyse how user involvement in health care quality control is defined and approached by the governments and the health sector;
- Identify existing formal and informal (non-institutional) mechanisms to bring about user involvement in quality control;
- Find out the extent to which participatory mechanisms for quality control are implemented;
- Find out the extent of users' willingness to participate and perceived ability to affect health services performance through the existing participatory mechanisms; their experiences with them, and their views on the quality of service provision;
- Examine how consumer organizations determine and represent consumer demands on health services quality;
- Identify what are the main conflict areas regarding health care quality between users and the health system; and the nature and extent of the health system responsiveness to such conflicts;
- Identify opinions, expectations, interests and influence of key actors in relation to policies aimed at increasing users involvement in health care quality control;
- Identify factors and actors that may hinder or enable the effectiveness of institutional participatory mechanisms for users' involvement in quality control, within and across countries.

Activities

- Study design and methods
- Document analysis,observation, group discussion and individual interview, case studies and survey.
- Analysis of research results will be firstly carried out for each country case study; secondly, a cross country analysis will take place.
- Quality control mechanisms: using different qualitative and quantitative methods.
- Identification of problems and feasibility.
- International workshops.

Expected outcome

This research will analyse and assess policies directed to increase citizen participation in health services quality control in two Latin American countries. These policies have been widely supported by national governments and international organisations as part of their increasing attention to issues of good governance. This research will contribute with policy lessons for promoting enabling environments for the emergence of strong and independent civil society organisations. These lessons, in turn, will benefit national efforts to achieve good governance and improved public management. The research will further develop appropriate methods to assess opportunities and process for improved participation in the health sector. The findings are expected to be conducive to better, efficient and effective use of channels and mechanisms of social participation as well as the responsiveness and quality of the health services.

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CYTOCHROME P450 AS A BIOLOGICAL MARKER OF SUSCEPTIBILITY AND EFFECT OF OCCUPATIONAL AND ENVIRONMENTAL EXPOSURE TO VOLATILE ORGANIC CHEMICALS (VOC'S), POLYCYCLIC AROMATIC HYDROCARBONS (PAH'S) AND PETROL-DIESEL HYDROCARBONS (DPH'S) IN LATIN AMERICA

Period: October 1998 to September 2001

Co-ordinator: Università di Padova, Padova, Italy (Maurizio Manno)

Objectives

The general aims of this project are *a)* to improve the scientific knowledge on the mechanisms of toxicity of volatile organic compounds (VOCs), polycyclic aromatic hydrocarbons (PAHs) and diesel/petrol hydrocarbons (DPHs) and *b)* to use this knowledge to assess the health risks deriving from human exposure in occupational and non-occupational environments of Latin America (LA) and Europe (EU). Specific objectives are *a)* clarification of the mechanisms of toxicity and bioactivation of specific VOCs/PAHs/DPHs, *b)* risk assessment of occupational and non-occupational human exposure to VOCs/PAHs/DPHs in EU and LA, and *c)* transfer of material, methodologies and personnel including exchange of samples, expertise and personnel among the partners and participation to congresses.

Activities

* Start up meeting to organize focal points for all partners;
* Clarification and comparison, in different *in vitro* animal systems of the metabolism of specific VOCs/PAHs/DPHs and determination of the metabolic rate constants to be used for PBPK studies;
* Determination of the effects of specific VOCs/PAHs/DPHs on P450 enzymatic activities and content;
* Identification and characterization of the human populations occupationally and non-occupationally exposed to be studied in the next phases;
* Acquisition and exchange of basic techniques among the different laboratories;
* Identification of the specific P450 isoform(s) responsible for the biotransformation and activation of specific compounds using various methods *in vitro*;
* Comparison of the metabolism of specific VOCs/PAHs/DPHs by human and animal enzymes;
* PBPK studies on species and sex differences in gas-uptake and metabolism of specific compounds;
* Environmental and biological monitoring of exposure to specific VOCs/PAHs/DPHs;
* Assessment of P450 phenotype and genotype in workers;
* Study of the metabolism of VOCs/PAHs/DPHs by human and animal enzymes;
* Validation of PBPK models using data obtained from humans exposed to VOCs/PAHs/DPHs;
* Detection of health effects/hypersusceptibility in subjects exposed to specific VOCs/PAHs/DPHs;
* Publication of the results and targeted training;

* Final meeting/Conference on bioethics.

Expected outcome

⇒ Integration of EM, BM and P450 GT/PT will provide information for screening human populations and may help to predict/prevent human health risks from exposure to these chemicals. Specific expected results include:

⇒ Development, on a collaborative basis, of *qualified human resources* (i.e. PhD, MSc and BSc graduates).

⇒ Application of new biochemical, pharmacological and molecular biology techniques to the risk assessment of occupational/environmental exposure to chemicals.

⇒ Increased *awareness* and more balanced *perception* of toxicological risks by the workers, the employers, and the other groups of the general population participating in this study.

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Contract number: IC18*CT980344

CHANGING HEALTH SYSTEMS IN LATIN AMERICA: PROMOTION AND PROTECTION OF HEALTH WITHIN THE DECENTRALISED SYSTEM

Period: November 1998 to January 2001

Co-ordinator: University of Manchester, Manchester, United Kingdom (Sarah Atkinson)

Objectives

- Define factors that, within a context of decentralised health systems, enable or hinder change towards a health care model of promotion, protection and disease prevention as advocated in current Latin American public health discourse;
- Identify criteria by which to evaluate the extent of a shift in the model of health care adopted by decentralised health systems;
- Assess whether and the extent to which local decentralised health systems with a policy directive for reorientation of health and health care have succeeded in making the new model operational;
- Identify factors that enable or hinder change in the health care model through comparison of SILOS in urban and rural regions within two different national systems of health care;
- Contribute to public health care policy debates in Latin America regarding the practical strategies and impediments in the implementation process of new visions and theories for local health care systems.

Activities

- Description of eight local health systems by five broad themes: activities, intersectorality, responsiveness, vision and awareness of the local population;
- Comparison of the eight local health systems at three levels: similar local health systems (urban or rural) within each national system; urban and rural local health systems within a national system; similar local health systems (urban or rural) across different national health systems (Chile and Brazil);
- Comparison of the eight local health systems according to nine features of health systems: policy intentions, system structures, complexity, intersectoriality, information systems, incentives, participation, communication and values;
- Data collection from policy and legal documents, local and national scale existing data, debates in local and national press, inventories of activities and resources, open interviews with key actors.

Expected outcome

⇒ Final research report in Portuguese, Spanish and English;
⇒ Papers submitted to international journals; conference presentations planned;
⇒ Meetings held with local governments and national ministries of health about the findings;
⇒ Development of a training module on policy implementation in practice for incorporation into in-service training of local health system managers.

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THE PRACTICE OF HEALTH CARE REFORM: LESSONS FOR THE FUTURE

Period: September 1998 to August 2001

Co-ordinator: Prince Leopold Institute of Tropical Medicine, Antwerpen, Belgium
(Wim Van Lerberghe)

Objectives

- Develop a framework for characterising and documenting:
  - the factors fundamental for an adequate understanding of past, current and proposed policy changes in the countries of the participating groups, both in Europe and in Developing Countries;
  - the strategies for implementing these reform exercises;
  - the (desirable and undesirable) achievements of these reform exercises.
- Develop a manual for systematic and comparable documentation of the reform process, with focus on
  - the identification of the paradigms underlying the reform agendas, and on
  - (institutional) strategies used to gain support for and overcome resistance against implementing these reform agendas.
- Provide systematic documentation of reform exercises in partner countries as a basis for a comparative analysis of the approaches and strategies to planning and implementing health care reform.
- Promote discussion and exchange of ideas on the manual and the framework through the establishment of a discussion group on the Internet.

Activities

This concerted action builds on a number of case studies. After completing a literature review and a review of experience with the ongoing reform exercises in the partner countries (Sweden, Belgium, Central-America – Nicaragua-Guatemala –, Lebanon, Morocco, Mozambique, Portugal, Sweden, Thailand), a provisional analytical framework for describing rationale, agendas and implementation arrangements of the reform exercises as well as a provisional framework for systematic documentation of the process of reform are agreed upon by the different partners at a first partner meeting. The various partners will utilise these draft frameworks to describe and document the reform process in their respective countries. The various country reports are compared at a closing meeting that produces the following deliverables: (i) a reform process documentation manual; (ii) case study descriptions; (iii) a comparative analysis of the case studies with identification of common patterns and the do's and don'ts in the practice of reform.

There are thus five major steps in the concerted action:

Step 1. a) literature review; partner meeting to b) draft an initial analytical framework for describing the problems, the principles and purposes, the proposals, the protagonists and the implementation arrangements of reforms; c) draft a framework for systematic documentation of the reform process; d) organise a discussion group on the frameworks on the internet.

Step 2. a) first round of documentation of the reform process in the participating countries, with b) structured peer validation of the observations, according to a methodology agreed upon during the first partner meeting.

Step 3. partner meeting to a) compare provisional results (validation, comparability, feasibility, congruence), b) review the framework through a consensus generating method; c) attempt a first draft of the manual, in preparation of the second round of documentation; and d) disseminate the new version of the framework and the draft manual through the Internet discussion group.
Step 4. a) second round of documentation of the reform process in the participating countries, with b) structured peer validation of the observations.
Step 5. partner meeting for a) collation and comparative analysis of the documented reform processes, for b) evaluation of the usefulness of the framework and manual; in order to c) produce their final version, taking into account comments obtained through the Internet.

Expected results and follow-up

Activities and deliverables	Month	Milestones
Step 1 Preparation		
1.1. Literature review	1-2	
1.2. Circulation of literature review	3-4	
1.3. Partner Meeting I. (Portugal) Draft analytical and documentation frameworks.	4	Partner meeting 1
1.4. Organisation of a discussion group on the Internet	4	
Step 2 First round of documentation of country reform exercises		
2.1. Documentation using the frameworks	5-15	
2.2. First country report (draft)	10	
2.3. Peer validation of country documentation through exchange visits	12-13	
2.4. Revised country report	14	Country reports round 1 available
Step 3. Mid term evaluation		
3.1. Partner Meeting II. (country to be decided) Revised frameworks	15	Partner meeting 2
3.2. Revised frameworks on the Internet	16	
Step 4. Second round of documentation of country reform exercises		
4.1. Further documentation using the revised frameworks	16-28	
4.2. Peer validation of country documentation through exchange visits	18-24	
4.3. Production of country reports	25-26	
4.4. Circulation of country reports	26-28	Country reports round 2 available
Step 5. Analysis		
5.1. Partner meeting III. (country to be decided) Evaluation of frameworks; comparison of country experiences	29	Partner meeting 3
5.2. Final reports: Documentation manual; Country case studies; Comparative analysis of documented country experiences	30-34	
5.3. Publication final reports	35-36	Final reports available

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HEALTH SYSTEM REFORM IN CUBA: ANALYSIS OF THE EFFECTIVENESS, COST AND ACCEPTABILITY OF THE NEW EMERGENCY CARE SUBSYSTEM

Period: November 1998 to October 2001

Co-ordinator: National Institute of Hygiene, Epidemiology and Microbiology, Ciudad Habana, Cuba (Mariano Bonet Gorbea)

Objectives

- Determine the effectiveness of the new emergency care subsystem to provide timely and appropriate medical care for emergency patients at different levels;
- Evaluate the efficiency of the system in terms of adequate flow of patients and costs;
- Assess its acceptability to users and providers, estimating the level of satisfaction and addressing behavioural changes;
- Compare the performance of the subsystem in different environments: urban, urban-rural and rural, and to define procedures for introducing changes and monitoring.

Activities

- A descriptive ambispective study in 3 urban areas, where the reform is being introduced since early 1997;
- A prospective quasi-experimental study in 2 semi-urban and 2 rural areas, where the changes will be introduced during the study period;
- Workshops for training and exchanging scientific information and results between the teams (Belgium, Mexico, Ireland and Cuba);
- Prepare papers to be submitted to national and international journals and organize a Regional Conference to disseminate intermediate and end-results.

Expected outcome

⇒ An improved emergency subsystem in terms of effectiveness, efficiency and acceptation by the population and information for evidence-based decision-making at local, provincial and national level;

⇒ A final report with detailed recommendations for national scaling up and monitoring to be discussed with the Ministry of Public Health of Cuba;

⇒ Development of tools for costing health care delivery that are adapted to the Cuban context;

⇒ Increased research capacity in teams participating in the project and health workers and decision-makers related to the system;

⇒ International dissemination of results relevant for Health System Reforms in other countries.

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Contract number: IC18*CT980350

UTILIZACIÓN DEL ANALISIS OPERACIONAL PARA MEJORAR LA INTEGRACIÓN DE LOS PROGRAMAS CONTRA LA TUBERCULOSIS EN LOS SERVICIOS DE SALUD EN AMÉRICA LATINA

Period: July 1998 to June 2002

Co-ordinator: Université Libre de Bruxelles, Brussels, Belgium (Bruno Dujardin)

Objectives

The general objective of this joint research project is to test the applicability of a method, called "operational analysis", designed to improve the integration TCP activities in both private and public health services.

Four specific objectives have been identified:

- ♦ Adapt and implement operational analysis in three different field contexts in Latin America (El Salvador, Nicaragua and Peru) so as to identify the bottlenecks related to the integration process of TCP;
- ♦ Propose recommendations (based upon results of objective 1) to improve this integration and test their acceptability;
- ♦ Introduce operational analysis as a training tool for the public health schools of the three countries;
- ♦ Promote at national and international level the use of this methodology by academic and other institutions i.e. the PAHO and the International Union Against Tuberculosis and Lung Diseases (IUATLD).

Activities

- ✶ Field experience of the health professionals involved in the TCP integration process will be documented and analyzed, through individual interviews and focus groups;
- ✶ In working group sessions, health professionals will together build an operational model with an action-research perspective. This model identifies the different steps, starting from the onset of active TB up to its cure. At each step, health professionals will identify bottlenecks and pitfalls, referring to their own experience;
- ✶ The major weaknesses identified will be investigated by further research and specific methodologies are foreseen;
- ✶ The final results will be presented at national, regional and international levels to disseminate results. Two meetings are scheduled to co-ordinate the work of the whole project.

Expected outcome

- ⇒ Improvement in the care given to TB patients in both the private and public sectors;
- ⇒ Better efficiency and quality of the integrated TCP activities in general health services;
- ⇒ Higher staff motivation and better teamwork between health professionals working in TBC programmes and general health services;

⇒ Dissemination and promotion of the operational analysis methodology at national, regional and international level (PAHO and IUATLD).

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STRATEGIES TO IMPROVE THE USE OF HEALTH SYSTEMS RESEARCH FOR SECTOR REFORM

Period: October 1998 to September 2001

Co-ordinator: University of Heidelberg, Heidelberg, Germany
(Ansgar Gerhardus, Rainer Sauerborn)

Objectives

General objective:

- Improve the use of research for more adequate and effective health sector reform.

Specific objectives:

- Enhance our understanding of the decision-making process regarding selected health sector reform issues;
- Develop a measure of "research use" in the decision-making process;
- Develop and evaluate strategies for better use of research to support evidence based decision-making;
- Strengthen the capacity of DC and EC research groups to communicate with stakeholders of the decision-making process in order to identify research needs and to enhance the use of research results.

Activities

- Case studies in seven countries (Thailand, Vietnam, Pakistan, Mali, Ghana, Burkina Faso, El Salvador) on the decision-making process regarding one health sector reform with specific attention to the role of Health systems research (HSR). Identification of strategies to improve the use of HSR;
- Development of indicators to measure research use;
- Capacity building workshops to strengthen;
  - In-country research;
  - Research advocacy and communication skills (addressed to researchers);
  - Demand and use of research (addressed to decision-makers);
- Intermediate conference in Bangkok and final conference in Brussels.

Expected output

⇒ Seven monographs on the decision-making process and the use of HSR;
⇒ ·Curriculum and trainer's manual for workshops (see above);
⇒ ·Peer-reviewed publications;
⇒ ·Publication of the proceedings of the final conference in book-form;
⇒ ·The entire material will be available via www.

Expected outcome

⇒ Researchers, having identified strategies to influence the decision-making process and acknowledging their role as stakeholders, will support rational decision-making by adequate promotion of their research results;
⇒ Decision-makers will consult and use HSR before formulating a health policy;
⇒ International institutions like WHO, EC, World Bank, and initiatives like Alliance for health policy and systems research, COHRED/ENHR, etc. will profit from our results to enhance rational decision-making in the health sector.

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Contract number: IC18*CT980356

DEVELOPMENT OF AN ODOUR-BAITED TRAPPING SYSTEM FOR USE IN CONTROL OF THE VECTOR OF CHAGAS DISEASE *TRIATOMA INFESTANS*

Period: November 1998 to October 2001

Co-ordinator: University of Greenwich, Kent, United Kingdom (Alan Cork)

Objectives

- Identify and synthesise the aggregation pheromone and arrestant produced by *T. infestans* in copula and the aggregation pheromone released from dry faeces;
- Develop an odour-baited trapping system incorporating synthetic attractants for population surveillance;
- Validate an infestation detection and population monitoring system based on the odour-baited trap for *T. infestans*.

Activities

- Collection of semio-chemicals;
- Behavioural bioassays of natural and synthetic semio-chemicals;
- Chemical analyses of biologically-active collections;
- Detection of biologically-active compounds present in collections;
- Chemical characterisation of biologically-active compounds present in collections;
- Synthesis of biologically-active compounds;
- Confirmation of electro-physiological and biological activity;
- Identification of chemical moieties that impart activity;
- Development of controlled release dispensers;
- Development of odour-baited traps;
- Application of odour-baited traps for monitoring vector population.

Expected outcome

⇒ Behaviourally-active compounds characterised and synthesised by the end of first project year;
⇒ Synthetic attractant for *T. infestans* developed by the end of second project year;
⇒ Controlled release formulation for dispensing semio-chemicals developed by end of first project year;
⇒ Synthetic analogues of semio-chemicals synthesised and tested by end of second project year;
⇒ Odour-baited trap for *T. infestans* developed by end of second project year;
⇒ Odour-baited trap evaluated in field trials against conventional methods by end of third project year.

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THE PENTOSE PHOSPHATE PATHWAY IN *LEISHMANIA* - A TARGET FOR CHEMOTHERAPY

Period: November 1998 to October 2001

Co-ordinator: University of Glasgow, Glasgow, United Kingdom (Michael P. Barrett)

Objectives

- Assess the pentose phosphate pathway and its components as targets for chemotherapy in *Leishmania* parasites, which cause a spectrum of disease world-wide;
- Identify and localise the pathway and assess its overall contribution to cell physiology;
- Purify and characterise in detail three key enzymes: Glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase & transketolase;
- Identify and clone genes from these three enzymes, then remove them from the genome and assess the impact of these mutations on cellular phenotype.

Activities

* (Glasgow) Characterisation of transketolase, cloning of its gene and gene knock out;
* (Brussels) Characterisation of G6PD, cloning of its gene and knock out;
* (Ferrara) Characterisation of 6PGDH and kinetic analysis of other enzymes;
* (Venezuela) Cloning of 6PGDH gene and gene knock-out;
* (Argentina) Identification & Localisation of enzymes of pathway, and assessment of role in cell physiology, protection against oxidant stress;
* (Nigeria) Contribution of the pathway to nucleotide metabolism.

Materials and information will be routinely transferred between laboratories and an exchange of personnel between laboratories will also occur.

Expected outcome

⇒ Characterisation of the pentose phosphate pathway in *Leishmania*. Sub-cellular localisation of the enzymes. Its contribution to cell physiology including defence against oxidant stress and nucleotide metabolism. An appreciation of its practical status as a target for chemotherapy;

⇒ Purification and detailed characterisation of the key enzymes glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase and transketolase;

⇒ Cloning of genes for each of these three enzymes and overexpression for facilitate analysis. Removal of these genes from the *Leishmania* genome to assess phenotype of mutants lacking these genes.

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Contract number: IC18*CT980358

PRODUCTION AND CHARACTERISATION OF MUTANT LEISHMANIA LACKING PROTEINASE GENES AS ATTENUATED LIVE VACCINES

Period: October 1998 to September 2002

Co-ordinator: University of Glasgow, Glasgow, United Kingdom (Graham H. Coombs)

Objectives

- Produce cysteine proteinase-deficient *L. mexicana, L. infantum* and *L. braziliensis*, by targeted gene disruption, that will be have potential as attenuated live vaccines;
- Assess the efficacy of these mutants as immunomodulators by:
  - Comparing the kinetics of the developing immune response following infection with wild type *L. mexicana*, *L. infantum*, *L. braziliensis* or cysteine proteinase-deficient mutants;
  - Monitoring disease progression and the evolving immune response following challenge with wild-type *L. mexicana/L. infantum/L. braziliensis* of animals vaccinated with cysteine proteinase-deficient mutants;
  - Determining if vaccination with the cysteine proteinase-deficient mutants can result in cross-species immunity;
  - Testing the efficacy of the potential vaccines against challenge via sandfly bite and the effects on the mutants of passing them through the sandfly vector.

Activities

- The project will use an innovative approach to the production of attenuated live vaccines based on the genetic engineering of *Leishmania* to generate mutant parasites that lack cysteine proteinases.
- Modern technology for cytokine analyses will be used to determine how the mutant parasites modulate the host's immune response.
- Host-parasite interactions and vaccine efficacy will be assessed using the latest methodology.

Expected outcome

⇒ This project will produce cysteine proteinase-deficient mutants of leishmania.

⇒ This investigation will provide key data on the potential of such mutant parasites as vaccine candidates.

⇒ The study will provide insights into how leishmania are able to modulate the host's immune response.

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SCHISTOSOMIASIS VACCINE NETWORK (SNV)

Period: November 1998 to October 2000

Co-ordinator: Institut Pasteur, Inserm U167, Lille, France (Gilles Riveau)

Objectives

- Develop a strategy for the development of schistosomiasis vaccines from bench to field;
- Find solutions to problems associated with clinical testing of schistosomiasis vaccines;
- Improve communication and to exploit opportunities for collaboration among those researching schistosomiasis vaccines;
- The ultimate objective of the Network is to publish guidelines for the evaluation of schistosomiasis vaccines in populations exposed to natural infections.

Activities

* The SVN will be based on exchange of knowledge through round tables involving members of the partner institutions and the different laboratories, which are or have been involved in INCO-DC Joint Research Projects related to schistosomiasis. A total of four workshops will be organized where both aspects of Bench and Field research will be discussed.
* The Core Group of Partners will prepare the work programme of workshops and be in charge of the edition of WebSite, reports and other publications related to the Network. The Core group will look after the correct functioning and timetable of the workshops.
* The Workshops will around three major points:
  1. To generate scientific discussions on the strategy and the progress towards a schistosomiasis vaccine;
     - Identification of the desired biological effects of a vaccine;
     - Identification of the vaccine candidates;
     - Selection of optimum vaccine formulations;
     - Selection of relevant experimental vaccine testing systems.
  2. To help in the development of human schistosomiasis vaccine trials;
     - To define the criteria for the identification of the target population for clinical trials;
     - To integrate vaccine trials into existing control programmes;
     - To identify and to standardise the criteria for evaluating the efficacy of a schistosomiasis vaccine under field conditions;
     - To examine infection and reinfection patterns and transmission schemes;
     - To exchange experiences regarding operations/logistical possibilities and difficulties related to field trials.
  3. Dissemination of information on schistosomiasis vaccine progress;
     - To suggest and plan courses relevant to schistosomiasis vaccine trials;
     - To diffuse information through a specific SNV WebSite and international media.

Expected outcome

⇒ The definition of the strategies towards the development of schistosomiasis vaccines;
⇒ The identification of the needs and the requirements for vaccine evaluation, and the edition of guidelines for clinical trials in endemic countries;
⇒ The improvement of communication and the exploitation of the opportunities for collaboration among those researching schistosomiasis vaccines, including laboratory researchers, field researchers, and clinicians.

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ADHESION OF *PLASMODIUM-FALCIPARUM*-INFECTED ERYTHROCYTES TO HOST GLYCOSAMINOGLYCANS AND DE-SEQUESTRATION STUDIES IN SAIMIRI MONKEYS

Period: December 1998 to November 2001

Co-ordinator: Karolinska Institutet, Stockholm, Sweden (Mats Wahlgren)

Objectives

The project aims at characterising the adhesion of pRBC to endothelial cells (cytoadhesion) and to uninfected erythrocytes (rosetting) and to design substances that reverse the binding. In particular the receptor chondroitin-sulfate A (CSA) on endothelial cells, the receptor heparan sulphate (HS) on the uninfected erythrocyte and the parasite-derived ligand *Plasmodium falciparum*-erythrocyte-membrane-protein-1(PfEMP-1) will be studied.
The reasons are several:
1) The binding of pRBC to CSA on endothelial cells correlates with certain aspects of complications of the disease;
2) The binding of pRBC to erythrocytes correlates with the occurrence of cerebral malaria and severe anaemia;
3) PfEMP-1 is the ligand involved in binding to CSA on endothelial cells and to HS on un-infected erythrocytes.

Activities

* Characterisation of CSA;
* Characterisation of the HS;
* Functional analysis the gene products responsible for CSA & HS binding by;
* Reversal of pRBC adhesion to CSA or to non-infected erythrocytes (HS) in vivo using the experimental model for human malaria the Saimiri monkey;
* Evaluation of the virulence of parasites with selected adhesive phenotypes (CSA, HS, ICAM-1, CD36, PECAM-1/CD31) using Saimiri monkeys;
* Examination of genetic and phenotypic prevalence of parasite encoded CSA and HS rosetting ligands (PfEMP1) in clinical isolates from malaria endemic areas;
* Analysis of mechanisms implicated in changes of cytoadherent phenotypes.

Expected outcome

The studies will lead to a deep understanding of the molecular mechanisms underlying endothelial- and erythrocyte adhesion. It should also give rise to new approaches for the treatment and prevention of complicated childhood malaria and malaria during pregnancy.

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Contract number: IC18*CT980364

ANALYSIS OF VAR GENE EXPRESSION FROM *P. FALCIPARUM* AND *P. VIVAX* IN THE FIELD

Period: January 1999 to June 2000

Co-ordinator: University of Heidelberg, Heidelberg, Germany (Michael Lanzer)

Objectives

- Investigate the function of *P. falciparum var* genes in the pathophysiology of tropical malaria;
- Assess the entire *var* gene repertoire of a parasite population in a defined field setting;
- Survey the expressed *var* gene repertoire in field isolates;
- Correlate the expression pattern found with case histories and specific disease symptoms;
- Explore the possibility of *var* gene homologues in *P. vivax.*

Activities

* Generate specific sequence tags for the majority of *var* gene variants present in the genomes of the *P. falciparum* clones Dd2 and 3D7;
* Generate specific sequence tags from *var* gene variants of several geographically dispersed *P. falciparum* field isolates, in particular those from India, Kenya, Brazil and Colombia;
* Generate specific sequence tags for such *var* gene variants that mediate defined adherent phenotypes;
* Employ DNA chip technology to generate a filter that contains the sequence tags of all *var* gene variants identified;
* Analyze the *var* gene expression pattern of field isolates using the *var* gene tag chip;
* Verify the existence of *var* gene homologs in *P. vivax* and clone the corresponding gene, if present;
* Verify antigenic variation in *P. vivax* in the field.

Expected outcome

⇒ Sequencing and collation of DBL regions from a range of *P. falciparum* isolates, with special emphasis on clinical isolates collected in Brazil, India, Kenya and Colombia;
⇒ A DBL microchip for rapid and easy screening of silent and expressed var gene variants in field settings;
⇒ Analysis of expressed *var* gene repertoire in field population by hybridization to DBL microarray;
⇒ Verification of *var* gene homologues in *P. vivax;*
⇒ Complete sequence of a sub-telomeric *P. vivax* YAC;
⇒ Verification of antigenic variation in *P. vivax.*

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Contract number: IC18*CT980366

LATIN AMERICAN NETWORK FOR RESEARCH ON THE BIOLOGY AND CONTROL OF TRIATOMINAE (ECLAT)

Period: December 1998 to November 2001

Co-ordinator: London School of Hygiene and Tropical Medicine, London, United Kingdom
(C.J. Schofield)

Objectives

This Concerted Action Programme is designed to provide a co-ordinated network promoting collaborative research on the biology and control of Triatominae, in support of Chagas disease control programmes throughout Latin America. In addition to basic and applied research, the network also acts as a focus for discussion and liaison between research scientists, operational personnel, and industries involved in Chagas disease control. Research is focused on the comparative population genetics and dispersal (gene flow) of primary and secondary vector species, especially in relation to recolonisation of treated communities and the adaptive mechanisms involved in colonising new domestic and peridomestic habitats. The following species groups are given priority in view of their current vector status and trend to adapt to become more highly domesticated:

a. - intraspecific studies (especially studies of gene flow and identification of population markers) *T.infestans; T.brasiliensis; T.dimidiata; T.rubrofasciata; P.megistus; P.geniculatus; P.rufotuberculatus; R.prolixus; R.pallescens* and *R.ecuadoriensis;*

b. - interspecific studies (especially genetic differentiation between populations) *R.prolixus* group; *T.infestans* group; *T.sordida* group; *T.phyllosoma/dimidiata* group; *T.barberi/protracta* group.

The network is also designed to promote research on less well known species of potential epidemiological significance, to clarify the adaptive processes involved, develop markers for entomological surveillance, and help assess the potential for control.

Activities

- Investigate intraspecific variation as a means to estimate rates of gene flow and dispersal of individual bugs between silvatic, peridomestic and domestic populations;
- Develop genetic and/or morphometric markers for identifying the source of domestic vector populations, especially in cases of reinfestation following control interventions;
- Measure rates of population growth and dispersal to help in planning sustainable epidemiological surveillance and control;
- Describe the ecological and genetic mechanisms that influence adaptation from silvatic to domestic habitats, so that such factors can be monitored in areas undergoing major ecological changes;
- Develop taxonomic indicators for species identification, in order to define regional control targets (this is particularly important for the *prolixus* group in Andean pact countries, and for the *phyllosoma/dimidiata* group in Mexico and Central America);

* Test the general hypothesis of speciation by radiative adaptation from a discrete source, and assess the role of recent human activities in promoting specific adaptations.

The network also has a series of technical objectives related to promoting scientific interchange and collaboration between different research groups, providing technical data and advice to control services, and stimulating research in countries with little experience in this field. We also seek to test the use of new techniques such as ribosomal and mtDNA sequencing, microsatellite analysis, and comparisons of salivary gland proteins and digestive enzymes, as additional markers for population genetics and studies of evolutionary processes within the group.

Expected outcomes

⇒ Support for control services:

Mexico - (joint ECLAT/Min. Health workshop being planned for early 1999); recommendations for national surveillance and control;

Peru – assistance with evaluation of control requirements in northern departments, and evaluation of *T. infestans* control in southern departments;

Colombia and **Venezuela** – clarification of domestic status of *R. prolixus* as target for eradication or control; recommendations for continued surveillance of *R.prolixus* and monitoring of secondary species (e.g. *T. maculata*);

Ecuador – development of control and surveillance recommendations for *T. dimidiata*; development of control and surveillance recommendations for Amazon species;

Central America – assistance with monitoring *R.prolixus* populations; development of control and surveillance recommendations for *T.dimidiata* and *T.nitida;*

Brazil – expansion of surveillance and control activities against *T.brasiliensis* and *T.pseudomaculata* (cf. contract number ERBIC18CT960042); surveillance studies of Amazon species.

⇒ Research outputs

- comparison of new techniques (DNA sequencing) with established techniques (isoenzymes, morphometry etc.); extension of SI-ECLAT software for sequence data and correlations with phenetic data; revision of procedures;
- *Rhodnius prolixus* – clarification of domestic status in Colombia and Venezuela; confirm origins of Central American strains;
- *Rhodniini* – complete phylogenetic analysis;
- *Panstrongylus* – preliminary phylogeny based on morphometry;
- *T.protracta/barberi* complex – geographical reconnaissance; revised distribution maps; initial population genetic studies;
- *T.phyllosoma/dimidiata* complex – geographical reconnaissance; revised distribution maps; initial population genetic studies;
- *T.rubrofasciata* – initiate collections and lab. colonies;
- Amazon species – geographical reconnaissance; revised distribution maps; initial population genetic studies;
- general description of evolutive processes in the Triatominae, with recommendations for monitoring these processes in areas experiencing major land use changes.

Contract number: IC18*CT980366

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Contract number: IC18*CT980371

ENZYMES INVOLVED IN STEROL BIOSYNTHESIS AS TARGETS FOR TREATMENT OF LEISHMANIASIS

Period: November 1998 to October 2001

Co-ordinator: Consejo Superior de Investigaciones Científicas, Granada, Spain
(Dolores González Pacanowska)

Objectives

- Establishment of specific aspects regarding enzymes involved in sterol biosynthesis in *Leishmania* that may aid in the design of new inhibitors;
- Explore the potentials for developing antileishmanial agents based on inhibitors of sterol biosynthesis inhibitors;
- Understand the mode of action of sterol biosynthesis inhibitors.

Activities

* Heterologous expression of HMGCoA reductase in bacteria;
* Detailed kinetic and functional characterisation of HMGCoA reductase;
* Cloning and heterologous expression of sterol C-24 methyltransferase, an enzyme not present in the vertebrate host;
* Antisense agents to establish the role of C-24 methenylation and HMGCoA reductase;
* Design and synthesis of new sterol biosynthesis inhibitors;
* Analysis of the interaction of already known and newly synthesised compounds with sterol biosynthesis enzymes;
* Testing of different combinations of HMGCoA reductase,C-24 methyltransferase and 14 α-demethylase inhibitors for inhibition of growth of *L. donovani*. *L. major* and *L. mexicana*;
* Identification and further optimisation of candidate inhibitors;
* Studies on the ultrastructural effects of sterol biosynthesis inhibitors;
* Detailed intracellular localisation studies of enzymes involved in sterol biosynthesis

Expected outcome

⇒ Cloning and overexpression of sterol C-24 methyltransferase;
⇒ New C-24 methyltransferase and HMGCoA reductase inhibitors. Inhibitors with differential specificity and active on cultured cells;
⇒ Determination of the ultrastructural effects of new inhibitors;
⇒ Establishment of the intracellular localisation of sterol biosynthesis.

Contract number: IC18*CT980371

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Contract number: IC18*CT980372

RATIONAL DRUG DESIGN IN LEISHMANIASIS: MECHANISM-BASED INHIBITORS OF TRYPANOTHIONE BIOSYNTHESIS

Period: October 1998 to September 2001

Co-ordinator: University of Antwerp, Antwerpen, Belgium (A. Haemers)

Objectives

The discovery of new and non-toxic antiparasitic drugs with a broad spectrum of activity against all diseases caused by leishmania. This proposal concentrates on the biosynthesis of trypanothione (N^1,N^8-(bisglutathionyl)-spermidine), more especially on two enzymes, glutathionylspermidine synthetase (GSS) and trypanothione synthetase (TS).

Activities

* Clone and express these enzymes for detailed inhibitor studies;
* Design and synthesis of a new group of leishmanicidal molecules, targeted towards the trypanothione synthesizing enzymes, using already developed inhibitors (Ki in the lower micromolar level) as lead compounds;
* Determine their inhibitory properties against both enzymes;
* Select the most interesting compounds against *Leishmania* species by *in vitro* and *in vivo* screening. Compounds will also be tested against *Trypanosoma b. brucei* and *T. cruzi*;
* Further study the structure-activity relationship of the compounds for optimization of both inhibitory activity and bioavailability as leishmanicidal agents.

Expected outcome

⇒ Pure enzymes: glutathionylspermidine synthetase and trypanothions synthetase;
⇒ Inhibitors of these enzymes;
⇒ Lead compounds for further development of leishmanicidal agents.

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Contract number: IC18*CT980373

GENETICS OF HUMAN SUSCEPTIBILITY TO SCHISTOSOMIASIS, VISCERAL LEISHMANIASIS AND CEREBRAL MALARIA

Period: November 1998 to October 2001

Co-ordinator: INSERM Unité 399, Marseille, France (Alain Dessein)

Objectives

- Test the existence of major gene(s) controlling visceral *Leishmaniasis (Leishmania donovani)* in a Sudanese population;
- Identify loci of susceptibility to cerebral malaria (*Plasmodium falciparum)* in a population of Mali;
- Identify and characterize the major gene (SM1) that controls infection by *Schistosoma mansoni* in a population of Brazil;
- Map the major gene (SM2) that controls Symmers fibrosis in a Sudanese population;
- Test whether the genetic controls of visceral leishmaniais, cerebral malaria, and hepatic fibrosis (schistosomiasis) correlate with specific cytokine or cytokine receptor phenotypes;
- Train young scientists from Brazil, Sudan and Mali in Genetics of infectious diseases;
- Set up a network of laboratories working on the Genetics of parasitic diseases.

Activities

- Epidemiological analysis (identification of environemental and behavioral risks factors in severe fibrosis caused by *S. mansoni* and in visceral leishmaniasis);
- Genetic analysis, (Segregation analysis, Sib pair analysis, linkage analysis using wide genome search), to identify major loci of suceptibility;
- Molecular Genetics (detection of polymorphisms, gene sequencing, analysis of the effects of the mutations at the molecular level) to identify gene of susceptibility to infection and disease;
- Immunological studies on cytokines and cytokine receptors in subjects resistant or susceptible;
- Training in Epidemiology, Genetics and Immunology.

Expected outcome

- ⇒ Identification of the genetic polymorphisms that play a major role in the determination of human susceptibility to infection by *S. mansoni*;
- ⇒ Identification of major gene control of human susceptibility to visceral leishmaniasis; localisation of the(se) gene(s) in human genome;
- ⇒ Identification of loci of susceptibility to cerebral malaria;
- ⇒ Linkage of the genetic effects with the cytokine response to these infectious agents;
- ⇒ Training of young scientists from Mali, Sudan, and Brazil in the field of Genetics, Epidemiology and Immunology of parasitic diseases;
- ⇒ These results should allow new approaches in the development of drugs and vaccines against schistosomiasis, leishmaniasis, and malaria. Diagnostic tests could be developed to identify susceptible individuals.

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Contract number: IC18*CT980374

MOLECULAR EPIDEMIOLOGY OF HUMAN RESPIRATORY SYNCYTIAL VIRUS INFECTIONS

Period: October 998 to March 2002

Co-ordinator: University of Birmingham, Birmingham, United Kingdom (Patricia Cane)

Objectives

We intend to continue previous work on the genetic and antigenic analysis of the attachment (G) protein of human respiratory syncytial virus (HRSV) isolates from European and South American countries and to evaluate the relevance of G protein epitopes in the post-infection immune response as well as to assess the incidence of HRSV infections in the general population. These studies should provide a better understanding of the epidemiology of HRSV infections and indicate control measures for prevention of the disease.

Activities

* Isolation of HRSV from clinical specimens (nasopharyngeal washes) and to evaluate the antigenic and genetic variability of the G glycoprotein;
* Analysis of the reactivity of antibodies from patient sera with G protein segments (fragments, synthetic peptides) of matched viruses;
* Evaluation of the relevance of G protein antigenic variation in the induction of cross-protective immune responses;
* Estimation of the incidence of HRSV infections in the general population by screening blood samples for antibody titres.

Expected outcome

Each of the specific objectives can be easily quantitated by the number of specimens, viruses and sera included in the different assays. It is estimated that 300-400 samples/year of diagnosed HRSV infections will be available in the different participating laboratories. From these, 50-80 viruses and matched sera will be obtained for the antigenic, genetic and serological assays. Several hundreds of blood donor samples will be collected yearly for screening of antibody titres to allow an estimation of levels of RSV antibodies in the general population. Finally, recombinant vaccinia viruses expressing the G protein of 3-4 well characterised viruses will be used to immunise groups of 5-6 mice. These will be challenged with HRSV isolates representative of the different antigenic groups and subgroups and give data on the degree of cross-protection in experimental animals.

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Contract number: IC18*CT980377

DEFINITION OF NOVEL *MYCOBACTERIUM TUBERCULOSIS* ANTIGENS FOR VACCINATION AGAINST, AND EARLY DETECTION OF TUBERCULOSIS

Period: January 1999 to December 2001

Co-ordinator: Leiden University, Leiden, The Netherlands (T.H.M. Ottenhoff)

Objectives

- Define novel antigens (Ag) of *M. tuberculosis* for induction of CD8 and CD4 T cell immunity in TB;
- Determine the immunodominance of these Ag for CD4 and CD8 T cells in human TB in an endemic area;
- Determine the functional programmes and subsets of Ag specific immune T cells in TB;
- Determine the antigenicity of novel specific Ag and peptides for human B-cells (IgA, IgG, IgM) in a TB endemic area.

Activities

- Protective immunity to TB is dependent on Ag specific, MHC II restricted CD4 T helper-1 cells as well as MHC class I dependent CD8 cells. The Ag that trigger human CD4 Th-1 cells have only been identified in part, whereas the target Ag recognized by CD8 T cells are essentially unknown. We propose to use recently developed technologies to identify Ag for CD8 and CD4 T cells. This will include DNA vaccination of HLA class I and II transgenic mice as a novel approach to define such Ag and their epitopes, and to establish their immunogenecity in the context of human MHC. Additional new approaches will be used to define novel Ag for human T cells, notably advanced mass spectrometry to identify *M.tuberculosis* stress Ag and combinatorial peptide libraries to identify novel epitopes and Ag for *M.tuberculosis* specific T cells.
- Examine whether the above identified Ag are efficiently recognized by polyclonal human T cells, in comparison to already known major Ag (hsp, early culture filtrate Ag) we will use well established human T cell assays (proliferation, cytokine production, expression of activation markers, intracellular cytokines, ELISPOT) to determine specific recognition by T cells from blood and pleural effusions from patients with various forms of disease as well as healthy individuals and carefully compare T cell responses to those induced by known dominant Ag.
- We will examine in detail the T cell subsets that are induced by relevant Ag in patients and healthy contacts by using cytokine production assays and recently identified Th1/Th2 related activation markers, as well as by determining the capacity of these T cells to induce killing of *M.tuberculosis* infected macrophages.
- We will investigate whether specific Ag or peptides, identified either by the approaches outlined in 1 or by scanning of the genomic sequence bank of *M.tuberculosis* using well-defined B cell epitope algorithms, can be used to develop novel and specific diagnostics tests that detect TB specific antibodies (IgG, IgM and IgA). Initially, ELISA will be used; in a later stage, promising peptides or Ag will be applied to a simple dipstick technique. Available sera from well defined TB patients and controls will be used.

Expected outcome

⇒ Definition of novel antigens and peptides for vaccine development and serodiagnosis.

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Contract number: IC18*CT980378

ISOLATION, CHARACTERIZATION AND MOLECULAR CLONING OF VARIANTS OF HEPATITIS A VIRUS (HAV) CIRCULATING IN SOUTH AMERICA, AND EXPRESSION OF ANTIGENS INVOLVED IN VIRUS NEUTRALIZATION BY RECOMBINANT DNA TECHNIQUES

Period: November 1998 to October 2000

Co-ordinator: Gruppo Romano Virologia Oncologica, Rome, Italy (Raoul Perez Bercoff)

Objectives

- Search for variants of hepatitis A virus circulating in South America;
- Isolation and characterization of these variants, the long-term goal of such endeavour being the development of safe, cheap polyvalent vaccines against Hepatitis A by recombinant DNA techniques.

Activities

To this end, the three Latin American laboratories will conduct an extensive molecular epidemiologic survey searching for circulating variants of Hepatitis A virus. This will include:

- Molecular Epidemiology Survey; <MI>;
- Search for HAV-like Viruses in Pathological Specimens: by molecular hybridization;
- Search for HAV Variants in the Environment: in samples of food, waste waters, etc. collected and treated by standard techniques;
- Tentative isolation of HAV Variants;
- Molecular Cloning of the Viral Genome(s);
- Nucleotide Sequencing and Phylogenetic and Statistical Analyses.

Expected outcome

Besides the training of Latin American scientists in up-to-date techniques, these studies are expected:

⇒ Tracing the origin of epidemic outbreaks;
⇒ Quantify the rate of evolution, and
⇒ Designing effective vaccines by choosing (when required) relevant variant antigens for vaccine formulation.

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Contract number: IC18*CT980387

SELECTION OF *P. FALCIPARUM* ANTIGENS FOR MPES VACCINE DEVELOPMENT

Period: February 1999 to May 2000

Co-ordinator: Institut Pasteur, Paris, France (Pierre Druilhe)

Objectives

- Compare various means of delivering the *P. falciparum* pre-erythrocytic antigen LSA3 in terms of the protection afforded against a virulent challenge;
- Assess the potential of a set of 10 new *P. falciparum* pre-erythrocytic antigens;
- Further the identification of surrogate markers of protection i.e. immune responses relevant to protection.

Activities

* The *P. falciparum* LSA3 antigen has been shown to be very immunogenic using a large range of antigen delivery systems. We will immunise simultaneously mice using these and new means of immunisation and compare the protection afforded after *P. yoelii* sporozoite challenge. The most consistent formulations will be thereafter employed in aotus monkeys and in chimpanzees and compared in terms of protection against a *P. falciparum* sporozoite challenge;
* A set of 10 new pre-erythrocytic antigens has been selected from 120 clones, representing ca. 20 genes. These will be recloned in histidine-tailed vector and Vical DNA vector ; using these formulations, the vaccine potential of these new antigens will be assessed by immunisation of Aotus and chimpanzees followed by a challenge by *P. falciparum* sporozoites;
* Previous work on irradiated sporozoites immunised chimpanzees, LSA3 immunised mice, aotus and chimpanzees has already supplied strong indications in favour of which types of immune responses are related to protection (and conversely, which are not). We will further these investigations in additional animals and with more detailed immunological analysis.

Expected outcome

⇒ It is already demonstrated that the *P. falciparum* LSA3 antigen can induce protection against *P. falciparum* challenge in chimpanzees and aotus and against *P. yoelii* in mice. Four types of antigen deliveries have so far led to reach protection, however, not always in 100 % of animals immunised. We expect from the above activities to identify an improved administration regime using one of these 4, or a novel formulation offering enhanced protection against challenge;

⇒ It is now clear that the pre-erythrocytic stages express a large number of antigens. Even though LSA3 shows great promise, we believe that it is vital at this stage to examine the potential of some of the remaining molecules, particularly those which are stage-specific

(not expressed in blood stages), as alternative candidates or molecules which could reinforce the protection afforded by LSA3 alone;

⇒ The identification of the mechanisms responsible for defence, or at least of an immunological parameter correlated to protection, (even though it may not be the effector arm directly mediating protection). This is critical to rationalise vaccine development and is relevant to all steps of pre-clinical trials in animals, at GMP grade production level and to monitor phases-I, II and III of vaccine development.

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INCO-DC

Natural Resources and Agriculture

Contract number: IC18*CT950002

Period: August 1996 to July 1999

CONTROL OF *TAENIA SAGINATA* AND *TAENIA SOLIUM* CYSTICERCOSIS THROUGH SPECIFIC DIAGNOSIS; SYSTEMATIC EPIDEMIOLOGY AND DEVELOPMENT OF RECOMBINANT VACCINE CANDIDATE

Co-ordinator: University of Edinburgh, Edinburgh, United Kingdom
(Leslie Jayne Stevenson Harrison)

Objectives

- Provide suitable sensitive and specific diagnostic tools in order to assess control of human, porcine and bovine cysticercosis via diagnosis and drug treatment in selected ecological zones. Of particular importance will be the detection of neurocysticercosis in man.
- Assay selected recombinant antigens as potential vaccine candidates.

Activities

* Transfer established diagnostic procedures from Europe to participating developing countries.
* Apply existing diagnostic tools to epidemiological monitoring and where appropriate control studies in participating developing countries.
* Clone, express and evaluate potentially diagnostic and protective antigens from oncospheres and immature metacestodes.
* Establish panels of defined sera for primary screening and evaluation of potentially useful recombinant antigens, both diagnostic and protective.
* Develop recombinant parasite antigen based detection assays.
* Carry out vaccine trials in pigs and cattle using potentially protective recombinant antigens.

Expected outcome

Taenia saginata and *Taenia solium* are responsible for public health problems in addition to creating financial losses to cattle and pig producers in endemic areas. This project aims at improving the available methods for control through improved diagnosis and immunoprophylaxis. The project is a second phase to a two-year INCO-DC STD-3 project. it will apply the technology and reagents developed in Phase 1 and those developed in this project to epidemiological and vaccination trials in a range of endemic countries. It will introduce work on the closely related *T. saginata*. It is possible to work on both species since they are so closely related that they contain many cross-reactive determinants and can be regarded as reciprocal models.

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Period: November 1996 to October 1999

A NEW APPROACH FOR DEVELOPING A SUSTAINABLE DISEASE MANAGEMENT SYSTEM FOR BEAN, BASED ON HEALTHY LEAF AREA DURATION AND PHOTOSYNTHETIC EFFICIENCY

Co-ordinator: Universität Hannover, Hanover, Germany (Bernhard Hau)

Objectives

- Develop methods for estimating disease severity and host parameters, like healthy leaf area duration and healthy leaf area absorption.
- Determine losses caused by single diseases and by combination of several diseases.
- Quantify the effects of control measures on the development of diseases and on plant growth.
- Determine which physiological parameters, like the photosynthetic efficiency of leaves, are affected by different pathogens.
- Model the development of diseases and their effects on bean growth and yield.
- Design a system for integrated bean-disease management.

Activities

- Field experiments on bean are carried out to assess the effects of single diseases and disease combinations on host growth and yield. Diseases studied: bean rust (*Uromyces appendiculatus*), anthracnose (*Colletotrichum lindemuthiamum*), angular leaf spot (*Phaeoisariopsis griseola*), Fusarium wilt (*Fusarium oxysporum* f. sp. *Phaseoli*), common bacterial blight (*Xanthomonas campestris* pv. *Phaseoli*), and bean golden mosaic (BGMV). In addition, experiments to quantify the effects of control on disease dynamics as well as on host growth are being conducted. Meteorological data, including radiation, are recorded by automatic weather stations. Besides the manual assessments of bean growth, measurements are taken with a radiometer and a ceptometer.
- Laboratory experiments permit to determine the influence of diseases, alone and in combination, on the relative net photosynthetic rate and on fluorescence. Moreover, chlorophyll fluorescence images are used to map the photosynthetic efficiency of leaves.
- The global analysis of all field data and the results of the laboratory experiments form the basis of the development of a model describing host growth and disease dynamics, as well as the effects of diseases on yield. A system for integrated disease management will be designed, using cost/benefit analyses from control experiments in the fields.

Expected outcome

The work carried out in this project will help to better understand the effect of diseases on host growth and yield. This will result in designing a system for integrated disease management on bean that will include not only the actual disease situation but also the status of the host plant. This system will help bean growers make better decisions on control measures. This will reduce yield losses and act as a safeguard against long-term risk of environmental pollution, hazards to human health, and reduced agricultural sustainability.

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Contract number: IC18*CT960038

Period: October 1996 to September 1999

NON-TIMBER FOREST PLANT RESOURCE ASSESSMENT IN NW AMAZONIA

Co-ordinator: Universiteit Amsterdam, Amsterdam, The Netherlands (Joost F. Duivenvoorden)

Objectives

- Carry out a market survey of NTFPP in NW Amazonia.
- Carry out a comparative assessment of NTFPP resource availability in different forest types in three pilot areas in NW Amazonia.

Activities

- Initial seminar for the project's participants: to get to know each other and to discuss relevant project issues at an initial stage, including organization, methods, publication policy, data handling, intellectual ownership, time schemes, benefits and goals.
- Market survey of NTFPP in NW Amazonia: comprising a reconnaissance of current level of commercialization of NTFP products in NW Amazonia, on basis of field visits to selected local, and regional/national markets.
- Assessment of potential NTFPP resource availability: comprising quantitative ethnobotanic and ecological research in plots of 0.1 ha and along transects in pilot areas in Ecuador (Yasuni area), Peru (basins of Ampiacu and Yaguasyacu rivers), and Colombia (middle Caquet river basin). Methods to estimate the potential usefulness of trees and lianas (down to 2.5 cm diameter) in forest types, recognised on recent aerial photographs and satellite imagery, will be compared and evaluated.
- Final book: includes editing of reports into chapters of the final book, which will be printed and distributed among all partners and collaborating communities.

Expected outcome

⇒ Improved knowledge of market situation for commercial NTFPP extraction in NW Amazonia.

⇒ Evaluations of NTFPP assessment techniques, with improved estimates of NTFPP usefulness of different forest types, also shown on maps.

⇒ Strengthening of research and fieldwork capacity of Latin American scientists regarding NW Amazonia forest ecosystems and NTFPP resource management.

⇒ Spanish book on NTFPP forest resources in NW Amazonia on the basis of the contributions from all researchers.

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Contract number. IC18*CT960044

Period: October 1996 to September 1999

CONTROL OF CITRUS VIRUS DISEASES IMPORTANT IN THE MEDITERRANEAN AREA AND SOUTH AMERICA: DEVELOPMENT OF MOLECULAR PROBES FOR QUICK DETECTION OF SEVERE STRAINS OF CITRUS TRISTEZA VIRUS (CTV) AND PSOROSIS-RINGSPOT

Co-ordinator: Instituto Valenciano de Investigaciones Agrarias, Valencia, Spain
(Pedro Moreno)

Objectives

- Develop quick and specific procedures to discriminate between mild and severe strains of Citrus tristeza virus (CTV) and set up a simple protocol for detection of severe CTV strains in field trees or nursery plants.
- Use this technology for:
  -- early screening of mild CTV isolates with protective capacity against severe isolates
  -- monitoring cross protection in the field.
- Develop quick and reliable methods to diagnose and characterise psorosis-ringspot isolates from the Mediterranean area and South America.
- Search for the natural vector of psorosis-ringspot in the South American areas where this disease naturally spreads.

Results so far

⇒ The complete genome sequence of a Spanish mild CTV isolate has been obtained. Sequence comparisons between isolates of different biotypes revealed some groupings that have been used to develop specific probes. Differences in the 5' end have allowed for the first time to classify CTV strains in three groups.

⇒ Quick hybridization and PCR systems have been developed for fast comparison of field CTV isolates. These are being used to assess genetic diversity of CTV populations and to monitor cross protection.

⇒ The genome of a psorosis-ringspot isolate has been partially sequenced and a PCR system for quick diagnosis of the disease has been set up. The procedure is being adapted to be used in field trees.

⇒ Improved antibodies to the virus have also allowed for the first time to detect the virus by ELISA.

Selected publications

García M.L., Sánchez de la Torre M.E., Dal Bo E., Djelouah K., Rouag N., Liosoni E., Milne R.G., and Grau O. 1997. Detection of citrus psorosis-ringspot virus using RT-PCR and DAS-ELISA. Plant Pathology. **46**: 830-836.

López C., Ayllón M.A., Navas-Castillo J., Guerri J., Moreno P. y Flores R. 1998. Sequence polymorphism in the 5' and 3' terminal regions of citrus tristeza virus RNA. Phytopathology **88**: (In press).

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Contract number: IC18*CT960063

Period: October 1996 to September 2000

FITTING MAIZE INTO CROPPING SYSTEMS ON ACID SOILS OF THE TROPICS

Co-ordinator: Universität Hannover, Hanover, Germany (Walter J. Horst)

Objectives

- Develop screening procedures for aluminium (Al) resistance in maize.
- Develop screening techniques for phosphorus (P) efficiency in maize.
- Select and breed maize cultivars with improved adaptation to acid soils high in Al and low in P content.
- Evaluate the site specificity of acid soil resistance of maize cultivars.
- Improve the in-depth knowledge of the physiological mechanisms responsible for Al resistance and P efficiency in maize.
- Screen a larger maize germplasm based on this physiological understanding.
- Improve the quantitative understanding of the comparative advantage of the genetic and agronomic approaches to solve the problem of maize production on acid soils.
- Develop agronomic techniques for good maize seedling-establishment in acid soils.
- Develop a simulation model allowing to predict the performance of a maize seedling in a specific acid soil.

Activities

- ***Plant improvement:*** Field screening of various genetic material on acid tropical soils, using standardized experimental conditions at several field sites in the tropics, carefully selected for their soil characteristics. Further improvement of quick laboratory screening techniques for Al resistance (root elongation, callose formation, hematoxylin staining) and correlation to field screening.

- ***Agronomy:*** Long-term field experiments are carried out at the tropical sites on acid soils. Factorial treatments comprise 2 lime rates, 2 P rates, 3 organic manure rates, and 3 maize cultivars differing in adaptation to soil acidity. Measurements to be taken each year are: analysis of soil chemical and biological characteristics, shoot and root growth, yield, nutrient status and uptake of maize. In addition, in field and laboratory studies, different factors affecting seedling viability in an acid soil environment such as localized lime application and N amount and source will be studied in more detail.

- ***Plant/soil interaction:*** A mathematical model based on two variable charge exchangers (soil, root) linked by the soil solution will be further developed and validated under controlled conditions and evaluated by comparison with data measured under field conditions. Possible interacting effects of N source, Mg deficiency, Mn toxicity, and organic soil compounds on Al toxicity will be taken into consideration.

- ***Physiology:*** The fundamental role of organic ligands and polyamines, and the possible role of polypeptides and proteins in Al toxicity and resistance of maize cultivars are studied under controlled conditions. A technique will be developed, which allows the rapid characterization of the P efficiency of maize cultivars, and possible physiological mechanisms of P efficiency in relation to Al resistance will be studied.

Expected outcome

It is expected that the project will contribute to the development of maize cultivars and accompanying agronomic technologies for natural recource-friendly, sustainable, and economic cultivation of maize on the acid soils of the world with special emphasis on South America.

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Contract number: IC18*CT960067

Period: December 1996 to November 1997

SUSTAINABLE PRODUCTION OF NATURAL RESOURCES AND MANAGEMENT OF ECOSYSTEMS: THE POTENTIAL OF SOUTH AMERICAN CAMELID BREEDING IN THE ANDEAN REGION

Co-ordinator: Ente per le Nuove Tecnologie, l'Energia e l'Ambiente, Roma, Italy
(S. Vinella)

Objectives

The broad objective of the research programme is the definition of an integrated intervention strategy for the sustainable development, in the Andean region, of the production chain related to fibres and meats of Domesticated South-American Camelids (DSC) (alpaca and llama).

Specific objectives are:

- Identification of regional policies of sustainable development connected to the productive use of DSC.
- Definition of an institutional and social approach to ecosystem management in the areas of DSC production
- Identification of resource-management methods and operative tools aimed at strengthening the potential of the DSC production chain, from the pastures and DSC stock-breeding to the manufacturing and trade of fibre and meat.
- Integration of the RTD activities with the development programmes ongoing in the Andean region.

Activities

* Providing an information framework to support the development of policies of sustainable management of natural resources in the Andean region. The work is carried out for selected DSC production areas, representative of the major ecosystems of the Andean altiplano. To this aim:
  - a basic information structure for the analysis of the interrelations between ecosystems and economic activities, to be used as an ecosystem management tool, will be developed
  - an analysis and assessment of the environmental impact of the DSC production chain in relation to specific and critical aspects/problems of ecosystems management will be performed.
* Understanding the relationship between socio-economic and policy factors affecting the agro-industrial activities and the management of natural resources in the Andean region and outlining policy recommendations for the improvement and growth of the sector, that would be compatible with local socio-economic and environmental conditions. To this aim, a methodology for the analysis of the socio-economic factors in the sustainable development of the DSC production chain will be set up? The methodology will be enhanced or documented by applicative solutions on specific aspects/problems.

* Improving the effectiveness of the DSC production chain without producing negative effects on the environment. The work will be carried out in the field, in selected areas of the Andean altiplano, and in experimental settings. To this aim:
  - methods of sustainable management of stock breeding will be developed
  - actions to improve the production of quality DSC fibre and meat will be devised
  - methods and systems of quality control of DSC fibre and meat will be studied.

Expected outcome

The work carried out should provide guidance for the management of all natural, technological and human resources involved in the development of DSC fibre and meat production. It is aimed at supporting ecosystems characterized by the existence of very-low income social groups, having poor economic growth expectations, and by environments the climatological, ecological and productive features of which are fragile and threatened by irreversible deterioration.

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Contract number: IC18*CT960068

Period: January 1997 to June 1999

ENVIRONMENTAL LAW AND LOCAL MANAGEMENT OF NATURAL RESOURCES. COMPARATIVE RESEARCH IN BRAZIL AND COSTA RICA

Co-ordinator: Groupe de Recherche et d'Echanges Technologiques, Paris, France
(Philippe Lavigne Delville)

Objectives

- Identify in the law texts of the two countries the competence of local communities and local governments in regard to environmental law, management of natural resources and land use.
- Identify the obstacles to the application of those rights (legal, technical, cultural, administrative, communicational - including the balance of power at the local level - , economic incentives or disincentives, using the "stakeholders analysis" method.)
- Promote the application of those rights in a sample of local communities or local governments (districts), using innovative participatory methods and local planning approaches.
- Based on the experimentation and comparison between sites and countries, make recommendations to adapt the national legal setting and environmental policies.
- Learn from experience in terms of consulting, participatory research, and training methodologies for the sustainable management of natural resources by local entities.

Activities

* Selective inventory of the laws concerning NRM.
* Choice of application and experimentation areas and concerned local entities and/or groups.
* Experimentation of participative approaches for natural resource management diagnosis, local planning and conflict-resolution methods involving the local groups or entities and the national government.
* Analysis of the processes and outcomes, examination of the difficulties or limits in the application of national laws, and of the potential and limits of local rule-setting.
* Cross-country comparison based on two international exchanges (field visits and seminars), one in Costa Rica and one in Brazil.
* Synthesis and recommendations.

Expected outcome

⇒ The work carried out will help thinking over such important topics for the management of natural resources as local planning and control. It will deal with taxes, land tenure, and ownership rights, creation of regional and municipal parks, commercial but sustainable use of resources, and biodiversity preservation. It will also provide new analyses of projects and methodologies for natural resources management and for communication about environmental rights and policies.

⇒ It will also stimulate exchanges between the representatives of the local organizations,

who will be associated to the field activities and to the final discussions and recommendations of the project, and have a significant training output both for the Brazilian and Costa-Rican researchers as well as for local leaders.

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Contract number: IC18*CT960069

Period: January 1997 to December 1999

CLIMATE IMPACT ON WATER RESOURCES AND DRYLAND AGRICULTURE (CLIWARDA)

Co-ordinator: Winand Staring Centre for Integrated Water Management in Arid Zones, Wageningen, The Netherlands (Massimo Menenti)

Activities

The network participants (fifteen, in eight countries) intend to establish a baseline, against which the impact of forecast climatic variability may be assessed, both by looking at the effects of past climate variability on farming systems (with special reference to irrigated agriculture) and on water resources, and by taking into account the history of land use, of farming systems and of socio-economic conditions. This approach will also provide useful insights into sustainable management of land and water resources in drylands.

Expected Outcome

The network is an attempt to collect and analyze information on hydrological variability in the recent past – 200-300 years – , and on the impact of this variability on agricultural production. The latter will focus on test areas, studied in detail by the network members, where a wealth of data and results exists already. The test areas are: Argentina: the watersheds of Rio Mendoza and Rio Atuel; China: the rims of the Taklimakan desert and the Hei He and Shiyang He watersheds in north-western China; India: the basins of the rivers Luni and Yamuna; Egypt: oases in the Western Desert of Egypt and the eastern Nile Delta; Niger: rainfed agricultural lands. These studies will provide the basis for assessing the sensitivity of these production systems to expected climate variability.
The network is an attempt to establish a unique co-operative mechanism among research organizations and countries with scarce opportunities for joint research efforts on specific subjects, notwithstanding the similar, and at times identical, scientific and development issues they face. The joint efforts will also be an opportunity to compare techniques used in the participating institutes:

1) analysis of historical archives and archaeological studies;
2) geomorphological studies;
3) use of tree-ring chronologies;
4) numerical modelling of regional hydrological processes and of crop growth in farming systems;
5) experimental and modelling studies of land- surface-atmosphere interactions in relation with land use (irrigated lands vs. deserts);
6) remote sensing and geographical information systems;
7) computational decision support tools for water management;
8) analysis of policy and institutional constraints on resource management

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Contract number: IC18*CT960073

Period: December 1996 to November 2000

GEO-ENVIRONMENTAL DYNAMICS OF PANTANAL-CHACO: MULTITEMPORAL STUDY AND PREVISIONAL MODELLING

Co-ordinator: Universitá degli Studi di Siena, Siena, Italy (Luigi Carmignani)

Objectives

- Identification of the relationships and connections among the factors that influence the environmental changes in the Pantanal-Chaco ecosystem through the multitemporal study of parameters which have changed during the last 30 years;
- Conceptual and mathematical modeling of evolutionary trends; forecasting of the ecosystem evolution;
- Exchange among the partners of techniques and know-how applied and developed during the project;
- Definition of a methodology for multitemporal analysis and monitoring of natural areas which are either threatened by human activities or affected by geological and climatic changes.

Activities

During the 1st year of the project, the following main activities have been carried out for the Rio Verde do MT pilot area (Eastern Pantanal):

* Digitization, geocoding and processing of existing topographic maps at scale 1:100,000 and 1:250,000. These activities have led to the realization of the Digital Elevation Model (DEM), hydrography network data bank and land cover data bank (for the year 1966), in a GIS environment (Esri Arc-Info®).
* Photointerpretation and classification of 1985 and 1996 Landsat TM images, by means of Erdas Imagine® and Rsde Cartha for Windows®, in order to produce two land cover data banks, also using radar images and aerial photographs;
* Field work during the dry period, mainly to check the preliminary interpretative keys adopted for the Landsat TM images and to collect data on geology, pedology, landforms, erosion-deposition processes, vegetation changes;
* GIS spatial and multitemporal analysis of the data banks from topographic maps and Landsat TM images;
* Analysis of official statistic data regarding: a) agricultural and zootechnical activities during the last 30 years; b) climate, hydrology and hydrogeology.

Results

- DEM and related Slope, Aspect and Hillshade data banks of the studied area;
- Geologic-geomorphologic data bank, underlining the distribution of the unconsolidated superficial formations which may experience rapid erosion.
- Multitemporal land cover data banks related to years 1966-1985-1996 describing the land cover changes occurred in the area during the last 30 years. The analysis of these data banks highlights that more than 50% of the studied area, originally occupied by shrubby vegetation and tropical forest has been deforested to create new areas for agriculture.

Follow-up

In the next years, the work methodology tested in the Rio Verde do MT pilot area will be applied to the whole project area. The new multi-temporal data banks and data resulting from field work will be analyzed through mathematical models in order to understand evolutionary trends and relationships between land cover variation and soil erosion in the highlands and increasing sedimentation in the Pantanal lowlands. A hydrological model of the upper and middle Paraguay-River basin will be developed, too. In some pilot areas evaluation of environmental damages associated with agriculture, zootechnics, urbanization and mining will be carried out.

Expected outcome

This project will provide a characterization of the geo-environmental dynamics of Pantanal-Chaco system and its probable future evolution with respect to the type and intensity of the anthropic activities. The evaluation of the evolutionary trends through the multitemporal approach will help both to manage the important cattle farming, agriculture and mining and to protect the ecosystem.

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Contract number: IC18*CT960076

Period: September 1996 to August 1999

DEVELOPMENT OF ENVIRONMENTALLY FRIENDLY PHOTOACTIVATABLE COMPOUNDS FOR TREATMENT OF MICROBIALLY POLLUTED WATER

Co-ordinator: Università degli Studi di Padova, Padova, Italy (Giulio Jori)

Objectives

- Develop a pilot plan for the decontamination of microbially polluted water by a novel photochemical technique, which is based on the use of visible light (or even sunlight) and porphyrin-type photosensitizers, i.e. a technique requiring a simple and cheap technology and having a low environmental impact.
- Identify porphyrin photosensitizers which exhibit an efficient and non-specific phototoxic action against a broad number of microbial species, including Gram-positive and -negative bacteria, yeasts and mycoplasma.
- Identify an inert water-swollen matrix (e.g. resin or inorganic bead) to which the photosensitizer can be covalently coupled without impairment of its photobiological activity.
- Define a protocol for the efficient photosensitizer inactivation of microbes in waters to be used for aquaculture and irrigation.

Activities

* Synthesis and photochemical/photobiological characterization of polymer- or inorganic bead-bound porphyrin photosensitizer.
* Development of protocols for large-scale photoinactivation of bacteria and other microbial species.
* Evaluation of the efficiency of a pilot plant for water decontamination, possibly in synergism with mechanical filtration.

Expected outcome

⇒ The final result should be the development and validation of a pilot plant for water photosterilization as well as the mise-au-point of optimal protocols for large scale decontamination of water from aquaculture systems.

⇒ The project should identify combinations of photosensitizer-inert support systems which are not noxious for the environment and allow one to achieve an efficient control of the microbial population without causing the selection of photoresistant species.

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Contract number: IC18*CT960087

Period: December 1996 to November 1999

ECOLOGICAL BASES FOR THE SUSTAINABLE MANAGEMENT OF FLOODED TROPICAL ECOSYSTEMS: CASE STUDIES IN THE LLANOS (VENEZUELA) AND THE PANTANAL (BRAZIL)

Co-ordinator: Universidad Complutense de Madrid, Madrid, Spain (Francisco Diaz Pineda)

Objectives

Characterise changes induced by land use intensification in the flooded neotropical savannahs, mainly through building a network of dams ("modulos"), for regulating surface run off:

- Relative weight of some major limiting factors (effects of water regime, fire management, wildlife and cattle herbivory) in the productive processes of flooded savannah ecosystems.
- Within a regional frame, establishment of patterns and relative proportions of main habitat types in the landscape.
- Elaboration of maps showing the major landscape changes during the last 30 years, resulting from the use of the dam systems for the control of surface run off.
- Analysis of the diversity of landscapes and communities, and the relationships between the natural environment and the areas under human influence.
- Measurement of water fluxes in a flooded savannah ecosystem: rainfall, soil water, depth of the water table, content and evaporation into non flooded and permanently flooded habitats.
- Establishment of flooded savannah's floristic composition, diversity and phenological behaviour and their changes in response to various grazing pressures and fire regimes.
- Estimation of above- and below-ground primary production under the current management practices by quantifying the above and belowground biomass in a flooded savannah site under three different stocking rates: protected, extensive grazing and overgrazing.
- Quantification of carbon and nitrogen stocks in vegetation, soil, megafauna and microbial biomass, in the aforementioned treatments. Evaluation of the soil's carbon flux through root and biomass respiration by in situ soil incubation and measuring of CO2 losses. Procurable indicative figures for certain soil-atmosphere nitrogen fluxes: losses through denitrification and gains by biological fixation (free and symbiotic).

Activities

- Landscape scale: a) Habitat heterogeneity and changes induced by land uses during the last 30 years. Consequences of the dams' system. Mapping and description of the study area using satellite imagery. b) Monitoring landscape changes resulting from land use and transformation processes over the last 30 years. Measurement of the rate of replacement of the habitat types. The temporal changes in habitat will be estimated through a GIS.
- Ecosystem: d) Productivity and forage quality of flooded savannahs under three different stocking rates. d1) Land resources inventory at plot level. Information on species characteristics, life forms, phenology, soil profile, floristic composition, crop of consumable biomass, production and its relationship with biodiversity, soil respiration,

mineral nitrogen and biomass'carbon and nitrogen, soil microbial biomass, denitrification and nitrogen fixation. d2) Animal ecology approach.

Expected outcome

Effect on the biodiversity. Proposal for development according to local and external demand, and for water management systems taking into account the local development (tourism) and the biological diversity maintenance. There is a great demand for ecological information by Governmental offices, environmentalist organisations, tourist services, and the producers themselves (ranchers). The expected results shall have a positive impact on regional development policies, and even on the legislation regulating land use. Scientifically, this project is fully justified as an integrated research combining various complementary approaches to obtain a global understanding of the functioning and dynamics of one of the less known tropical ecosystems, mainly, its diversity, and carbon and water cycles.

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Contract number: IC18*CT960089

Period: November 1996 to October 1999

GENETIC ANALYSIS AND ENGINEERING OF ALUMINIUM TOLERANCE IN MAIZE AND IN MODEL PLANTS.

Co-ordinator : Consejo Superior de Investigaciones Científicas, Barcelona, Spain
(Pere Puigdomenech)

Objectives

- Mapping of genes responsible for aluminium tolerance in maize by using genetic and molecular methods.
- Identification of genes associated with aluminium tolerance in maize, and analysis of their expression.
- Identification of Arabidopsis ecotypes tolerant and sensitive to aluminium, and analysis of rhizosphere modification.
- Production of transgenic plants altered in the expression of the genes involved in the biosynthesis of organic acids.
- Analysis of specific root epidermis promoters, in transgenic tobacco and maize plants.

Activities

- Mapping of maize genes involved in aluminium tolerance, using available segregant populations and molecular marker methodology.
- Identification of genes involved in aluminium tolerance by differential display and differential screening between tolerant and sensitive maize lines, in the presence or absence of aluminium. Analysis of the structure and expression of selected clones.
- Production of transgenic tobacco and maize plants overexpressing bacterial citrate synthesis, PEPC, and MDH Analysis of variations in organic acid production and secretion in root exudates. Study of the tolerance of the transgenic plants to aluminium.
- Screening of *Arabidopsis thaliana* ecotypes for aluminium sensitivity.
- Analysis of root exudates and initial genetic analysis.
- Analysis of promoters encoding root-specific malic enzyme in tobacco and maize, and analysis of the specificity of expression in the epidermis.

Result so far

⇒ Construction, with the promoter of the gene coding, of a malic enzyme expressed in the embryo root epidermis. Transgenic plants are being produced. The promoter of a gene coding for a homologous protein expressed in the adult root epidermis is being analyzed.

⇒ Transgenic tobacco plants overexpressing a bacterial citrate synthesis have been shown to increase aluminium tolerance (see article by the Irapuato group in Science, 276, (1997), 1566-1568). Tobacco plants overexpressing PEPC have also been obtained, and the exudation of organic acids is being studied.

⇒ Bulk segregation analysis of aluminium-tolerance lines by RFLP is in progress. Two regions marking the short arms of chromosomes 6 and 10 were detected.

⇒ Differential display analysis of roots under aluminium treatment was carried out. A cDNA coding for a glycine-rich sequence has been identified.

⇒ Ecotypes from *Arabidopsis thaliana* have been analyzed for aluminium tolerance. At the moment, 9 ecotypes have been classified as highly sensitive, 12 showed intermediate sensitivity, and 3 ecotypes have a high tolerance. This material offers a potential for further analysis of this character in *Arabidopsis*.

Expected outcome

- The project intends to provide tools for the analysis of aluminium tolerance in maize, such as molecular markers and cDNA clones corresponding to genes induced or repressed in different aluminium conditions.
- A screening in a model plant - *Arabidopsis thaliana* - will be carried out in order to obtain tolerant and sensitive ecotypes.
- Transgenic tobacco and maize plants with altered organic-acid biosynthesis will be obtained, and they will be analyzed for aluminium tolerance. Transgenic plants will also be used to analyze promoters specific for root epidermis.

Selected publications

de la Fuente J.M., Ramírez-Rodríguez V., Cabrera-Ponce J.L. and Herrera-Estrella L., 1997. Aluminium Tolerance in Transgenic Plants by Alteration of Citrate Synthesis, Science. **276**:1566-1568.

Lopez Becerra E., Puigdomenech P. and Stiefel V., 1998. A gene coding for a malic enzyme expressed in the embryo root epidermis from zea mays. Plant Physiol. In the press (accession no. AJ224847).

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Contract number: IC18*CT960090

Period: November 1996 to November 1999

CONCERTED ACTION FOR THE EVALUATION OF THE ENVIRONMENTAL SUSTAINABILITY OF AGRICULTURAL SYSTEMS IN THE SOUTHERN CONE OF LATIN AMERICA

Co-ordinator: Red Internacional de Metodología de Investigación de Sistemas de Producción, Santiago, Chile (Julio A. Berdegue)

Objectives

The general objective of this project is to improve the scientific capability to evaluate *ex ante* the environmental sustainability of agricultural systems.

The specific objectives are:

- To define a conceptual framework and methodological protocol to guide the comparison of different methods to measure and evaluate the sustainability of agricultural systems across agroecosystems and socioeconomic environments.
- To measure and evaluate the sustainability of selected agricultural systems in each country, using several qualitative and quantitative methodologies.
- To compare the results obtained with each method in each agricultural system.
- To disseminate the resulting methodologies and to support their integration into the regular programs of the participating institutions and other research organisations.

Activities

- Standardisation of existing data sets from Brazil, Argentina, Chile and Peru, according to a common conceptual and methodological framework agreed upon by the six participating institutions.
- Integration of the standardised data sets in a distributed database on the Internet.
- Analysing the sustainability of the agricultural systems from which the data sets were derived, using two qualitative and two quantitative methods (Participatory Rural Appraisal, Rapid Appraisal of Agricultural Knowledge Systems, Multiple Objective Programming, and Farm Simulation Modelling).
- Comparing the results of the analyses conducted with each method, and finding opportunities for their integration into a common methodology.
- Dissemination of partial and final results by means of electronic publication on the Internet, and in journals; presentation in scientific meetings and newsletters.

Results so far

⇒ A common conceptual and methodological framework was define in a workshop held in 1997;

⇒ Data sets from each agricultural system have been largely adapted and completed to fit that common framework;

⇒ Specific qualitative and quantitative methods have been pilot-tested in three methodological workshops held during 1997;

⇒ A web site has been established, at **http://www.rimisp.cl/europa.htm.** It contains information and documents produced by the Concerted Action.

Selected publications

Memorias del Primer Taller de Evaluación de la Sostenibilidad de los Sistemas Agrícolas del Cono Sur de América Latina [Proceedings of the First Workshop on the Evaluation of the Sustainability of Agricultural Systems in the Southern Cone of Latin America]. Jaguariuna, Sao Paulo, Brazil: RIMISP – CIRAD – INTA – IIED – ISG – ECOFORCA. 28 – 30 April 1997. Published electronically on the Internet at **http://www.rimisp.cl/memoriae.htù**

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Contract number: IC18*CT960096

Period: November 1996 to October 1999

DEVELOPMENT AND APPLICATION OF SOIL PRODUCTIVITY INDEXES FOR CENTRAL AMERICA

Co-ordinator : Universität für Bodenkultur, Vienna, Austria (Nicola Rampazzo)

Objectives

- Standardization of field and laboratory methodologies.
- Testing of the applicability of existing Productivity-Index (PI) models for Central America.
- Improvement of selected modelling relationships between soil loss and soil functions based on process-oriented factors.
- Development of an extended soil PI model with special reference to application for countries of Central America.
- Testing and modification of the European Soil Erosion Model (EUROSEM) for tropical environments.
- Application of the extended PI model for selected watersheds.
- Linkage of EUROSEM to the extended PI model to produce a physically-based approach to modelling erosion - soil productivity relationships.
- Application of the SPIES model on catchment scale in Central America using GIS.
- Simulation of change of distributed soil sustainability through application of a soil-erosion model (EUROSEM) and the extended PI model, using different scenarios of management exemplary in Costa Rica.

Activities

- Develop a methodology to estimate the relationship between soil erosion and soil productivity for Central American conditions. Based on this knowledge, an extended Productivity-Index model will be linked to the EUROSEM, which first will be validated under conditions of Central America.
- Once established, the combination of the extended PI model EUROSEM (SPIES) will serve as a tool to predict the sustainability of agricultural land use according to different environmental scenarios.
- To provide useful tools for decision-makers, SPIES, combined with a GIS will be applied on catchment scale. The results of this approach will also be supported by the development of maps and animation techniques in order to assess the time horizon in which a current land use can be performed at a certain productivity level.
- The activities during the three-year project will be co-ordinated through several workshops and field trips in each of the participant countries. Staff exchange with diploma and doctorate theses will be performed.

Expected outcome

⇒ Issuance of a standardized methodology handbook for collection of PI data.
⇒ Selection of an applicable PI model for Central America and its adaptation for Central American production conditions.
⇒ Evaluation of the suitability of other models for further development.
⇒ Production of an extended PI model for Central America
⇒ Application of EUROSEM for tropical environments
⇒ Validation of the extended PI model on watershed scale
⇒ Linkage of EUROSEM to the extended PI model to the SPIES model.
⇒ Application of SPIES at a larger scale by GIS, and development of maps for different scenarios.
⇒ Estimation of productivity risks exemplary for Costa Rica, using GIS.

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Contract number: IC18*CT960097

Period: September 1996 to February 1999

IPM IN MAIZE : SUSTAINABLE PEST CONTROL FOR SMALL-SCALE LATIN AMERICAN FARMERS

Co-ordinator: University of Southampton, Southampton, United Kingdom (David Goulson)

Objectives

Our main objective is to develop a low-input integrated pest management programme for the key pest of maize in Latin America, *Spodoptera frugiperda*, based primarily on use of a baculovirus insecticide backed up with biological and cultural control measures. Hence, we will reduce the current dependence on synthetic pesticides which, at present, represent an acute toxicological hazard, with regular poisoning incidents among growers and consumers.

Activities

- ✶ To characterize and assess pathogenicity of strains of baculovirus pathogenic to *S. frugiperda.*
- ✶ To quantify *S. frugiperda* larval behaviour and feeding rates at varying ages and on varying growth stages of maize to enable optimization of control measures.
- ✶ To assess the efficacy of baculoviral insecticides in various formulations and at various application times and rates, using replicated field trials.
- ✶ To assess the efficacy of baculoviral insecticides in various formulations and at various application times and rates, using replicated field trials.
- ✶ To assess the control potential of egg and larval parasitoids (either via in-field management or mass release) and the potential for integration of parasitoid and baculoviral controls.
- ✶ To screen the available pesticides for toxicity to man and beneficial insects, and to examine possibilities for integration of biological controls with a reduced chemical input.
- ✶ To integrate all of the above into a practical control programme.

Results

- ⇒ A Nicaraguan isolate of *S. frugiperda* has proved to have the highest pathogenicity (lowest LD50) and has thus been selected for use in field trials. We have also finished quantifying the yield of virus provided per infected larvae with various isolates.
- ⇒ Larval feeding rates have been quantified across instars; in conjunction with LD50 studies across instars, this enables us to predict which ages are most likely to become infected in the field. The incidence of cannibalism and its role as a means for transmission of the virus has also been assessed, but despite high levels of cannibalism it seems that avoidance of infected larvae as victims renders this an unlikely route for substantial virus transmission.

Six large-scale field trials were completed during the summer of 1997 in Mexico and Honduras. These compared the efficacy of biological and viral control versus chemical insecticides in controlling *S. frugiperda* in maize. We found that viral control was as effective as use of synthetic insecticides. In conjunction with natural levels of larval parasitism (which were absent in insecticided plots) virus-treated plots achieved the same yields as those treated with insecticides. The cost of use of viral insecticides versus chemical control has also being quantified and is approximately equal.

Follow-up

Experiments to examine whether the performance of biological controls could be improved further by manipulating the timing and amounts of virus sprayed or by augmenting parasitoid numbers are currently planned for 1998.

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Contract number: IC18*CT960104

Period: December 1996 to November 1999

CLEAN WATER WITH CLEAN ENERGY - DRINKING WATER PROVISION IN REMOTE REGIONS WITH DECENTRALISED SOLAR POWER SUPPLY

Co-ordinator: Fraunhofer Institut Solare Energiesysteme, Freiburg, Germany (Klaus Preiser)

Objectives

- Get an overview of the situation of drinking water provision in rural regions of Latin America.
- Get an overview of water treatment technologies applied in rural regions of Latin America, and of commercially available devices for decentralized drinking water purification.
- Make suggestions for sustainable water management in rural villages in Latin America.
- Set up design guidelines for decentralized drinking water purification systems powered by solar energy.
- Couple treatment devices for decentralized drinking water provision to photovoltaic systems, and to develop by this method stand-alone water purification systems.
- Install two water purification systems powered by solar photovoltaics in two pilot-villages; one in the province of San Juan (Argentina) and the other one in the State of Mexico (Mexico).
- Disseminate the expertise gained with the project.

Activities

- Assessment of the water pollution, needs of water and needs of drinking water purification, together with the legal background in rural regions of Latin America.
- Isolation of typical problems regarding water provision in rural Latin America.
- Elaboration of a catalogue of applicable purification technologies and available devices.
- Performing laboratory-and field tests with promising drinking-water purification devices.
- Analysis of water management practices in different villages in Argentina and Mexico from a socio-technical point of view.
- Elaboration of general design guidelines for solar-powered water purification devices.
- Development of at least two water purification systems operated by solar photovoltaics.
- Implementation of two drinking-water purification systems in two villages in Argentina and Mexico. Important steps: selection of pilot villages, preparation of these villages, installation and evaluation.
- Elaboration and organization of three seminars in Latin America for the dissemination of the projects results.

Expected outcome

The work carried out in this project together with the World Health Organization (WHO) as external partner should provide suggestions for how to improve drinking water provision, and therefore how to improve living conditions and health of people settling in rural areas of Latin

America. Recommendations for design, implementation and operation of solar-powered water purification systems will help to achieve this goal.

Selected publications

Parodi O., Preikschat K., Preiser K. 1997. PV contra *Coli-Bacteria* - Suitability of UV-Water Purification Devices for PV-Systems. 14th EU PV Solar Energy Conference. Barcelona, Spain

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Contract number: IC18*CT960118

Period: January 1997 to December 2000

EVALUATION AND UTILIZATION OF PINEAPPLE GENETIC RESOURCES FROM THE AMAZON TO BREED RESISTANT VARIETIES

Co-ordinator : CIRAD-FLHOR, Montpellier, France (Geo Coppens)

Objectives

The long-term objective is recovering or breeding pineapple varieties to improve production systems and provide the South American markets with larger quantities of high-quality fruits, so contributing to the sustainable development of this region and the tropical small farming systems. The present project aims at obtaining knowledge and tools for the development of varieties resistant to the main diseases in the region, by :

- characterizing and evaluating available genetic resources,
- studying the genetic structure of the genus Ananas,
- developing resistance screening techniques,
- studying the heredity of agronomic traits,
- testing the potential of partial inbreds in breeding.

Activities

- Characterize the germplasms collected in a previous series of projects (botanical and agromorphological description, nuclear and cytoplasmic DNA characterization); study the structure and genetic diversity of the genus *Ananas*, evaluate the potential for breeding or direct use in small farmers systems.
- Create a standard database to promote information and germplasm exchange between the partners, and, later, between all the existing pineapple germplasm collections.
- Develop techniques to screen for varietal resistance to fusariosis, black spot, thecla and nematodes, four of the main diseases and pests in the area, and apply them on the germplasm.
- Develop new breeding schemes from the results obtained in the project. Selfing cycles will result in fixing main traits and expressing new recessive traits. Prebreeding among these inbred families will be associated with inbreeding depression studies.
- Establish a reference genetic map of molecular markers for pineapple; analyse the heredity and recombination of chromosome fragment of interspecific hybrids; study the heredity (and gene mapping) of agronomic traits.

Expected outcome

⇒ Common germplasm inventory allowed exchanges and repatriation of lost germplasm. The list of descriptors has been revised. Multivariate analysis of the first morphological description produced consistent results. Molecular markers have been developed for characterization and genetic mapping. New sources of resistance to fusariosis have been identified.

⇒ Characterization studies will provide key information to assess the genetic diversity of pineapple and understand its structure. The project will favour new uses of neglected or

traditional varieties, so widening the genetic base of pineapple cultivation. The development of new breeding schemes associated with gene mapping should greatly improve breeding efficiency, allowing to transfer resistance and thence promote integrated and more environment-friendly control of pests and diseases.

Selected publications

Cabral J.R.S., de Matos A.P. and Coppens d'Eeckenbrugge G., 1997. Segregation for resistance to fusariose, leaf margin type and leaf colour from the EMBRAPA pineapple hybridization programme. Acta Horticulturae, **425** : 193-200.

Coppens d'Eeckenbrugge G., Leal F. and Duval M.F., 1997. Germplasm resources of pineapple. Horticultural Reviews, **21** : 133-175.

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Leal F., Coppens d'Eeckenbrugge G. and Holst B., 1998. The genera Ananas and Pseudananas. I. An historical review. Selbyana (special issue on bromeliads). Submitted for publication.

Noyer J.-L., Lanaud C., Coppens d'Eeckenbrugge G. and Duval M.F., 1997. RFLP study on rDNA variability in Ananas genus. Acta Horticulturae, **425** : 153-160.

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Contract number: IC18*CT960124

Period: October 1996 to September 1999

ENGINEERING MONOCOTYLEDONOUS PLANTS FOR A HIGHER TOLERANCE TO ABIOTIC STRESS

Co-ordinator: Vlaams Interuniversitair Instituut voor Biotechnologie, Gent, Belgium
(Marc Van Montagu)

Objectives

- To assess the importance of catalase in cellular defence against environmental stress using a catalase-deficient barley mutant.
- To analyse the sequence of molecular events that occur during abiotic stress in a catalase-deficient barley mutant.
- To characterise the antioxidant defence response in maize against drought stress, in barley against UV-B stress and in rice against salt and pathogen stress.
- To establish a genetic transformation methodology for barley.
- To produce transgenic maize, rice and barley with increased levels of catalase and to assess stress tolerance of transgenic versus control lines.

Activities

* Expression analysis of antioxidant defence proteins during environmental stress in maize, rice and barley will be assessed by measuring enzyme activities and mRNA levels. Partial cDNAs of the major antioxidant genes have been cloned from maize by RT-PCR to be used as probes in the mRNA analysis. As part of this research, the first iron superoxide dismutase-form monocotyledons was cloned. Antioxidant gene expression will also be followed in catalase-deficient barley in order to understand the molecular mechanisms that are activated specifically by H_2O_2 stress. Low molecular-weight antioxidants such as ascorbate and glutathione will be measured by HPLC with simultaneous UV and electrochemical detection.
* Development and/or improvement of methodologies. Significant progress on barley regeneration from immature embryos was made. Research will now focus on barley transformation using Agrobacterium tumefaciens, and on methods for monitoring H2O2 and oxidative damage (lipid peroxidation, protein carbonylation). Catalase overexpression constructs for monocot transformation by physical methods have been developed and transformation of rice has been initiated. Stress tolerance will be assessed towards drought, salt stress and pathogens. Salt stress will be imposed in hydroponic cultures, Magnaporthe grisea strains P2, 87eP2 and 86 will be used as fungal pathogens.

Expected outcome

The work carried out in this project should identify the role of catalase in the defence against various stress factors and will address the feasibility of improving stress tolerance by increasing the levels of catalase in monocotyledonous plants. Comparison of oxidative damage and catalase overproduction during stress in C3 and C4 plants will contribute to elucidating the role of photorespiration in photooxidative processes evoked by environmental

adversity. Establishment of a barley transformation and regeneration system will be extremely valuable for technology-based improvement of this important crop.

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Contract number: IC18*CT960126

Period: October 1996 to September 1999

DEVELOPMENT OF TRANSGENIC POTATO CULTIVARS WITH COMBINED PROTECTION AGAINST VIRUS AND FUNGAL PATHOGENS

Co-ordinator: Institut National de la Recherche Agronomique, St. Paul-lez-Durance, France
(Christophe Robaglia)

Objectives:

- Production of transgenic *Solanum tuberosum* carrying simultaneously artificial viruses and fungi-resistance genes.
- Mapping and cloning of a natural resistance gene against Potato Virus X (Rx gene) located in the genome of the wild potato species *Solanum commersonii*.

Activities:

- Construction of multigene cassettes based on the following artificial resistance genes:
  - genes for resistance against Potato Virus Y, PVY (based on the Lettuce Mosaic Virus coat protein), Potato Leaf Roll Virus, PLRV (based on the PLRV replicase).
  - genes for resistance against phytopathogenic fungi coding for: Barley type I ribosome inactivating protein (RIP), Barley class II chitinase, thaumatin-like AP24 protein.
- Combination of multigene cassettes in the genome of different local and international *Solanum tuberosum* varieties, using *Agrobacterium tumefaciens* mediated and selection with multiple selectable genes (coding for kanamycin and hygromycin resistance).
- Field tests of the resulting new potato genotypes in several locations in South America (Argentina, Brazil, Uruguay).
- Crosses between Rx and rx *Solanum commersonii* and search for Rx-associated genetic markers using AFLP analysis of bulked segregant populations.
- Construction of *Solanum commersonii* BAC library and screening of BAC clones for Rx candidates using AFLP defined probes.

Expected outcome

⇒ This work should improve our knowledge of the feasability of incorporating multiple agronomically useful genes in the genome of an important crop plant. The resulting new potato varieties are expected to display an enhanced resistance against some of their most significant diseases with consecutives benefits concerning yield and reduced use of pesticides.

⇒ This work will also improve our knowledge of natural mechanisms for virus resistance in wild plants.

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Contract number: IC18*CT970140

Period: November 1997 to October 2000

DESIGN OF ENVIRONMENTAL DECONTAMINANTS USING CALIXARENES

Co-ordinator: University of Surrey, Guilford, United Kingdom (Angela F. Danil de Namor)

Objectives

- Design new calixarene derivatives containing aliphatic and alicyclic amines and mixed donor atoms (sulfur and nitrogen) in which the distance between the phenolic oxygens and the amine nitrogens (or sulfur) is increased (-CH_2-CH_2-instead of -CH_2-) in order to :
  - induce selective complexation with polluting cations (mercury, cadmium and lead) while reducing the accessibility of the phenol oxygens to interact with essential cations (Na^+, K^+, Ca^{2+}, Mg^{2+});
  - use the protonated calixamines and thiocalixamines for anion complexation with polluting anions (AsO_4^{-3});
  - investigate the hosting properties of the hydrophobic cavity for interaction with polluting chlorinated aliphatic and aromatic compounds.
- Incorporate these macrocycles in solid supports (natural products of Latin America, alumina, polymeric frameworks) for the development of recyclable materials and in membranes for the production of sensors for the detection of inorganic polluting ions.
- Put calixarene based decontaminants into action by testing their capabilities on polluted natural resources (water) and to compare them with the currently used ion exchange methods.

Activities

* Synthesis and characterisation of calixamines and thiocalixamines : phase transfer catalysis for the synthesis of derivatives will be fully explored. The new compounds are to be characterised by spectrometric techniques and X-ray crystallography. Experimental work will be assisted by computer modelling studies.
* Thermodynamic and kinetic studies : essential to the development of design protocols which will guide the synthetic programme in the later stages and so provide effective calixarenes as environmental scavengers is the understanding of the ion-solvent and ligand-solvent interactions embodied in the complexation and release processes involving macrocyclic ligands with metal cations, anions and neutral species. Calorimetry (thermodynamics) and flow methods (kinetics) will be the methods used.
* Comparative studies with ion exchange resins : for cation removal, based on the thermodynamics and kinetic investigations, assessment of ion-exchange selectivity will be tested with different commercially available resins while anion exchanges will be used to test anion selectivity.
* Incorporation of calixarene derivatives in supports and membranes : the strategy adopted aims to anchor calix(4)arene derivatives on solid supports (swollen cross-linked chloromethylated polystyrene matrices using Merrifield polymers of different mesh sizes and various contents of DVB and natural materials from Latin America) without affecting the active sites for interaction with polluting ions or with neutral species. Physico-

chemical characterisation involve the use of a variety of techniques including thermogravimetric analysis and calorimetry. Considerable emphasis is to be placed of the recycling of these materials.

Expected outcome

⇒ Availability of recyclable solid materials for water purification of higher efficiency than the ones currently used based on the use of ion exchange systems.
⇒ The development of new sensors based on these new materials for the detection of toxic metal cations in aqueous medium.
⇒ Quantitative information regarding the amount of polluting agents in contaminated waters stored in a database prior and after treatment with new materials.

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Period: November 1997 to October 2000

SUSTAINABLE USE, CONSERVATION AND RESTORATION OF NATIVE FORESTS IN SOUTHERN MEXICO AND SOUTH-CENTRAL CHILE (SUCRE)

Co-ordinator: University of Edinburgh, Edinburgh, Scotland (Adrian Newton)

Objectives

The overall aim of the project is to define sustainable approaches to forest use, and to develop innovative approaches to forest rehabilitation on degraded sites, to promote the conservation of native forests. This will be achieved by a multi-disciplinary analysis of the ecological impacts of forest use, to define the key thresholds and indicators of sustainability, and to identify the primary constraints to recovery of degraded forest ecosystems. The results will be used to develop guidelines for sustainable use and conservation of native forests by local communities and other stakeholders.

Activities

* Defining the relationship between different intensities and types of forest use and the key ecological processes which determine sustainability. This will be achieved by the establishment of a minimum of 10 experimental plots in native forests subjected to different land use histories, in each of two areas in both southern Mexico and South-Central Chile. A detailed comparative analysis will then be made of the following ecological characteristics: soil properties including fertility, the regeneration capacity of selected tree species, biodiversity of the vascular plant flora, and the genetic structure of selected tree species of high economic and conservation value.
* Integrating the results from the ecological analyses, using multiple regression and modelling techniques, to identify the key constraints to sustainable forest use, to explore interactions between different ecological processes, and to identify and evaluate suitable indicators of sustainability in each of the forest study areas.
* Examining the process of forest rehabilitation on degraded sites, by establishing a minimum of one field experiment in each of the experimental areas, designed to identify the key ecological processes limiting forest recovery. Experimental treatments will include encouragement of natural regeneration, artificial seedling establishment of threatened tree species and modifications of current land-use practices as a method of achieving forest restoration.
* Assessing the impact of forest use and habitat fragmentation on the viability of populations of a minimum of four tree species of international conservation concern, and to develop guidelines for their continued monitoring and effective conservation.

Expected outcomes

Guidelines for the conservation and restoration of native forest in each of the four study areas, both for relevant policy-makers, and for the development of sustainable forest management plans by local communities.

- Dissemination of project results through a minimum of eight scientific publications in international refereed journals and six research reports.

- Strengthening of the research capacity of all partner organizations through a technical exchange programme.
- Training of at least eight young scientists through existing postgraduate programmes of the partner organizations.

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Contract number: IC18*CT970147

Period: November 1997 to October 2000

EFFECTS OF CHANGES IN LAND USE AND LAND MANAGEMENT PRACTICES ON LAND DEGRADATION IN FOREST AND GRAZING ECOSYSTEMS

Co-ordinator: Universidad de Aveiro, Aveiro, Portugal (Celeste de Oliveira Alves-Coelho)

Objectives

- Investigate the changes in land degradation with increasing forest and grazing activities in the western Mediterranean region: North Africa (Morocco and Tunisia) and the Iberian peninsula).
- Assess the current and likely future land-use and land-management practices that contemporary socio-economic trends, national/regional policies, the 2010 free-trade zone, and the EU-CAP Aid Scheme for Forestry may induce in areas that are vulnerable to land degradation and desertification.
- A holistic research approach combining both natural environment, societal, and socio-economic dimensions, will be adopted in order to improve the basis of policies supporting sustainable development.
- Determine models, both conceptual and semi-quantitative, that describe the relationship between hydrology, vegetation, land-use, and socio-economic constraints.
- Contribute to the definition of criteria for evaluation and mitigation of land degradation and desertification.
- Transfer the results of this project into action-guiding instruments adapted to land-users and managers in the western Mediterranean region.

Activities

- Studies of policies affecting forest and grazing systems. This will be conducted simultaneously at the field level and at regional and international scales. It will emphasize the comparison between objectives and real effects of policies on land degradation and desertification.
- Field studies based on (1) structured interviews and a questionnaire survey of land-users and key actors, on their views of current/future land-use and land-management practice trends and problems, and their responses to national initiatives and policy. Investigation of their reactions to policy alternatives, and conservation measures and techniques. (2) gathering of environmental data on hydrological, erosion, soil property, and vegetation characteristics of the land-use/land-management practice types within each of the studies areas.
- Development of models for the different land-use/land-management practices using data (socio-economic and physical) gathered in demonstration areas. Different evolution scenarios will be defined from this modelling.

Expected outcome

⇒ Contribute towards sustainable development in rural areas affected by drought and over-

exploitation of natural resources, by integrating the views of local populations as an efficient part of land- and water-conservation strategies.

⇒ Building scenarios for feasible alternative land-uses/land-management practices, under different socio-economic conditions, with the help of GIS techniques.

⇒ Establish thresholds for sustainability and degradation limits for the present status, and for mitigation measures proposed by the various groups acting in the field (land-users, government officials, researchers, etc.)

⇒ Prepare a handbook on techniques and methodology on the physical and socio-economic aspects developed in the project, that could be used elsewhere in the Mediterranean or in other areas.

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Contract number: IC18*CT970148

Period: October 1997 to September 2000

POLICIES FOR SUSTAINING ENVIRONMENTS AND LIVELIHOODS IN MOUNTAIN AREAS

Co-ordinator: University of Leeds, Leeds, United Kingdom (David Preston)

Objectives

- The main aim of the research is to encourage the uses of environmental resources (particularly vegetation, soils and water) for the benefit of all people.
- A second aim is to improve the understanding of past and present environmental deterioration. We will study the changes that may be a consequence of farming, pollution from towns and mines and the frequency of natural hazards (such as floods, landslides and earthquakes).
- We want to see how people make use of the environment in order to live, and how they maintain its quality.
- The recent changes in land use will be studied to discover patterns of environmental change which may be associated with such use and whether community and regional inequalities have increased.
- Sector policies which influence these changes will be investigated to see if they can be improved for the benefit of all.

Activities

* The study will be conducted in the Quebrada de Humahuaca (North-Western Argentina), the central Tarija valleys (Southern Bolivia) and the Colca and Puquina river basins (Southern Peru). A Geographical Information System (GIS) will be established for each study area to show changes in erosion, land use and vegetation over a 10-20 year period and to identify sub-catchments for detailed study.
* Geomorphological maps (1:10,000 scale) of each field site will be produced. Different parts of river basins will be surveyed periodically to monitor change. Geomorphological investigations will establish erosion histories during at least the past 10,000 years. We shall assess the impoverishment of soils as a consequence of erosion. Downstream from mining areas heavy metal (e.g. lead) contamination in soils and sediments will be assessed. Levels of toxic metal pollution in irrigation and drinking water supplies will also be measured. The ways in which vegetation is changing will be studied and related to soils and erosion, as well as to present and past human use.
* The research will cover populations and environments at a household, community and regional level. The livelihood differences associated with resource accessibility will be investigated, using oral and historical records. This will create a human as well as environmental history. Organisations at a community and regional level that influence change will be identified. Histories of key organisations and ways of managing stress will be recorded. Of special interest in Bolivia will be how the people have responded to the new policies on Decentralisation and Popular Participation. Many environmental issues (e.g. human responses to past and present droughts) will need to be studied by both natural and social scientists.

Expected outcome

⇒ Identification of human use factors underlying positive and negative environmental changes.
⇒ Accurate data on how environments have been affected by natural and environmental change factors.
⇒ Natural hazards and related coping strategy identification.
⇒ High quality findings on policy options.
⇒ Delineation of institutional structures coping with environmental changes and stresses.
⇒ Identification of resource uses associated with positive and negative environmental change.
⇒ Assessment of impact of sector policies at a community and regional level.
⇒ Identification of intensive resource uses and their impact on households and environment.

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Contract number: IC18*CT970149

Period: November 1997 to January 2001

ASSESSMENT OF LEVELS AND DYNAMICS OF INTRA-SPECIFIC GENETIC DIVERSITY OF TROPICAL TREES FOR CONSERVATION AND SUSTAINABLE MANAGEMENT

Co-ordinator: Institute of Terrestrial Ecology, Penicuik, United Kingdom (Julia Wilson)

Objectives

This project applies new molecular techniques to studies of intra-specific diversity in a range of tropical tree species. The main objectives are:

- To describe and compare the level and distribution of genetic diversity in a range of tropical-tree species from different types of forests.
- To describe the dynamics of this diversity.
- To identify the effects of specific human impacts on the diversity.
- To derive suitable conservation strategies for selected tree species.

Activities

* The project focuses on the following tree species:
  - *Cedrela odorata, Vochysia ferruginea, Hyeronima alchorneoides, Lonchocarpus costaricensis* (Central America).
  - *Astrocaryum* sp. *Qualea rosea, Symphonia globulifera, Ocotea rubra, Monorobium coccinea* (French Guiana).
  - *Tabebuia heterophylla* (Caribbean Islands).
  - *Eugenia uniflora, Anacardium occidentale, Swietenia macrophylla, Pseudobombax munguba, Ceiba pentandra* (Brazil).
* The key activities are:
  - Development of 'universal' molecular markers to enable comparisons of levels and distribution of diversity to be made between different species. Studies will focus on AFLP techniques and the development of a database of AFLP fingerprints, RFLP markers of cpDNA, and co-dominant DNA markers (SAMPL).
  - Examination of the diversity of a range of species at the regional level, using AFLPs and cpDNA RFLPs
  - Examination of the spatial distribution of diversity at the local level (a few hectares), using AFLPs and cpDNA RFLPs.
  - Estimation of the outcrossing rates for species.
  - Estimation of the extent of gene flow through seed-dispersal studies utilizing maternally inherited cpDNA, and studies of pollen movement by relating the distribution of rare alleles in seedlings to their occurrence in mature trees, using SAMPL data.
  - Examination of the effects of domestication on species' genetic diversity through comparison of wild and cultivated individuals.

- Evaluation of the effects of forest fragmentation on genetic diversity, comparing different sizes of fragments, neighbour distances, and time since fragmentation occurred.
- Evaluation of the effects of logging comparing plots that have been logged at different intensities,with control plots, and exploited and unexploited species, to enable the assessment of direct and indirect effects of logging.

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Contract number: IC18*CT970150

Period: January1997 to December 1999

FLUXES OF ENERGY, WATER AND CARBON OVER DISTURBED SAVANNA ECOSYSTEMS AND THEIR APPLICATION AS INDICATORS OF SUSTAINABILITY AND CARBON SEQUESTRATION (SAVAFLUX)

Co-ordinator: University of Edinburgh, Edinburgh, United Kingdom (John Grace)

Objectives

- Establish a coherent picture of the pattern of variation in structure and biodiversity of savannas in S. America and to establish an e-mail network of savanna experts.
- Link structure and biodiversity to function, by measuring and modelling fluxes of energy, water, CO2 and trace gases over the range of savanna types. These measurements will be made with a mobile eddy covariance system.
- Determine the capacity of these systems to sequester carbon following disturbance and to explore the link between carbon sequestering capacity and (I) biodiversity and (ii) sustainability.

Activities

- Construction of two mobile flux-measuring systems to measure CO2, H2O and energy fluxes over intact and disturbed savannas in Brazil and Venezuela. To build a related system to measure gaseous fluxes from the soil.
- Comparison of sites on a wide geographical scale, their carbon stocks, structure, leaf area index, seasonality and species composition. The use of models which represent these features and enables the simulation of the fluxes of carbon; the development of these models to study the dynamic changes in carbon stocks on a large scale as a result of different management practices.
- Selection of criteria for verification that particular management practices are sustainable; the production of indicators and indices of sustainability.

Expected outcome

There will be an improvement in the knowledge base on the biological and biophysical properties of savanna ecosystems, with a view to managing savannas sustainably. We expect to contribute to the ongoing discussion about the fundamental nature of sustainability. Carbon inventory data (stocks and fluxes) will be obtained for different stages of regrowth, to enable examination of the role of savannas in the regional carbon balance;also to explore the possible participation of savanna farmers as role-players in the global sequestration of carbon. We will obtain data on the surface characteristics of the landscape such as the short wave reflectance and surface resistance, to assist modellers and interpret remotely sensed imagery. There will be an enhancement of appropriate training to maintain this sort of activity when the project has ended.

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Contract number: IC18*CT970156

Period: January 1998 to December 2000

UNIFICATION OF INDICATOR QUALITY FOR ASSESSMENT OF IMPACT OF MULTIDISCIPLINARY SYSTEMS

Co-ordinator: IACR-Rothamsted International, Harpenden, United Kingdom (Janet Riley)

Objectives

- To bring together developing country and European groups working on renewable natural resources (RNR) programmes at national and regional levels to standardize methods and indicators for assessing the impact and sustainability of farming systems, ecosystems converted to agricultural use, and degraded ecosystems.
- To spread this knowledge to appropriate national and regional agricultural research institutes, extension agents, research planners, and policy-makers to promote improved management of soil, water and biotic resources.

Activities

The concerted action revolves around three man themes:

* Rural and peri-urban farming systems
* Ecosystems converted to agricultural use
* Degraded ecosystems where attempts to convert natural ecosystems to sustainable use have been unsuccessful.

A strong socio-ecoconomic component is incorporated into the examination of each system. Researchers from different regions, countries and sectors are brought together at workshops to discuss and rationalize the choice of indicators and their impact, as well as sustainability-assessment methodologies with regard to the above themes. They are then involved in post-workshop concertation activities to provide the dissemination documents.

Expected outcome

⇒ Links between partners have been created via an electronic network and the world-wide web. The first newsletter has been distributed.

⇒ The first UNIQUAIMS workshop was held from June 3 to 6, 1998 at IACR-Rothamsted. The theme of this workshop was "Rural and peri-urban farming systems to improve agricultural productivity". Regional Theme Managers were identified at this workshop. Needs for different research foci and measurement scales were clarified. Database and aggregation issues were addressed. Baseline and impact indicators were identified, and guidelines for this theme were prepared.

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Contract number: IC18*CT970157

Period: January 1998 to June 2001

AQUACULTURE MANAGEMENT AND ECOLOGICAL INTERACTION OF NOXIOUS PHYTOPLANKTON IN SOUTHERN LATIN AMERICA

Co-ordinator: IFREMER, Plouzane, France (Geneviève Arzul)

Objectives

- Determine the effects of fishfarming on the marine ecosystem in terms of coastal water quality (including dissolved substances and seston) and benthos disturbance.
- Identify the factors involved in the development of phytoplankton, considering mineral nutrients as measured in seawater near the fish farms and dissolved organics coming from excess fish granules.
- Define the effects of UV-b radiation on the growth of phytoplankton populations,: examining cell stress, protection strategy, stimulation of PSP synthesis and photodegradation of organic nutrients.

Activities

- Field studies on the influence of fish farming on the pelagic component. Three typical cases are considered: a fjord, a bay and a channel. The hydrobiological parameters: conductivity, temperature and depth, light, seston and chlorophyll fluorescence are measured in real time by *in situ* probes (CTD Seabird, laser granulometer Cilas, fluorimeter). Seawater sampling for calibration, chemical analyses of nutrients and phytoplankton determination will be made according to the probes data.
- Modelling the dispersion of particles and dissolved nutrients in the water mass around the fish farms. The model provides information on nutrients flux from the cages and the associated chlorophyll cells.
- Field studies on the influence of fish farming on the benthic component. Vertical profiles of the carbon and nitrogen content of sediment beneath the fish farms, benthic macrofauna and diversity currentmetry and bathymetry provide a complete description of the seafloor characteristics.
- Modelling the particulate organic loading of the seafloor. The new model to be used and adapted to the conditions along South American coasts allows quantitative prediction of carbon loading on underlying sediments, considering spatial and temporal scales.
- Bioassays of phytoplankton growth regulation by nutrients. The role of exogenous inputs due to fish farming (feed waste) as well as natural substances caused by dense fish population (urea, ammonia, nitrate and phophate) is tested on phytoplankton growth. The bioassays are performed in the laboratory, on plurispecific algal populations and monospecific cultures.
- Bioassays of phytoplankton growth regulation by UV-b radiation. The selective effect of natural radiation on phytoplankton species and the effect of UV-b intensity will be estimated through a specific and chemical study of the irradiated populations. The consequence of

adding organic substances (feed waste) to phytoplankton cultures under UB-b radiation will be studied, as well as the photodegradation of organic substances. The response to UV-b stress of a toxic dinoflagellate responsible for shellfish contaminations will be studied, according to the concentration of mycosporine-like aminoacids and PSP production.

Preliminary results

⇒ Up to now, the results of the field study confirm the importance of stratification in the accumulation of seston, chlorophyll particles and ammonium in the upper layers in the fjord. From the complete pelagic, benthic and *in vitro* studies, we will intend to define an index of tolerance for the media, considering the risk of hazardous monospecific blooms, and particularly the presence of noxious cells and the substances stimulating them.

Expected outcome

⇒ In view of possible future expansion of salmon farming, the results will help forecast the risk and prevent irreversible degradation of the environment.

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Contract number: **IC18*CT970164**

Period: November 1997 to May 2000

TRANSMISSION AND ADAPTATION OF ENVIRONMENT KNOWLEDGE IN INDIGENOUS AND MIXED-BLOOD COMMUNITIES

Co-ordinator: Université Libre de Bruxelles, Brussels, Belgium (Pablo Isla Villar)

Objectives

The main objective was to carry out a systematic study of the mechanisms of transmission, adaptation and change of the ethnic knowledge about the environment, and its management.

Activities

* The research was conducted among two groups of Panoan Indians and one of "caboclos" rubber-tappers in the Amazonian rainforest of Brazil and Peru. In each country an interdisciplinary team was created, which included anthropologists, (ethno)botanists and zoologists, and in Brazil a remote-sensing team, active in both regions. Their work was complementary: the remote-sensing team was responsible for the preliminary evaluation of the biodiversity, with use of IGS, systemic contextualisation and verification *in situ* in collaboration with the biologists in order to identify the material grounds of the human elaborations, and to prepare the evaluation of the anthropic action.
* With the help of (ethno)biologists, the anthropologists had to identify the ecological knowledge of the different community members, in order to explicit their logical and symbolic structures, and especially the variability between individuals and situations.
* In the current context of increasing cultural, economic and demographic pressures, changes were increasing rapidly, in a way we could not exactly identify. The activities were thus threefold :
  1) explicit the global knowledge system;
  2) evaluate the sustainability and the adaptability of its contents and it articulations;
  3) check the pertinence of the methodological tools (systemic analysis).

Expected outcome

According to the information provided by anthropologists, botanists and zoologists had to evaluate the effects of anthropic action on the culturally most important resources - animal and vegetal - and to analyze the sustainability of their cultural management. Subsequently, the interpretation was more incumbent to anthropologists. Through the contextualization of all information they analyzed the variations they had observed and elaborated systemic patterns: first at a local level then at the intercommunity level. The purpose was to identify systemic indicators in order to make predictions about dynamism and the evolution of the underlying structure.

Follow-up

If the pertinence of the methodological tools is verified, the concept could be extended to other regions in order to monitor the adjustement of regional, national and international

programs for sustainable development or for conservation of the natural and genetic resources to the local communities underlying dynamic systems of values.

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Contract number: IC18*CT970175

Period: September 1997 to August 2001

PLACING FISHERIES RESOURCES IN THEIR ECOSYSTEM CONTEXT: COOPERATION, COMPARISONS, AND HUMAN IMPACT

Co-ordinator: North Sea Centre, Hirtshals, Denmark (Villy Christensen)

Objectives

- Produce scientific methodology toward ecosystem management of marine resources through the construction and analysis of mass-balance models of exploited marine ecosystem of the Atlantic coasts of Europe, Sub-Saharan Africa and Latin America, of the Caribbean, and of the Pacific coast of Latin America to gain information of the resources and their interaction.
- Compare mass-balance models along gradients and transects guaranteeing the highest possible differences, including latitude, ensuring strong ecological (cold vs. warm), and socio-economic (industrialized vs. developing) gradients.
- Evaluate the impact of human exploitation on marine and coastal ecosystems with the purpose of setting criteria for eco-labelling of fishery products, which will thus accrue additional benefits as a consequence of this concerted action.

Activities

* Linking researchers in Europe, Africa, the Caribbean and Latin America working with mass-balance models of exploited marine ecosystems through open, voluntary and proactive co-operation.
* Support of and co-operation between the partners in the model parametering, analysis, and description.
* Arranging four regional training workshops to ensure that a sufficient number of representative ecosystem models are prepared. The workshops will ensure that all partners share a common methodology and information base with regards to ecosystem modelling.
* Conduct two international synthesis workshops covering the two major regions involved (Atlantic and Caribbean, and Eastern Pacific, respectively), where the ecosystem models can be presented, discussed between participants, and analyzed comparatively. The workshops will provide an opportunity for active researchers to discuss and compare their ecosystem analysis, North-South transects, gradients of industrialized / non-industrialized exploitation, and ecosystem criteria for eco-labelling of fishery products.

Expected outcome

⇒ An Internet website will be published including description of modelling approach, a database of models, parameters and partner information.

⇒ Subsequent to the international workshop the models will be published in edited proceedings.

⇒ An interactive CD-ROM will be published containing all data, methodologies, model descriptions, etc.

⇒ Establishment of capabilities for multispecies management of fisheries, and ecosystem modelling in many partner institutions.

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Contract number: IC18*CT970180

Period: December 1997 to November 2001

REDUCTION OF THE CHEMICAL INPUTS IN A VEGETABLE CROP BY THE USE OF BENEFICIAL RHIZOSPHERIC MICRO-ORGANISMS

Co-ordinator: Institut National de la Recherche Agronomique, Dijon, France
(Philippe Lemanceau)

Objectives

- Select strains of fluorescent pseudomonads for their rhizospheric competence and glomalean fungi for their symbiotic competence in Argentine and Uruguayan soils.
- Further select among these strains those which are able to suppress soil-borne diseases in tomato and to promote tomato plant growth.
- Characterize the modes of action responsible for the beneficial effects of the selected micro-organisms and to describe their interactions with the host-plant in order to determine conditions for their use.

Activities

- Establishment of a large collection of fluorescent pseudomonads and glomalean fungi. Isolation of bacteria and glomalean fungi from Argentine soils either used for tomato cultivation or not.
- Characterization of microbial diversity in order to select fluorescent pseudomonads for their rhizosphere competence. Bacterial traits allowing the discrimination of soil and rhizospheric populations will be used to preselect rhizosphere competent strains. Preselected strains will be tested subsequently for their rhizospheric competence in different soil types from Argentina and Uruguay. Strains of glomalean fungi will also be selected for their symbiotic competence. The effect of soil characteristics on the rhizosphere and on the symbiotic competence of the selected strains will be assessed.
- Selection of strains for their ability to promote plant growth and/or to suppress tomato diseases among the rhizosphere and symbiotic competent strains. The beneficial effects of the selected strains will be further tested in commercial conditions especially in naturally infested soils in Argentina. The compatibility of these strains with other cultural practices will be checked.
- Evaluation of the effects of the selected micro-organisms on root development of the host plant, infected or not with pathogens. Description of the colonization pattern, and cellular and molecular interactions between the host-plant, the pathogens, and the beneficial micro-organisms. Characterization of microbial metabolites and activities responsible for the beneficial effects on plant growth and plant health.

Expected outcome

The work carried out in this project should provide microbial strains able to reduce the use of pesticides in tomato crops in Argentina and Uruguay. Inoculation of tomato with these strains should improve plant growth and health. The selected strains should be compatible with other

cultural practices. The modes of action of the beneficial micro-organisms and their interactions with the host plant will be analyzed in order to identify the most favourable environment for the expression of beneficial effects. Guidelines on application of the micro-organisms to improve their efficacy will then be given to the farmers.

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Contract number: IC18*CT970181

Period: October 1997 to September 2000

SUSTAINABLE IMPROVEMENT OF NEMATODE RESISTANCE IN COFFEE CULTIVARS (*COFFEA ARABICA* L.) OF CENTRAL AMERICA: ENHANCED USE OF GENETIC RESOURCES BY THE DEVELOPMENT OF MARKER-FACILITATED SELECTION PROGRAMMES

Co-ordinator: IRD (Ex-ORSTOM), Montpellier, France (Philippe Lashermes)

Objectives

⇒ Enlarge the narrow genetic base of cultivated coffee-trees (*Coffea arabica* L.) and contribute to the development of cultivars combining high quality and resistance to root-knot nematode.

⇒ Overcome most of the limitations faced by conventional coffee breeding in using the genetic resources through the development of molecular marker-facilitated selection programmes.

⇒ Investigate and control the repercussion on coffee quality of wild trait introgression into *C. arabica*.

⇒ Extend and implement molecular marker technology in relation to coffee breeding in Central America.

Activities

* Evaluation for coffee quality and resistance to nematode populations of a wide range of plant materials including major cultivars, F1 hybrids as well as F2 segregating progenies.
* Genetic analysis. This study will be conducted to specify the genetic determinism of coffee tree resistances to two nematode species (*Meloidogyne exigua* and *M.* sp. of Guatemala). It will look for easily scored genetic markers linked to nematode resistance sources. Furthermore, activities will be oriented to determine the type, importance and consequences on quality (fertility, biochemical..) of chromosome exchanges during the introgression of desirable traits into *C. arabica* from wild relative *Coffea* species.
* Development of molecular markers suitable for large scale application in arabica coffee genetics (i.e. DNA microsatellite markers).
* Formulation and set up in connection with the regional breeding programme, of marker-facilitated selection programmes optimising the use of genetic resources.

Expected outcome

Enhanced use of genetic resources through the development of molecular marker approaches will lead to the production of improved coffee cultivars. In particular, this project would make possible to associate root-knot nematode resistance traits in single cultivars, without reducing coffee quality and within an acceptable time frame. It would therefore contribute to

the sustainable improvement of coffee production which constitutes a major economic and social activity in Central America. The strategy and molecular tools developed during this project could also be used for other important agronomic traits (Coffee Berry Disease, Leaf Rust), and by other coffee breeding programmes world-wide. In addition, the project will significantly contribute to the strengthening of research capabilities through training and technology transfer.

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Contract number: IC18*CT970182

Period: September 1997 to December 2000

DEVELOPING LATIN AMERICAN FRUITS USING THE YEAST *KLUYVEROMYCES MARXIANUS* AND ITS SECRETED PECTINOLYTIC ENZYME ENDOPOLYGALACTURONASE

Co-ordinator: University of Bath, Bath, United Kingdom (A. Wheals)

Objectives

- Produce strains of the yeast *Kluyveromyces marxianus* that over-express the gene for the pectinolytic enzyme endopolygalacturonase (endo-PG).
- Study and optimise natural cocoa fermentations using these strains.
- Produce and purify the enzyme endo-PG from these strains.
- Using the endo-PG enzyme, study and optimise the processing of indigenous Latin America fruits which have not yet been commercially exploited.
- Isolate from these indigenous fruits yeasts which have superior enzyme production and fermentation characteristics.

Activities

- Create endo-PG overproducer strains of *K. marxianus* by conventional means. Use these yeast strains to produce stable, dried yeast for use in cocoa fermentations on the farm. Clone and sequence the endo-PG gene and then overproduce the enzyme using genetic modification techniques for large-scale production purposes.
- Use both fresh-pressed and freeze-dried genetically modified yeast strains to study altered cocoa fermentations both in laboratory-scale experimental fermenters and in small-scale field trials and determine the physiological parameters to optimise the fermentation.
- Develop a production protocol that integrates, in one step, fermentation and purification using the genetically modified *K. marxianus* yeast strains for bulk enzyme production. Scale-up the process to produce food-grade, partially purified and concentrated endo-PG.
- Analyse the biochemical and organoleptic characteristics of eighteen indigenous Latin American fruits. Choose those with pectinaceous pulp and other desirable characteristics and determine their suitability for producing fruit juice (which will be tested in local consumer trials) as well as juice concentrate and fruit nectars.
- Investigate these fruits for their suitability for producing a fermented alcoholic beverage using natural microflora which will be isolated from these fruits and taxonomically and physiologically characterised.

Contract number: IC18*970182

Expected outcome and results

The work carried out should enable existing cocoa fermentations to be optimised which will lead to the production of chocolate of more reliable and improved quality. Some under-exploited Latin American fruits will be developed and be brought to market initially as fruit juices. An enhanced range of microbiological strains will be both created and characterised for use in these and other fruit fermentation industries. A new and independent source of pectinase will be developed for food use on Latin American fruits.

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Contract number: **IC18*CT970185**

Period: December 1997 to November 2000

DEVELOPMENT OF BIOPROCESSES FOR THE CONSERVATION, DETOXICATION, AND VALORISATION OF COFFEE PULP (BIOPULCA)

Co-ordinator: ORSTOM, Montpellier, France (Maurice Raimbault)

Objectives

To recycle coffee pulp and coffee husk by biotechnological processes:

- Transformation of fresh coffee pulp into a stable and detoxified lactic-acid silage product
- Utilization of the lactic-acid silage for food and feed, and for metabolite or enzyme production
- Elimination of an agro-industrial pollutant in Latin America
- Recycling of coffee husk for mushroom and metabolite production
- Biodegradation of toxic compounds (caffeine, ployphenols)
- Diversification of employment in rural coffee-growing areas
- Optimization of infrastructure and employment in coffee-growing areas.

Activities

* Microbiological studies of coffee-pulp silages (microbial ecology, biodiversity, isolation and selection of strains, physiological studies, inoculum production).
* Improvement of fungal strains for the production of tannases, decaffeinases, pectinases, cellulases and hemi-cellulases.
* Production of efficient mixtures of fungal enzymes (cellulases, pectinases, caffeinases, tannases, etc.) by SSF (Solid Subtrate Fermentation).
* Studies of the effect of composite inocula (lactic acid bacteria + fungal enzyme cocktails) on the fermentation process: stabilization and detoxification of the coffee pulp.
* Vapour and chemical pretreatment of dry coffee husk in liquid and solid fractions, and application of bioprocesses for mushroom or metabolite production.
* Breakdown of caffeine, tannins and polyphenols by fungi and lactic-acid bacteria.
* Biodigestibility of fermented coffee pulp and further utilization in food and feed industries: i) balanced feed for ruminants, ii) fish, iii) production of *Pleurotus*.
* Economic analysis and technical feasibility studies of coffee-pulp silage in Mexico, Brazil and Latin America in general.

Expected outcome

⇒ Mexican applications will concern the pulp produced by wet processing. Brazilian studies will use coffee husk produced by dry processing, with or without steam-pressure treatment. Improvement in coffee agro-industry processes leading to less contamination of the environment, and products of increased quality and diversity are a priority for Mexico and Brazil.

⇒ For Brazil, it is important to examine the potential utilization of coffee-husk residues before and after the steam-explosion process that allows an increase in biodigestibility and possible applications in mushroom production.

⇒ For Mexico, ensiling of coffee pulp and its further utilization could avoid the rapid degradation of the material and its rapid conversion into an important source of water pollution in coffee-growing areas. The stabilized and detoxified coffee pulp could then be used during the non-crop season as animal feed or for mushroom, fungal enzyme or metabolite production.

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Contract number: **IC18*CT970188**

Period: December 1997 to November 2000

IMPROVEMENT OF SCALLOP PRODUCTION IN RURAL AREAS

Co-ordinator: Rijksuniversiteit Gent, Ghent, Belgium (Patrick Sorgeloos)

Objectives

- Determine the effects of nutrition, microbial flora, genetic background and water quality on the survival and quality of larval and juvenile stages of scallops derived from hatchery cultivation and natural recruitment.
- Characterize and identify bacteria that play a role in seed production, either beneficial (improving growth, survival and/or settlement success) or detrimental (pathogens).
- Determine the critical nutrients (mainly vitamins and lipid compounds) in hatchery rearing of scallops (broodstock, larval and postlarval stages).
- Assess the genetic diversity in wild and cultivated stocks using both traditional (allozymes) and innovative (microsatellites and mitochondrial DNA) genetic markers.
- Improve microbial control (use of probionts), nutrition (through live algae as well as through the use of artificial supplements), and genetic aspects (through broodstock management strategies, triploidy induction) in scallop rearing.
- Evaluate adapted zootechniques on an experimental scale in rural hatcheries and their effect on grow-out success in the field.

Activities

- ***Nutrition***: Development/preparation of specific supplement diets to supply essential/limiting nutrients to scallop broodstock and larvae, development of feeding regimes for artificial diet supplementation to live algae, verification of the use of supplementation diets for local species in Latin-America, nutritional status and potential of seed collected in nature versus hatchery-produced seed.
- ***Microbiology***: Characterization of microflora, confirmation of pathogenicity (challenge test), confirmation of beneficial/detrimental bacterial strains, evaluation of the potential use of selected bacterial strains under the conditions of rural hatcheries, microbiological analysis of the environment, microbiological safety.
- ***Genetics***: Assessment of genetic resources in wild and cultivated scallops using allozyme techniques and novel biotechnological markers, optimization of the genetic component of broodstock management strategies, microsatellite loci and mitochondrial DNA techniques, triploidy induction development and evaluation.

Expected outcome

⇒ Supporting an activity that has a great potential as a sustainable source of income for rural communities that have recently switched from artisanal fishermen to mollusc growers.

⇒ Improving the predictability and sustainability of scallop seed production.

⇒ Better understanding of the various nutritional, microbiological and genetic factors determining the success of scallop larviculture. In particular the genetic work will provide

baseline data for genetic improvements of scallops stocks and the conservation of genetic resources.

⇒ Collaboration between European and Latin-American partners provides an opportunity for a high degree of training in methodology and strengthen the research capability both of the young researchers involved and their host institutions.

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Contract number: IC18*CT970192

Period: December 1997 to November 2000

SIGATOKA DEFENSE GENES OF BANANA CULTIVARS AND WILD *MUSA* SPECIES IN LATIN AMERICA

Co-ordinator: Johann Wolfgang Goethe Universität, Frankfurt-am-Main, Germany
(Günter Kahl)

Objectives

- Establishment of cell-suspension cultures from the Yellow Sigatoka-resistant somaclonal Cavendish mutant CIEN BTA-03 and its susceptible mother plant Brasilero.
- Development of an analytical HPLC method for *Musa* phytoalexins and a DNA marker system to measure (semi)quantitatively the response of *Musa* cells after elicitation by crude elicitor preparations of both pathogenic *Mycosphaerella* species.
- Application of the newly developed marker system(s) to select the fungal crude preparation with the highest elicitation activity, and to characterize *Mycosphaerella* isolates from different geographical origins for their elicitation potential.
- Systematic use of *M. fijiensis* and *M. musicola* elicitors to discover differences of gene expression patterns between the resistant CIEN-BTA-03 mutant and the corresponding mother plant BRASILERO.
- Use of *M. fijiensis* and *M. musicola* elicitors to analyse differentially expressed cDNAs in the *Musa acuminata* ssp. *burmanicoides* (Calcutta IV), a wild BLACK Sigatoka-hypersensitive banana, to isolate a fast fungus-inducible promotor.
- Design and assembling of *Musa* promotor/reporter gene constructs, stable transformation of susceptible banana cultivars, and *in vivo* testing of the reporter gene induction by local *Mycosphaerella* populations in greenhouses.

Activities

- Elicitation of Yellow - and Black Sigatoka-resistant and -susceptible plants, RNA-isolation and cDNA synthesis, performance of differential display RT-PCRs and subtractive hybridizations to isolate and characterize differentially expressed cDNAs.
- Establishment of a BAC library from a resistant banana and use of differentially expressed cDNAs to isolate corresponding *Musa* defense genes, and to characterize their promotor sequences.
- Assembling of promotor/reporter gene constructs, stable transformation of susceptible banana cultivars, sampling and single-sporing of *M. fijiensis* and *M. musicola* from Latin America, and infection of transgenic banana plants using conidiospores.

Expected Outcome

⇒ The main goal of this project is the isolation of several fungus-inducible *Musa* defense gene promotors for genetic engineering of Black/Yellow Sigatoka resistance in *Musa* cultivars.

⇒ Novel data on the induction of different banana defense genes by a range of fungal elicitor preparations and the time course of their transcriptional activity will be gathered.

⇒ A *Musa* BAC library will be available for future use (e.g. cloning of resistance genes).
⇒ Finally, a transgenic *Musa* cultivar with improved resistance to *M. fijiensis* and *M. musicola* is expected.

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Contract number: IC18*CT970194

Period: December 1998 to November 2001

EVALUATION AND UTILIZATION OF *CALLIANDRA CALOTHYRSUS* MICROSYMBIONT BIODIVERSITY FOR OPTIMIZING FORAGE PRODUCTION ON SMALL FARMS IN HUMID REGIONS

Co-ordinator: ORSTOM, Dakar, Senegal (Didier Lesueur)

Objectives

The main objective of the project is to optimize the forage production of *C. calothyrsus* on small farms by inoculation with highly efficient strains of rhizobia and/or mycorrhiza strains selected under laboratory, greenhouse, and field conditions. In order to achieve this objective we will investigate the main symbiotic characteristics of *C. calothyrsus*, evaluate a range of potential inocula, and attempt to produce a suitable microbial inoculum for inoculating plants under field conditions.

Activities

- Collection of microsymbionts of *C. calothyrsus*. The project will establish a large collection of rhizobiae and mycorrhizae isolated respectively from the nodules and roots of this species harvested in its native range (Honduras, Costa Rica and Mexico), and in humid countries where it has been successfully introduced (Cameroon, Kenya and New Caledonia). After evaluation of the biodiversity within the collection of microsymbionts, a symbiotic screening will be carried out in the laboratory and greenhouse in order to select the most efficient strains for inoculation under field conditions.
- Field inoculation of *C. calothyrsus*. Existing methodologies for producing rhizobial inoculum and inoculating plants under field conditions will be developed further for inoculation in field stations, and finally, under farm conditions.

Expected outcome

To produce and dispense selected strains of microsymbionts suitable for use on farms in order to increase forage production. Researchers involved in the project from both European and third country institutes will have the opportunity to train in technical areas, such as inoculum production, and in scientific areas, such as survey of the biodiversity of rhizobia strains and study the persistence of rhizobia after inoculation in soil.

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Period: October 1997 to September 2000

NEW TECHNOLOGY FOR CONTROLLING INSECT PESTS OF OIL PALM AND COCONUT CROPS: RESEARCH AND DEVELOPMENT IN SELECTIVE TRAPPING USING SYNTHETIC ATTRACTANTS

Co-ordinator: CIRAD-CP, Montpellier, France (Dominique Mariau)

Objectives

The project aims at the sustainable improvement of oil palm and coconut production in the circum-Pacific area. This will be achieved by developing cost-effective and environment-friendly technology. In particular, Integrated Pest Management (IPM): the selective mass trapping of the major insect pests of these crops, *Rhinoceros* beetles (Rbs) and Palm weevils (Pws), using synthetic attractants, pheromones and plant synergists.

Activities

The project will depend for success on constant field and laboratory exchanges. Prior to any laboratory work, attraction to insect or plant sources will be determined by field experimental trapping. Such attractions will be determined by olfactometry studies in the field. In the laboratory, the most recent techniques for trace volatile isolation and analysis will be used: Gas Chromatography (GC) and Mass Spectometry (MS). Large-size olfactometers adapted to the insect pests and GC-Electroantennography (GC-EAG) coupling will be developed for accurate screening of bioactive compounds. Organic synthesis will provide synthetic pheromones, while dispensers and traps will be designed and calibrated in collaboration with industry to screen compound bioactivity in the field. Simplification of plant-derived mixtures will be undertaken according to subtractive-additive procedures. The mass trapping tools (attractants, dispensers, traps) will be optimized and developed taking into account local economic and bioclimatic conditions for rapid use in integrated pest management.

Expected outcome

The achievement of efficient control of Red-Ring disease (RRD), vectored by Pws is expected in Colombia and in the whole neotropical region by the mass trapping of *Rhynchophorus palmarum* and *Metamasius hemipferus*. RRD is presently hardly controlled, resulting in dramatic economic losses. The mass trapping of *Rhynchophorus bilineatus* is expected to improve coconut protection in Papua New Guinea, together with the control of local Rbs, especially *Scapanes australis*. The achievement of efficient and cost-effective control of the Rbs is expected in the three developing countries involved in the project, and by extension, in the whole coconut growing area, by the mass trapping of *Oryctes rhinoceros*, *Strategus australis* and *Strategus aloeus*. A 50% decrease in the cost and in insecticide application is expected in Indonesia for *O. rhinoceros* control. New coconut development is expected in Papua New Guinea which has been impossible up to now because of *S. australis* damage.

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Contract number: IC18*CT970201

Period: January 1998 to December 2000

PHA PRODUCTION FROM SUGAR CANE DERIVATIVES

Co-ordinator: Westfälische Wilhelms-Universität, Münster, Germany
(Alexander Steinbuchel)

Objectives

- Develop a fermentation process for the production of biodegradable PHAs (polyhydroxyalkanoates) from cheap renewable substrates employing a previously isolated bacterial strain and agroindustrial products.
- Initiate a screening program to detect and isolate new bacterial strains with the capabilities to produce PHAs with different properties for future applications from renewable substrates.
- Conduct basic studies on the biochemistry and genetics of PHA granules associated proteins in the bacterial strains mentioned above.

Activities

* Employ batch cultures in stirred tank reactors for growth of bacterial cells on sucrose to high cell densities with maximum PHA content. The feeding regime, temperature, agitation, aeration rate, pH, medium components, dissolved oxygen concentration will be optimized for this purpose.
* Proteins associated with the PHA granules will be isolated and characterized, and the corresponding genes will be cloned and sequenced.
* In addition, various microscopic and electron microscopic methods such as confocal scanning laser microscopy, transmission electron microscopy and scanning electron microscopy will be employed to characterize the strains and to identify more suitable production strains.

Expected outcome

This project should reveal a process for production of PHA at low costs. The proposed research will also enhance the research level and the training of human resources in the participating developing countries. This will be achieved not only by an extended exchange of information and knowledge between the participating laboratories, and by the provision of strains and genes, but also and in particular by increasing the mobility of researchers of all participating laboratories as a result of giving researchers (PostDocs) or Ph. D. students of the laboratories of the developing countries the possibility to visit the laboratories of the other partners and to learn sophisticated methods. Vice versa, researchers from the latter laboratories will visit the participating laboratories of the developing countries for training courses and for laboratory research.

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Contract number: IC18*CT970202

Period: January 1998 to June 2001

ANALYSIS AND MANAGEMENT OF ORGANIC MATTER AND NITROGEN IN AQUACULTURAL PONDS FOR A MINIMAL WASTE PRODUCTION AND OPTIMAL EFFICIENCY

Co-ordinator: Wageningen Agricultural University, Wageningen, The Netherlands
(Johan Verreth)

Objectives

- The general objective of the present project is to develop a management system for feed driven fish/shrimp ponds that minimizes the accumulation of dischargeable products in the system. The working hypothesis is that this can be achieved when the concentrations of organic carbon, nitrogen and phosphorous in the system are balanced to each other. This will result in a maximal conversion of these nutrients into bacterial biomass which, in turn, may be harvested by the fish.
- The specific objectives of the project are geared towards the collection of the empirical data needed to construct a model for organic carbon and nitrogen fluxes in feed driven fish ponds and to use and validate this under different management procedures.

Methods and activities

- The work will be conducted through various work packages that coincide with the different steps in the process of model development and model testing under practical (management) conditions. To develop the model, basic information on the processes steering the nutrient fluxes in the pond must be collected. In this regard, the following topics will be addressed : (1) physical and biological characterization of the flocculant layer; (2) the kinetics of organic matter breakdown and the influence of its C:N ratio's on it; (3) the sedimentation to and resuspension from the flocculant layer of organic matter; (4) diffusion rates of nutrients at the flocculant layer - water interface; (5) the contribution of autotrophic and heterotrophic production to fish/shrimp production; (6) the indirect stimulation of the algae growth either through the ammonia excretion by the fish or by ammonia and phosphorous diffusing into the water column from the flocculant layer; (7) uptake, digestibility and growth of tilapia/shrimp fed material from the flocculant layer; and (8) upscaling of the results to pilot farm conditions and development of demonstrator.
- The characterization of the flocculant layer (physical and biological parameters, diffusion of material), data on sedimentation and resuspension etc. will be collected at Technion, Haifa in Israel. The kinetics of organic matter breakdown and of its conversion into fish will be investigated at Wageningen, The Netherlands. Pilot studies in shrimp ponds and studies on algae growth will be conducted at CIAD Mazatlan in Mexico. The Costarican counterpart (Universidad Nacional, Heredia) will carry out pilot experiments in tilapia ponds and develop a simulation model together with the support of the Wageningen University (Water Quality Group and Fish Culture & Fisheries Group).

Expected outcome

The project started on January 1998. During 1998, the project management has to be mounted, detailed experimental designs will be developed, the model will be conceptualized and parameterized and the first experiments on flocculant layer characterization and bacterial kinetics will be carried out. At the end of the project, the expected outcome is a detailed knowledge and understanding of the nutrient exchange processes at the soil-water interface in stratified and stagnant ponds; an operational and validated dynamic simulation model describing nutrient conversions in intensive fish and shrimp ponds; and an outline in which direction practical pond management should develop to make intensive fish/shrimp farming more environmentally friendly.

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Contract number: IC18*CT970203

Period: October 1997 to September 2000

QUALITY IMPROVEMENT OF SUGAR CANE FIBRES FOR THEIR USE AS RAW MATERIAL IN THE PRODUCTION OF PAPER AND ANIMAL FEED

Co-ordinator: Vlaams Interuniversitair Instituut voor Biotechnology, Gent, Belgium
(Wout Boerjan)

Objectives

The goal of this project is to produce, by genetic engineering, transgenic sugar cane varieties of which the bagasse can be efficiently used for paper pulp production and for livestock feed. Our strategy involves the reduction of the lignin content or a modification of its composition in the fibre by reducing the production of the enzymes cinnamyl alcohol dehydrogenase (CAD), bispecific caffeic acid/5-hydroxyferulic acid-*O*-methyltransferase (COMT) and/or cinnamoyl-CoA-reductase (CCR) by antisense technology. As an alternative strategy, lignin content and composition will be altered through the modification of the *p*-coumaric acid (and its aldehyde and alcohol derivatives) content. Both strategies would increase the quality of the bagasse pulp by facilitating lignin extraction during the pulping process in the paper industry. In addition, the proposed modifications would increase the nutritional value of the bagasse forage through improvement of its digestibility.

Activities

* Isolating cDNAs for CAD, COMT and CCR;
* Making chimeric sense and antisense constructs for all three genes;
* Transforming sugar cane varieties of high economic interest;
* Generating polyclonal antibodies for CAD, COMT and CCR;
* Evaluating the transgenic lines by molecular analyses;
* Isolating genes responsible for the catabolism of *p*-coumaric acid from *Pseudomonas*;
* Overexpressing these genes in sugarcane;
* Overexpressing this gene in transgenic sugarcane that overproduces COMT;
* Evaluating all transgenic lines for lignin modifications;
* Evaluating the transgenic lines for their digestibility;
* Evaluating the transgenic plants in the field.

Expected outcome

The sugar industry generates hundreds of millions of tons of bagasse that are unused or simply burned. The work carried out in this project should result in transgenic sugar cane varieties of which the bagasse can be used in the pulp and paper industry and as livestock feed. In addition, basic knowledge on the regulation of lignin biosynthesis in grasses will be gained.

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Contract number: IC18*CT970204

Period: October 1997 to September 2001

OPTIMIZATION OF NEW BREEDING STRATEGIES FOR BANANA FOR LOCAL MARKETS

Co-ordinator: Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD-FLHOR), Montpellier, France
(Hugues Tezenas du Montcel)

Objectives

- Synthesis of AAB interspecific triploid, cross-bred banana hybrids, aiming at a better understanding of the transmission of specific characteristics of *M. acuminata* and *M. balbisiana* to their hybrid offspring, notably for resistance to yellow cercosporiosis and to black-stripe disease.
- Production and characterization of AAB interspecific hybrids obtained by fusing *acuminata* diploid (AA) protoplasts with *balbisiana* haploid (B) protoplasts. These somatic hybrids will be compared with zygotic hybrids obtained by cross-breeding.
- Better knowledge of cercosporioses: Study of pathogenic populations by using neutral molecular markers. These studies are carried out on different reference banana plants.

Activities

* Creation of AAB hybrids using cross-breeding techniques.
* Multiplace assessment of the hybrids created by cross breeding.
* Study of the transmission and heritability of the agronomic and cercosporiasis-resistance characteristics.
* Preparation of *M. balbisiana* haploid protoplasts and interspecific fusion of these protoplasts.
* Comparison of the zygotic hybrids with the somatic hybrids obtained.
* Study of the genetic structure of -pathogenic populations of cercosporioses.
* Characterization of the partial resistance of the hybrids obtained.

Expected outcome

⇒ This project will enable us to obtain banana hybrids resistant to cercosporioses.
⇒ The comparison between the hybrids obtained by using two different pathways but the same parents will help us understand how the main agronomic characteristics are transmitted; among others, those linked to resistance to black-stripe disease.
⇒ The study of pathogenic populations of cercosporioses in Latin America, the Caribbean and Africa will permit the selection of banana hybrids with sustainable partial resistance with regard to the evolution of pathogenic populations and their pathogenic strength.
⇒ The development of early, *in vitro,* tests will make much easier the selection of cercosporiosis-resistant banana which currently makes a heavy demand for field work

Results

⇒ CIRAD has obtained first populations of hybrids by cross-breeding, as a result of the tetraploidization of the four *acuminata* diploids used as male parents.

⇒ Embryogenic calluses were obtained at CATIE, and/or from CIRAD's samples in Guadeloupe for the four diploids. These embryogenic calluses were transmitted to Orsay University, as a BZK (German partner) subcontractor.

⇒ Samples of pathogenic populations of black-stripe disease were sent to CIRAD in Montpellier to start a study on pathogenic populations.

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Period: September 1997 to August 2000

DEVELOPMENT OF NEW PROCESSES FOR THE EXTRACTION OF OILS AND ACTIVE PRODUCTS FROM NON CONVENTIONAL OILSEEDS AND VEGETABLES FOR THE PHARMACEUTICAL AND FOOD INDUSTRIES

Co-ordinator: Universidad de Santiago de Compostela, Santiago de Compostela, Spain
(Juan M. Lema)

Objectives

The aim of this project is to obtain valuable products from oilseeds and vegetables by applying an environmental sustainable technology, based on efficient extraction processes either aided or not by enzymes. The main objectives are:

- Improvement of the oil extraction yield attained in the conventional pressing process by means of an enzymatic treatment.
- Development of extraction processes using non toxic, renewable solvents (ethanol, water) emphasizing in the use of efficient non polluting technologies and economically feasible, improving the yields by means of an enzymatic treatment and enzyme reuse.
- Modeling enzyme action of cellulases and kinetic evaluation based in models of enzyme behaviour on synthetic substrates and characterization of the tissue modification in the treated seeds.
- Integral use of the raw materials by using the defatted meals as a source of protein and dietary fiber.

Activities

* Characterization of the vegetable substrates and selection of the best enzymes for enhancing the oil extractability.
* Basic research on enzyme technology including composition of the enzymatic complexes and optimization of the combination fragmentation/ hydrolysis in order to maximize oil extractability and minimize liberation of small molecules. Studies of adsorption-desorption and enzyme inhibition by polyphenolics present in the seeds are needed for practical purposes of reutilization of the enzymes.
* Optimization of the operational conditions for the enzymatic treatment at intermediate moisture before the pressing stage to improve the oil yields and to enhance the meal quality.
* Development of the alcoholic extraction process. This study will be focused on the solubility of the oil soluble fraction from the seeds in ethanol, the effect of liquid:solid ratio, particle size and flow pattern, miscella retention and kinetics of the batch extraction processes. At the end industrial operation will be simulated.
* Study and development of an enzyme aided extraction process based in an aqueous one or two phase system. Optimization of the operational conditions affecting both the enzyme efficiency and extraction is required : sample size and eventual pretreatment, enzyme reaction conditions, reaction system, solid:liquid ratio, type of reactor and extraction and separation of phases.
* The extraction of the protein and/or fiber contained in the extracted meal for feed or food products will be studied using the meal from the different oil extraction processes developed in this project by the utilization of these purified protein and fiber as ingredients in human foods.

Expected outcome

Results derived from the improvement of conventional technology will provide higher oil extraction yields and/or higher productivity of the equipment, without altering the properties of the oil and even improving those of the meal (enhanced oil extractability requires lower temperatures in the press) for use in food or feed. Development of alternative processing either improved or not by biotechnological means (application of enzymes) and the use of alternative extraction processes using renewable solvents will be useful for processing other oilseeds. The obtention of high-added value products from seeds is expected to improve the economy of the agricultural areas in DC, since the proposed processing methodologies can be implemented either in the existing equipment for extracting other oilseeds or in relatively economical equipments, batch, versatile, and useful for processing other seasonal crops or these seeds indigenous of the Latinoamerican area.

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Period: November 1997 to October 2001

ALLEVIATING ABIOTIC AND BIOTIC SOIL CONSTRAINTS BY COMBINING ARBUSCULAR MYCORHIZAL FUNGI WITH BANANA AND PLANTAIN MICROPROPAGATION SYSTEMS

Co-ordinator: Université Catholique de Louvain-la-Neuve, Louvain-la-Neuve, Belgium
(Bruno Delvaux)

Objectives

- Determine the impacts of AM fungi on the *in vitro* cultivation systems of banana and plantain, their subsequent effects on the acclimatization phase of the plantlets in the nursery and their performance under field conditions.
- Assess the effects of AM fungi on the alleviating of major soil constraints: mineral deficiencies or toxicities and root parasitism.
- Develop AM fungal inoculum production and application strategies consistent with current banana and plantain production systems.

Activities

* Establishment of a collection of *AM fungi* originating from banana and plantain fields in distinct soil conditions in Colombia, Cuba, the French West Indies and Cameroon: Andosols, Ferralsols, Acrisols, Vertisols.
* Improvement of monoxenic inoculum technologies of *AM fungi*.
* Determination of the *AM fungi* formulation and inoculation procedures for its implementation in micropropagated banana and plantain production systems.
* Determination of the effects of *AM fungi* on banana and plantain growth under abiotic stress (nutrient depletion and mineral toxicities) using nutrient flow condition.
* Assessment of the interactions between mycorrhized bananas and plantains and their main root parasites: the *fungi Cylindrocladium* spp. and *Fusariumoxysporum* var. *cubense* and the nematodes *Radopholus similis, Pratylenchus goodeyi, Pratylenchus coffeae, Helicotylenchus multicinctus* and *Meloidogyne*spp.
* Study of the field performance of mycorrhized bananas and plantains under current production systems.

Expected outcome and results

⇒ A successful association of *in vitro*-produced *AM fungi* with micropropagated bananas and plantains is foreseen. A faster plant growth is expected during the acclimatization of the young plantlets in the nursery and, hopefully, a reduction in the use of chemicals. Increasing the mineral uptake rate in nutrient-depleted acid soils and the plant tolerance to root parasites concerns both extensive and intensive cropping systems. Information on the interest and effects of *AM fungi* inoculation programs for bananas and plantains should be improved.

⇒ So far, it has been demonstrated that Al at low concentration induces a net decrease in root/shoot production, particularly large for AAA bananas as compared to plantains. Inoculation of banana roots by *AM fungi* has been successful in nutrient solution on a sandy (inert quartz) substrate allowing future experiments on the effect of AM fungi on alleviating the Al stress. Progress has been made on the establishment of models involving AM fungi (plant) nematode or pathogen *fungus* interactions. Isolations from fields have been initiated.

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Contract number: IC18*CT970209

Period: September 1997 to August 2000

CHARACTERIZATION OF IMMUNE EFFECTORS IN PENAEIDS: APPLICATION TO PROPHYLAXIS AND SELECTION OF RESISTANT SHRIMP

Co-ordinator: IFREMER/CNRS, Montpellier, France (Evelyne Bachère)

Objectives

- Increase basic knowledge on penaeid immunity by creating an open collaboration network between research laboratories involved in shrimp and other invertebrate immunity specialists ;
- Improve communication and collaboration between ongoing research projects in European, Asian and American countries ;
- Initiate new ideas and collaborative supported projects ;
- Transfer and exchange information and results to other related areas like nutrition, reproduction, genetics and toxicology or environment, in order to develop strategies aimed at prophylaxis and shrimp disease control.

Activities

* Biochemical and genetical characterisation of defence effectors involved and expressed in response to pathological injuries in penaeids.

Expected outcome

⇒ Within the framework of the project, immune effectors are characterised leading to the development of quantitative assays for evaluating and monitoring the immune state of shrimp. The establishment of regular health controls will permit to detect shrimp immunodeficiencies and so to prevent disease, but also to control and improve the quality of the environment

⇒ With regard to medium- or long-term application, the identification of immune effectors and the evaluation of the defence reactions will be related to genetic characterisation of shrimp strains for further application in selection programs of disease-resistant shrimps.

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Period: September 1998 to December 2002

BUFFER ZONES FOR THE SUSTAINABLE USE OF RAINFOREST BIODIVERSITY: THE EXAMPLE OF THE EASTERN SLOPE OF THREE ANDEAN COUNTRIES

Co-ordinator: Justus-Liebig-Universität Giessen, Giessen, Germany (Reinhard Kaufmann)

Objectives

The purpose of the project is to determine the effectiveness of buffer zones in protected rain forest areas and their regional surroundings using an interdisciplinary scientific approach. The Project will analyze the structure and development of buffer zones in their ecological and socio-economic contexts, as well as the administrative, planning and legal instruments to establish and maintain them. Study sites surrounding protected areas will be selected on the eastern slopes of three Andean/Amazonian countries: Bolivia, Peru, and Ecuador.

Buffer zones are expected to:

- Facilitate economic development through the use of planned and sustainable land practices;
- Conserve biodiversity outside as well as inside the protected area and reduce the negative impact of development in the nuclear zone of the protected area;
- Demonstrate the minimum requirements for political and social acceptance.

Activities

The eastern slope of the Andes has great biological diversity. As in many forested areas in the tropics, this region is also experiencing rapid changes related to development. Research sites in the lowland and montane areas will be studied with the expectation that the results of this pilot project will be transferable to other similar regions in South America. This interdisciplinary approach highlights the identification of ecological indicators in the planning phase and the consideration of social and economic needs in ecological research.

The following activities will be performed at each of the study sites:

* Analysis of current land use including suggestions to improve the current use and possible development of alternatives.
* Ecological analysis to determine the effect of the land use on biodiversity; a biomonitoring system with ecological indicators will be developed to identify inappropriate land uses.
* Identification of variables to evaluate local acceptance of government policies for environmental protection.
* Analysis of the current economic structure on environmental protection; the existing planning instruments will be evaluated for their efficiency.
* Study of the political and institutional expectations and their influence on sustainable resource use in buffer zones.

Expected Outcome

The expected result of the project is an interdisciplinary concept for buffer zone management that includes:

- Agroforestry systems to improve sustainability of land use and the income of local people which in turn is expected to raise the acceptance of protected areas.
- Accumulation of data about changes in biodiversity of tropical forests by land use category.
- A biomonitoring system to evaluate the impact of land use patterns on biodiversity within and near protected areas.
- Identification and evaluation of administrative, political, social, and economic factors that are related to the success/failure of resource management.

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Contract number: IC18*CT980262

Period: October 1998 to November 2001

THE SUSTAINABLE MANAGEMENT OF WETLAND RESOURCES IN MERCOSUR

Co-ordinator: Università degli Studi di Siena, Siena, Italy (Claudio Rossi)

Objectives

- Create the tools and methodology for a management of wetland resources that are socially, economically and environmentally acceptable.
- Carry out a long-term study of the impacts of large-scale regional modifications in economic activities, population density, transportation and energy production on wetland resource quality and wetland ecosystem stability in the Mercosur.

Activities

* A resource quality monitoring programme that includes two permanent sites for the collection and transmission of chemical, physical and biological data for long term monitoring of the wetland by regional and local authorities as well as the international scientific community. Temporal and spatial modifications of key wetlands factors will be monitored to determine the impact of anthropic activities as well as the natural variations in the ecosystem stability.
* A geographical information system as a basis for the construction of mathematical models that will be used in the study of the sustainable use of the wetland resources. Information on the structural and functional characteristics of the ecosystem will be systematically organised, incorporating data from past studies with new research into biological, chemical, hydrological meteorological and ecological characteristics of the ecosystem.
* Socio-economic, chemical, physical and ecological models to predict the consequences of growth in demand for and pressure on natural resources in the area. A historical study of the natural resource use in the economic activities of communities living near the wetland will be made.
* An analysis of potential resource uses and management scenarios integrating modelling and analysis techniques to examine overall impacts to the resource quality, ecosystem stability and local socio-economic situation.
* A management system package (software and written) for key natural resources (water and identified fauna and flora species) based on selected global and ecological modelling approaches for the sustainable use of natural resources in wetlands.

Expected Outcome

The project should create appropriate tools necessary to manage and monitor the natural potential of the region's wetlands without compromising the future availability of these resources. Innovative approaches to monitoring of wetlands will be developed and tested using state of the art monitoring techniques combined with remote sensing capabilities. A diversified team of modellers will integrate biological, hydrological, ecological and economic modelling approaches into a workable methodology for the evaluation of future scenarios and

continued management efforts. A final packet of instruments should including, monitoring protocols, goal function analysis, ecological economic models and ecological modelling. The instruments will be developed based on the study of the Esteros del Ibera wetland in northern Argentina but will be transferable in other wetlands in the Mercosur region. The project is further designed to make available to the international scientific and business communities a rich source of physical and ecological data, together with the mathematical models that could be utilised in their analysis.

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Period: October 1998 to September 2001

FERTILITY MANAGEMENT IN THE TROPICAL ANDEAN MOUNTAINS : AGRO-ECOLOGICAL BASES FOR A SUSTAINABLE FALLOW AGRICULTURE (TROPANDES)

Co-ordinator: Consejo Superior de Investigaciones Científicas, Santiago de Compostela, Spain (Tarsy Carballas)

Objectives

- Improve farm income and, consequently the standard of living of the rural population practising fallow agriculture in the North and Central Andes.
- Analyse the soil organic matter dynamics in the *paramos* of Venezuela and the *punas* of Bolivia as the agroecological base of the functioning of fallow agriculture, which is extensively practised in the tropical high Andes and which is characterised by a short period of potato crops, a fast decline in fertility and the need for a prolonged fallow period to restore fertility.
- For socio-economic reasons, develop tools allowing the exploitation of possibilities of improving the current management of the crop-fallow practices and the evaluation of short- and long-term consequences.

Activities

* At the regional scale: Field studies to identify and characterise, in selected regions of both countries, the areas where fallow agriculture is practised.
* At the farm scale: Studies to determine, in two selected zones or geomorphological units, the dynamics of land use, and to analyse the different factors which influence the length of the fallow period and to use this information in the elaboration of models simulating land use dynamics at the farm scale.
* At the ecosystem scale: i) Studies to elucidate the mechanisms of fertility loss during the crop period and its progressive restoration during the fallow period, in the hypothesis that the fast loss of fertility during the crop period is not due to the deficiency of nutrients but to their low availability and that fertility restoration would result from the progressive and slow remobilisation of N and P and its cycling through plant succession during the fallow period; ii) Development of integrated simulation models of agroecosystem functioning and scenario studies integrating the farm system.

Expected Outcome

This project should provide the necessary knowledge on which to base sustainable management of soil fertility in the high tropical Andes and to optimise the balance of fallow-crop systems without degrading the natural environment. Another outcome will be using the database and the integration models to elaborate simple and useful tools for the regional teams to explore other scenarios and alternatives and to formulate working hypotheses for promising future research, such as: fractionation of inorganic fertilisation, use of organic amendment, favouring successional species, introduction of secondary crops, etc.

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Contract number: IC18*CT980264

Period: August 1998 to July 2001

TROPICAL AND SUB-TROPICAL COST-EFFECTIVE TOOLS FOR AN INTEGRATED RISK ASSESSMENT OF WETLANDS (TROCA)

Co-ordinator: Universidade de Coimbra, Coimbra, Portugal (Amadeu Soares)

Objectives

The project will focus on two study sites: in the lower reaches of the Paraguaçu River estuary (Brazil) and in the Bay of Campeche (Mexico), adjacent to agricultural cash crop areas. By applying baseline information from the study sites, this project aims to develop cost-effective tests and other environmental diagnostic tools that can ultimately be used in an integrated risk assessment model for the management of tropical and sub-tropical wetlands. To attain this general objective, we will:

- describe land use and agricultural patterns in the study areas, and evaluate the agricultural potential of the regions in view of deriving policy options on alternative use of natural resources.
- investigate the environmental and human impacts of agriculture practices on aquatic systems by evaluating the amount of bioactive compounds (fertilisers and pesticides) released into the environment, investigating possible socio-economic changes derived from the introduction of agricultural practices based on an intensive use of agrochemicals, carrying out experimental research to generate data on chemical fate and effects on soil and aquatic life, through laboratory and *in situ* ecotoxicological tests (adapted to the conditions of tropical and sub-tropical environments) and the use of biomarkers.

Activities

* Field studies to collect baseline data on the watersheds and socio-economical information, on both study sites, located in Brazil and Mexico. The study will address the chemistry and biology of both studied systems; the collected information will be the basis for the other project components.
* Ecotoxicological assessment of both sites with local species. During a first phase, short-term (i.e. acute) single-species toxicity test methodologies will be used to determine toxic effects of relevant agrochemicals, using novel procedures developed in a previous EU Environment Programme project. The single-species tests will run under conditions relevant for the tropics. A recently proposed approach regarding *in situ* testing, where standard and local species are exposed in the field, will be followed. Test chambers will be built and respective protocols will be developed. Along with the comparison between laboratory and *in situ* testing results and between local and standard species, biomarker methodologies for use with local species will be developed and adapted.
* The full data set will be integrated in a risk assessment model, to allow the drawing of recommendations / guidelines for a more sustainable use of resources, hence a better management of tropical wetlands.

Expected Outcome

This project should provide indications regarding the use of appropriate diagnostic tools for use in an Integrated Risk Assessment Model for the Management of Tropical and Sub-Tropical Wetlands. To achieve this, it should define a suitable ecotoxicity test battery and evaluate the potential use of biomarkers in ecological risk assessment of chemicals in the tropics. An indication will be given regarding the use of local species for ecotoxicity testing. Appropriate strategies and specific technologies for implementing *in situ* toxicity assays will be recommended.

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Contract number: IC18*CT980271

Period: October 1998 to September 2000

INTELLIGENT MANAGEMENT SYSTEM FOR WATER AND ENERGY MINIMISATION IN LATIN AMERICAN FOOD INDUSTRIES (WATERMAN)

Co-ordinator: Universitat Politecnica de Catalunya, Barcelona, Spain (Luis Puigjaner)

Objectives

- Establish new criteria and develop the methodology and tools aiming at improving water management in food processing industries as an integral part of energy-efficient, environmental respectful ,and economically sound process operations.
- Reduce water consumption by efficient management and control of process operations, and optimize material and energy balances of the process by applications of advanced optimization strategies aiming at waste reduction.
- Integrate optimization and production planning techniques in conjunction with real-time plant measurements and control for product quality and reduction of losses.
- Enhance intelligent support to the operator by application of knowledge-based decision-making procedures to select those options which best protect the environment.
- Validate the different methodologies and associate supporting software in two representative agroindustrial sectors: the sugar industry and citrus processing (concentrated juice and essential oils).

Activities

* Evolutionary modelling framework of process operations using neural network structures specifically designed for multi input/output modelling applications and recurrent non-linear back propagation connections for control applications, leading to the establishment of real-time models that will address operational problems and support decisions.
* The modelling structures developed will be embedded in an integrated plant information framework for an enhanced understanding of the operations and support intelligent decision-making
* New optimisation methods of MINLP models with differential and algebraic constraints will model the importance of water / energy savings and environmental protection in the design / retrofit and production decisions.
* An expert system shell will embrace the methods and structure realised under second step and will have access to the optimisation systems developed. Thus, intelligent monitoring and control of the process operations will be achieved with overall performance indexes (on energy, water, product quality, etc.).
* The above methodologies and algorithms will be implemented in a software prototype that will incorporate user-oriented management tools in an expert guide mode.

Expected Outcome

The project is addressed to the food sector (sugar, fruit processing) of strategic importance in all LA. Both sectors involve complex water/energy intensive operations with waste and by-

product generation. Expected benefits are reductions over 50 % in water and up to 30 % in energy consumptions, which will have a direct implication in making environmentally more benign processes and will contribute significantly to reduce the water contamination in the region. Additional benefits are expected in controlling process variables in on-line hierarchically structured control to reach optimum decisions at all plant management levels. This is vital for industry in order to survive and prosper in today's competitive and aggressive market.

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Period: October 1998 to 30 September 2001

EVALUATION OF THE USEFULNESS OF BACTERIOPHAGES AS MODEL MICRO-ORGANISMS FOR THE ASSESSMENT OF WATER TREATMENT PROCESSES AND WATER QUALITY

Co-ordinator: Universidad de Barcelona, Barcelona, Spain (Juan Jofre)

Objectives

The project is expected to lead to the evaluation of the usefulness of different groups of bacteriophages as model microorganisms for the assessment of water treatment processes and water quality. The ultimate objective of the project is to prevent the transfer of infectious disease by water. The goals are:

- Verify whether bacteriophages serve as model microorganisms for the presence of faecal pathogens in the water environment.
- Verify whether bacteriophages are useful tools for determining the performance of the multiple "barriers", natural and artificial (water treatments), that pathogens and model microorganisms find in their way from faeces to drinking waters.
- Determine which of the three groups of bacteriophages studied (somatic coliphages, F-specific RNA bacteriophages and bacteriophages infecting *Bacteroides fragilis*) is more suitable as model por the purposes mentioned above.
- Verify whether the bacteriophages chosen as models are applicable in distinct geographical areas of the world as different as the Mediterranean area and Central Europe in Europe and the "Altiplano Andino" and the "Vertiente Atlántica del Cono Sur" in South America.

Activities

* Setting up of operating principles during collection and analysis of samples in the four laboratories in order to obtain data of known and defensible quality for all the partners. These principles will be established for those microbiological methods (bacteriophages, bacterial indicators and protozoa) for which standardised procedures and reference materials are available.
* Quantification of bacteriophages and the microorganisms included in the study in raw sewage and natural freshwaters (surface water and groundwater) from the countries of the four partners.
* Quantification of the removal of bacteriophages and the other microorganisms in wastewater treatments of different characteristics in the countries of the four partners.
* Quantification of the removal of bacteriophages and the other microorganisms in drinking water treatments of different characteristics in the countries of the four partners.

Expected Outcomes

⇒ Data to evaluate the usefulness of bacteriophages as model microorganisms for the assessment of removal by nature and treatments of faecal pathogens from water.

⇒ Data to evaluate the usefulness of bacteriophages as indexes of water quality.
⇒ Data on the incidence of different faecal micro-organisms in very different areas of the world.
⇒ Additional and complementary information and tools for water sanitation and integrated watershed management that may even be useful for the elaboration of new guidelines for water quality.
⇒ Transfer of know-how to microbiology laboratories from Latino America.

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Contract number: IC18*CT980284

Period: December 1996 to May 2000

INNOVATIVE STRATEGIES FOR THE PRESERVATION OF WATER QUALITY IN THE MINING AREAS OF LATIN AMERICA (WAQUAMINAR)

Co-ordinator: Universitá degli Studi di Cagliari, Cagliari, Italy (Luca Fanfani)

Objectives

- Protect water resources in the mining areas of developing countries both when exploitation activity is planning and when long time mining may have compromised water quality.
- Develop an integrated methodology for the evaluation of the hazard of chemical contamination of water resources in mining areas; the methodology will be based on deposit mineralogy and geochemistry, geologic environment, hydrodynamic conditions, and technologies employed for the exploitation, processing and waste disposal.
- Create decision tools in order to avoid contamination or to determine the best means of remediation or mitigation of the environmental damage caused to water resources.
- Propose technologies relatively inexpensive and based on an adequate support to natural processes of immobilization of toxic elements.

Activities

* Determination of metal contents, mineralogical and chemical forms and their bioavailability in solid waste materials.
* Description of mineralogical changes capable of modifying the mobility of toxic elements during weathering processes in wastes.
* Development of models describing the vertical and lateral migration of toxic metals in the hydrological systems and capable of determining their content, solubility and speciation through direct measurements and thermodinamic computations.
* Set-up of prevention devices and possible relatively inexpensive and quasi-natural remedial procedures.

Expected Outcome

This project should define appropriate guidelines for the assessment of the hazard of water pollution in mine areas (including sampling, geochemical and hydrogeological analyses, metal speciation and leaching tests). It should provide criteria to be put at the basis of prevention and remediation strategies in order to prevent or contain water pollution in mine areas, and suggestions for the extension of economical investigation and remediation techniques to other situations. Thematic maps representing the development of the water pollution hazard in risk-areas will be drawn.

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Contract number: IC18*CT980290

Period: January 1999 to June 2002

QUANTITATIVE INDICATORS AND INDICES OF ENVIRONMENTAL QUALITY; A EURO-LATINOAMERICAN NETWORK FOR ENVIRONMENTAL ASSESSMENT AND MONITORING (ELANEM)

Co-ordinator: Universidad de Cantabria, Santander, Spain (Enrique Frances)

Objectives

The general objective is to contribute to build a network of research and training institutions in Europe and Latin America, in the field of natural resources assessment and environmental planning and management.
More specific objectives are:

- developing a set of indicators which can be used for assessing the quality of different environmental components;
- developing integrated indices significant for expressing environmental quality and which allow meaningful comparisons among different types of environments;
- applying those indicators and indices to determine the condition and changes in the quality of the environment in selected areas, in order to identify existing trends;
- identify changes in environmental quality in the study areas and comparing those trends with the pressures affecting the areas and with the level of societal response;
- analysing certain processes of environmental degradation in specific study areas, in order to determine their contribution to changes in environmental quality.

Activities

- Field studies for surveying, mapping and indicator determination on the following types of environments/activities: evolution of environmental quality in coastal areas (a biosphere reserve and a sector under intensive tourism development); desertification and its influence on environmental quality; influence of mining activities on environmental quality; impact of sugar cultivation and refining activities on environmental quality; urban-agricultural problems in temperate humid plains and analysis of sustainability of human activities in a biosphere reserve in a mountain environment; natural hazards and environmental quality in relation to urban activities; modification of earth surface processes as a result of human activities.
- Study areas will cover industrialised and developing countries, northern and southern hemispheres, oceanic temperate, Mediterranean, arid and humid tropical areas in coastal, mountain and plains regions.
- Development of models and indices for space and time comparisons of environmental quality, with respect to a common standard, and design of a monitoring procedure.
- Training of young researchers, through exchanges among participating centres and organisation of joint graduate courses.

Expected Outcomes

⇒ A methodology for determining and monitoring environmental quality.
⇒ An annotated list of indicators including a description of their nature, significance, method of measurement, etc.
⇒ A set of indices of environmental quality with a description of the method and parameters used to compute it, environments and scale for application, etc.
⇒ A map and report for each study area, including a description of the environmental units identified and the values of the indicators determined, as well as a diagnosis of its condition and a CD-ROM with databases for the study areas.
⇒ An assessment of the changes in the state of the environment experienced by each study area, with reference to local and global trends.
⇒ A proposal of policies and actions which could be implemented to address the different environmental quality issues identified in the study areas, in order to mitigate existing problems.

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Contract number: IC18*CT980293

Period: November 1998 to October 2001

OCCURRENCE OF TOXIC CYANOBACTERIA WATERBLOOMS : IMPACT ON AQUATIC ENVIRONMENTS AND POTENTIAL HUMAN HEALTH RISK. ENVIRONMENTAL, PHYSIOLOGICAL AND GENETIC MECHANISMS INVOLVED IN TOXINS PRODUCTION.

Co-ordinator: Université Paul Sabatier - Toulouse III, Toulouse, France (Alain Dauta)

Objectives

Develop a set of methodologies:

- to determine the toxicity of blooms in water bodies
- to assess the toxicity risk in order to avoid human health problems
- to initiate a monitoring process of a crisis period, in relation to:
  - the strain of cyanobacteria implicated (genetic definition),
  - the steps of growth (ecophysiology) and the environmental conditions (ecology).

Activities

* Ecology of cyanobacterial strains: i) made *in situ* in reservoirs or rivers, ii) mainly done on strains isolated from natural blooms occurring in water supplies, iii) data provided by thorough ecophysiological studies taking mainly into account environmental factors, iv) and use of data from literature. The modern molecular biological methods have made possible to compare cyanobacteria strains in genetic level and study their phylogeny.
* Toxin obtention directly from i) *in situ* sampling (water supplies), ii) mass cultures under controlled conditions (directly connected with ecophysiological factors knowledge).
* A whole study of toxins (effects, toxicity threshold, toxicity tests,...) to define a range of toxicity effects, levels and strength. Intoxication by food chain must be considered. Chronic intoxication by sublethal levels of hepatotoxins needs to be further investigated and a maximum acceptable concentration for oral consumption proposed considering the environmental characteristics and data base of each country.
* Study of the chemical structure and of the ways of action of the various toxins (chemical modelling). The chemical stability of each toxin in a water body needs further investigation. In this case, studies on tropical conditions need to be considered. In order to be able to measure the toxic compounds one should be able to identify them. New toxic compounds should be purified for the structure determination and toxicological analysis. Standards of different microcystins are needed for quantification. The purification of peptide toxins will be performed by well established HPLC and TLC methods. Chemical structure will be performed by NMR. Mode of action of the different toxins will be assayed on several enzymes known to be their potential target: acetylcholinesterase and phosphatase.

Expected Results

⇒ The applied objective is to propose a set of various tests specially devoted:
- to the early detection of toxic cyanobacterial blooms,
- to the determination of the probability of toxicity evolution in the case of massive bloom.

⇒ The scientific work will enhance the knowledge on cyanobacteria, with strain collection, physiological description and data of molecular taxonomic characterisation (*in situ* and in laboratory), toxin production and toxic effects, toxin standards, databank of cyanobacterial 16sRNA gene sequences, data of molecular characteristics of the strains and knowledge of distribution of microcystin genes among isolates.

⇒ A predictive model to assess the risk of toxin production, integrating information from both field and laboratory.

⇒ All the data and publications will be available on a Web site, with also a protocol for toxic risk assessment, guidelines for toxins studies (identification and toxic effect) and classification of toxins.

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Contract number: IC18*CT980296

Period: December 1999 to November 2002

MEASURING, MONITORING, AND MANAGING SUSTAINABILITY : THE COASTAL DIMENSION

Co-ordinator: Tata Energy Research Institute (TERI), Panaji, India (Maria Ligia Noronha)

Objectives

- Develop a system for the integrated analysis of the economic, biological, geological, ecological, and human dimensions of coastal use.
- Examine the policy and the institutional matrix within which development in coastal areas occurs in the country.
- Develop a framework for decision making for coastal management that incorporates the concept of sustainability.

Activities

* A survey of the available policy and scientific literature on the state of development and the environment of coastal India. This along with a literature survey of what constitutes stressed and vulnerable environments will enable the development of Indicators of `Relative Vulnerability'.
* These indicators will be used to rank the coastal districts on the East and the West Coast of India into `hot spot districts'
* Within the 6 selected coastal districts, the villages around the coastal regulation zones as delimited by the state authorities will be used to actually select the regions which will be investigated intensively to study the interrelationships between population, development and the environment. These locations will be the sites for intensive primary data collection, study and analysis, and an assessment of the future options for economic and social development.
* Integration: A systems dynamics model will be developed comprising of submodules corresponding to the studies above. The integrated framework will be particularly useful in determining the impact of sector-specific policies on the entire eco-system. of the location being studied.

Expected Outcome

⇒ A methodology to identify environmental implications of coastal projects.
⇒ An identification of the environmental implications of coastal projects based on a detailed study of a representative number of locations.
⇒ A system to measure, monitor and manage sustainable use of coastal resources.

Partners

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Contract number: IC18*CT980297

Period: October 1998 to March 2002

APPROPRIATE MARINE RESOURCE MANAGEMENT AND CONFLICT RESOLUTION IN ISLAND ECOSYSTEMS. TEST CASE: MARINE INVERTEBRATES AND THE CO-EXISTENCE OF CONSERVATION, TOURISM AND FISHERIES INTERESTS.

Co-ordinator: Heriot Watt University, Stromness, Orkney, United Kingdom (Jonathan Side)

Objectives

The general objectives for the islands of the Galapagos, Ecuador and San Andres, Colombia are defined as follows:

- Investigate the potential for increased co-operation between different stakeholders in the pursuit of policies and actions which aim to promote conservation and sustainable use of available resources for the benefit of all involved without jeopardising conservation priorities or the biodiversity of marine ecosystems.
- Strengthen and promote local capabilities for conflict resolution and co-management of resource utilisation and conservation.
- Strengthen and promote local capacity in parallel with education, science and management.
- Provide sound technical information for informed conservation and management decisions.

Activities

* Technical studies including an assessment of spiny lobster stocks; interaction and conflict between conservation, tourism and fishing; the environmental impact of coastal tourism development and fishing activities on fragile marine resources and habitats; and existing and proposed legal instruments and policy measures.
* Stakeholder assessments through the establishment of new or the use of existing public discussion forums. These will allow the continued participation of local industries and other interested stakeholders in the research.
* An evaluation of the application of the conflict assessment tool AGORA (Assessment of Group Options with Reasonable Accord) to marine conservation in South America.
* Training of Ecuadorian and Colombian personnel in Europe.

Expected Outcome

The project should provide a database of resource utilisation related to tourism, fisheries and conservation, their contribution to the local economies and environmental impact. The activities that create conflict will be clearly defined and through the application of AGORA it is expected that the existing participatory management process in the Galapagos will be strengthened while a similar process has been established in San Andres with future measurable objectives in place. It is also planned to explore the potential establishment of a Latin American Peripheral Island Network (LAPIN) specialising in marine resources.

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Contract number: IC18*CT980298

Period: November 1998 to October 2001

AMAZONIA 21: OPERATIONAL FEATURES FOR MANAGING SUSTAINABLE DEVELOPMENT IN AMAZONIA

Co-ordinator: OEAR - Regionalberatung GmbH, Fehring, Austria (Robert Lukesch)

Objectives

- Compare different European and Panamazonian countries' (PAC) approaches and to elaborate a joint approach to assessing and measuring sustainable development, especially sustainable land and resource use in the basin of the river Amazonas/Solimões.
- Integrate these approaches and instruments into PAC university training programs and study schemes.
- Elaborate recommendations for innovative actors and policy makers and to establish long term relationships between scientific institutions and these actors for promoting sustainable development in the PAC.

Activities

* Theoretical and empirical development of a set of measurable parameters and indicators for sustainable socio-economic processes, which give practical political and economic orientations for actions directed to shift actual economic activities towards a more sustainable use of Amazonian biodiversity.
* Probation of the applicability of material and energy flow accounting in the PAC on local, regional, interregional and national levels.
* Case studies upon structural change and ecological modernisation in four key sectors of the Amazonian economy (biomass production, mining, crude oil extraction, energy production) with respect to sustainability parameters.
* Structural analysis of three innovative actions and policies in selected Amazonian regions in order to identify patterns of sustainable practice in human development, land and resource use, combined with technical assistance to these actors on local and regional levels thus establishing long term relationships between practicioners and scientists.
* Capacity building in sustainable development project elaboration and land and resource management by integrating the academic and field research into a university training programme.

Expected Outcome

This project should provide integrated recommendations for the operationalization of sustainable development in PAC. It should define appropriate strategies for a more sustainable use of land and natural resources from the local to the transnational level. It should also help to consolidate the academic and technical cooperation between Amazonian and European institutions committed to research, training and action for sustainable development. The results of the project will be presented at a final public conference in Belém, Brazil. The conference will be attended by the project partners, external field research partners, other scientists, representatives of international organisations, non-governmental

organisations and governmental officials from PAC. Both, the results and experiences from the project and the inputs from the conference participants, will be published as a project reader.

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Contract number: IC18*CT980303

Period: February 1999 to April 2002

DEVELOPMENT OF NEW BIOSEPTICIDES FOR ENVIRONMENTALLY-FRIENDLY INSECT CONTROL

Co-ordinator: Plant Genetic Systems N.V., Gent, Belgium (Jeroen Van Rie)

Objectives

- Identify effective protein toxins against selected insect pests of economic importance in Central and South America: *Helicoverpa zea, Epilachna varivestis, Agrotis ipsilon* and *Premnotrypes vorox.*
- Select insecticidal proteins to be used in *Bacillus thuringiensis* (Bt) -based products compatible with resistance management strategies.
- Evaluate the effectiveness of prototype Bt products under field conditions.
- Increase scientific collaboration between European and South American institutes and to enable technology transfer.

Activities

* Screening crystals from 3 different *Bacillus thuringiensis* (Bt) strain collections against the different pests. In order to focus our efforts on potentially interesting strains and increase the success of the screening, we will select Bt strains based on a molecular characterization of these collections, as opposed to the 'brute force' screening that has been applied in the past.
* Analysis of the activity of proteins present in the supernatant of Bt cultures: secreted proteins appear now to be a novel source of insecticidal proteins, besides the well known family of insecticidal crystal proteins (ICPs).
* Construction of a number of hybrid ICPs, based on functional data of available ICPs and exchange of ICP domains. Using this approach, it has been possible to transform a previously inactive ICP into an active one.
* Study of the binding characteristics of the identified active proteins. This information will be very valuable with respect to resistance management strategies, since one of these strategies involves the use of proteins that bind to different receptors in the same insect species.
* Small scale field trials with prototypeBt products.

Expected Outcome

⇒ This project should result in the identification of a number of Bt strains and Bt proteins that are active against one or more of the selected insect pests and that are compatible with resistance management. We also expect to have data on the field efficacy of at least one prototype product, based on a natural Bt strain. Such payable Bt products could be produced locally and be integrated as a new technology in small communities, so that environmentally friendly alternatives to synthetic insecticides can be used for pest control.

In this way the environmental hazards and health risks associated with current insect control practices in this region can be reduced.

⇒ Other project results will establish a basis for the development of future Bt-based products (e.g. transgenic plants) to be developed in Central and South America, as well as in other regions where the above pests represent serious threats to crop productivity.

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Contract number: IC18*CT980317

Period: January 1999 to December 2001

STUDY OF THE GENETIC VARIABILITY OF THE PATHOSYSTEM COMMON BEAN: ANTHRACNOSE AND IDENTIFICATION OF DURABLE RESISTANCE SOURCES TO REDUCE BEAN YIELD LOSSES IN LATIN AMERICA AND AFRICA

Co-ordinator: Univesidad National de Costa Rica, Heredia, Costa Rica,
(Carlos Manuel Araya)

Objectives

- Improve the current knowledge on the biology and pathology variability of *Colletotrichum lindemuthianum,* causal agent of bean anthracnose, in Latin America and Africa.
- Study the pathosystem components from both cultivated and wild beans in the two gene pools (Andean and Mesoamerican) and the six centres of diversity of bean.
- Identify sources of durable resistance to the pathogen, and their phenotypic and molecular markers.
- Transfer the information of this project to breeding programs in Latin America and Africa in order to accelerate the process of selecting and releasing tolerant varieties of bean.

Activities

* Field trips to the two gene pools of bean to collect samples of commercial and wild beans, as well as isolates of *C. lindemuthianum* from both geographic regions. A collection of pathogen isolates will be established to study their pathogenic variability and geographic distribution.
* Identification of physiologic races of the anthracnose pathogen using both the standard and a special set of differentials. The first set will provide information about the actual races. The second one will give information on the specificity of the isolates and thus ligths on the coevolution process.
* Field studies to determine the main sources of variability of *C. lindemuthianum.* The effect of environment factors and host resistance on selecting new pathtypes will be studied also.
* Determination of molecular markers of resistance factors and genetic differences among anthracnose isolates. These markers will help in selecting sources of resistance in breeding programs, and differentiate resistance factors to specific pathiotypes distributed in limited geographic areas.

Expected Outcomes

⇒ This interdisciplinary project will provide information on the geographic distribution and specificity of the pathogenic variability of *Colletotrichum lindemuthianum,* as well as resistance sources to this pathogen from both commercial varieties and landraces of bean. Data will allow a better understanding of the biology of the fungus and the factors that promote genetic variability. Further, breeding programs in Latin America and Africa will be able to work on gene deployment to reduce yield losses.

⇒ The identification of phenotypic and molecular markers in both components of the pathosystem can accelerate the selection process of new bean varieties. Knowledge on the

sources of variability of *C. lindemuthianum* is a useful tool to improve the durability of resistance cultivars. A new strategy on bean breeding will help reduce yield losses and thus, increase the familiar income of small landholders in Latin America and Africa.

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Contract number: IC18*CT980318

Period: November 1998 to October 2002

ENRICHMENT OF POTATO BREEDING PROGRAMMES IN LATIN AMERICA AND EUROPE WITH RESISTANCE TO LATE BLIGHT (*PHYTOPHTHORA INFESTANS*) ECOPAPA

Co-ordinator: DLO–Centre for Plant Breeding and Reproduction Research (CPRO-DLO), Wageningen, The Netherlands (Leontine Colon)

Objectives

- Incorporate durable resistance to late blight (*Phytophthora infestans*) in the germplasm employed in the potato breeding programs of the Latin America and Europe.
- Transfer marker-assisted breeding for the resistance in this germplasm to scientists in Latin America and Europe.

Activities

* Supply the potato breeding programs of the participating countries with 30 additional 2x and 4x parental genotypes with potentially useful late blight resistance genes.
* Identify sources of resistance to foliage and tuber blight in these 36 genotypes effective against the local late blight populations and under local conditions of daylength, temperature, rainfall and irradiation of the participating countries.
* Estimate the virulence and aggressiveness against these sources of resistance of the late blight populations occurring in the participating countries, and analyze the population genetic structure of the pathogen in order to predict future developments in virulence and aggressiveness.
* Develop and exchange molecular markers for foliage and tuber blight resistance
* Initiate marker-assisted selection programmes for the resistance of these sources, in order to accelerate the further development of late blight resistant germplasm, including training.
* Initiate the development of new parental lines and locally adapted commercial cultivars with durable resistance to late blight.

Expected Outcome

This project should significantly stimulate the breeding programs in the participating countries in the area of potato, a regional priority for Latin America in the 1997 call. Potential new cultivars that will result from these breeding programs, some of which may generate from the project directly, will answer the local farmers' great need of late blight resistant potato cultivars. Many of the farmers in Latin America are small farmers, for whom potato is the major crop. Resistant cultivars will decrease farmers' dependency on fungicides and reduce their costs. The project should have a large environmental impact through significant reductions in the use of fungicides. In this way, ECOPAPA should support the sustainable development of agriculture in the region.

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Contract number: IC18*CT980320

Period: November 1998 to October 2001

EXPLOITATION OF THE GENETIC BIODIVERSITY OF WILD RELATIVES FOR BREEDING POTATOES WITH SUSTAINABLE RESISTANCE TO LATE BLIGHT (*PHYTOPHTHORA INFESTANS*)

Co-ordinator: University of Tübingen, Tübingen, Germany (Lieselotte Schilde)

Objectives

- Produce breeding material with sustainable resistance to late blight (*Phytophthora infestans*) by incorporating genetic material of Latin American wild species into regionally adapted breeding material in order to reduce yield losses caused by the pathogen, to lower inputs in form of fungicides and thus improve the economic situation of the farmers in LAC and especially the andean highlands, in addition, to reduce hazards to the health of people and to the environment due to fungicide applications both in Europe and LAC.
- Combine biotechnological and conventional breeding methods for this goal and make these techniques available.
- Conserve the locally available richness of genotype biodiversity of cultivars in LAC by utilizing these genotypes for direct combinations or backcrossing.
- Gain more understanding of the pathogen and its interaction with different sources and components of resistance.
- Increase the participative research and interaction of the two potato networks, PRECODEPA and PRACIPA and their member countries for the benefit of the local farmers, for which this crop in most cases represents a subsistence and cash crop at the same time.
- Intensify contacts between LAC and European partners for further interaction in research and training.

Activities

* Establishment of standardized testing methods for *Phytophthora* resistance testing and evaluation at the corresponding places.
* Identification of wild species carrying valuable resistance traits.
* Combination of wild species with breeding lines/cultivars adapted to the different agroecological regions by sexual and somatic hybridization.
* Backcrossing of hybrids obtained to local cv/breeding lines.
* Evaluation of the progenies for resistance to late blight under different ecological conditions, selection for agronomic characters and acceptability by local farmers.
* Identification of the portions of the corresponding wild species genome of resistant genotypes by molecular markers.
* Analysis of the presence and nature of „sustainable resistance“ and start to determine possible components.

Expected Outcome

The results of this project will increase the understanding of the pathogen - plant interaction, the importance of environmental factors, and lead to the identification of components of resistance against this fungus. In addition the establishment of standardized test systems for resistance at the places of the different partners will facilitate the exchange of data and information also in the future. For the LAC countries valuable breeding material with adaptation to the corresponding zone and acceptability by farmers as well as information on sustainability of the resistance will be available. The techniques of protoplast fusion will be established in the region and the application of marker assisted breeding reinforced. EC agriculture could benefit in different aspects: widening the genetic base of an important food and industrial crop, which would result in the reduction of production costs and of environmental pollution. For the potato producing farmer the ultimate outcome will be an increase in income in the long run and thereby the improvement of standard of living.

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Contract number: IC18*CT980321

Period: September 1998 to August 2001

EXPLOITING THE BIODIVERSITY OF RHIZOBIA FOR THE SUSTAINABLE IMPROVEMENT OF COMMON BEAN CROPS IN SOUTH AMERICA

Co-ordinator: Universidad de Sevilla, Sevilla, Spain (Manuel Megías Guijo)

Objectives

- Propose solutions to limitations found in the nitrogen-fixing symbiosis and productivity of bean crops in Argentina and Brazil.
- Understand better the basis for successful nodulation of bean as related to the bean rhizobial community present in soil, and to the rhizobial genotype.
- Examine the patterns of nodulation gene expression (*nod* genes) and of production of nodulation factors in rhizobia isolates having different degree of competitiveness and efficiency for nitrogen-fixing, under environmental extreme conditions such as acidity, high temperature, droughtness.

Activities

* Isolation of bean rhizobia in natural populations. Broad collection of bean rhizobia isolated from the bean producing areas from Argentina and Brazil comprising a wide diversity of strains representing different climatic or edaphic characteristics. To expand the collection we plan to perform field campaigns in the bean cropping areas of each country, in particular where poor nodulation has been observed in spite of the presence of indigenous populations of bean rhizobia.
* Genomic, taxonomic and phenotypic characterization of isolates. Analysis of the variability in the production (nature and amounts) of nodulation factors and as influenced by the various genotypes and by different environmental conditions. Plant test will be able to assess the nitrogen fixation effectiveness of the different isolates. Bean varieties used by local farmers will be used in these tests of effectiveness.
* Analysis of the respective contributions of background genome and symbiotic plasmid genome to effectiveness and competitiveness of bean nodulating isolates. One approach to examine this aspect consists in the construction of hybrid strains in which a strain deleted of its symbiotic plasmid (pSym) and a pSym from a different strain are combined.
* Field inoculation experiments with selected strains. The experiments will take place during successive growing seasons in the most important bean cropping areas of Argentina and Brazil.

Expected Outcome

A positive correlation would allow to elaborate new criteria for the selection of more efficient and competitive starins for certain environments, based on taxon and genomic characterization. A main impact of this project is likely to be the inoculation technology that will be made available to farmers, policy decision markers and agricultural agencies. We envisage to attain this goal, by exploiting the extension capabilities of domestic agri-agencies

INTA (Argentine Institute of Agrotechnology) and Embrapa (Brazilian Enterprise of Agricultural Research).

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Contract number: IC18*CT980323

Period: October 1998 to September 2001

TREE RESOURCES OUTSIDE THE FOREST: DEVELOPMENT OF METHODS FOR ASSESSMENT AND MONITORING OF NATURAL RESOURCES TO SUPPORT REGIONAL PLANNING, WITH STUDY AREAS IN CENTRAL AMERICA

Co-ordinator: Albert-Ludwigs-Universität Freiburg, Freiburg, Germany (Barbara Koch)

Objectives

- Tree resources outside the forest represent a wide class of tree formations, ranging from natural occurrence of scattered trees to systematically managed trees in agroforestry systems. It is expected that those trees serve a number of ecological and socio-economic functions, similar in principle, but different in extent to the functions of forest. Moreover, tree resources outside the forest are little recognised in natural resources assessments, particularly on a regional level. It is only recently that this topic emerges as a systematic research issue.
- The project's objective is to develop a technique to inventory, assess, analyse and present data on tree resources outside the forest on a regional basis. The technique to be developed will provide regional statistics and maps. The results are to be compiled in an information system, which complement the data base for the regional planning of natural resources.
- Three partners from Central America and three from Europe co-operate in the project.

Activities

The scientific approach chosen combines different physical information sources:
1) Field data give information on biomass, species composition, tree characteristics and functional aspects,
2) air photos describe spatial arrangement on a local bases and
3) satellite images serve to extrapolate those findings to a larger area. The link between the different data sources will be made through statistical modelling.

The following key activities will be carried out, arranged in 9 work packages:
* Development of an unambiguous and clear classification scheme for the assessment in the field and by means of remote sensing. Among the classification criteria will be type, quantity/density, spatial distribution, functions of trees outside the forest.
* Along with the classification the functions of the different classes will be researched into.
* Detailed mapping of a number of study areas and field sites in Costa Rica, Honduras and Guatemala. These data will be the input for simulation studies with respect to sampling techniques, and to remote sensing classification and interpretation procedures.
* For the new generation of high resolution satellite images new algorithms and procedures will be applied like image segmentation and fusion.
* Development and adaptation of modelling and sampling strategies for biomass assessment, remote sensing classification rules, regional estimation.

* Design of a geographic information system that allows to adequately present data coming from the information sources mentioned.

Expected Outcome

⇒ Main outcome is the presentation of an assessment technique that can be fully integrated into general large area assessments of natural resources. The technique(s) identified as efficient will be described and discussed in detail, including all steps in field sampling, remote sensing data processing and information system building.

⇒ For the study areas statistics and maps will be produced that will serve as an example of the statistical and map output that can be achieved with the technique developed.

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Contract number: IC18*CT980324

Period: October 1998 to September 2001

CASFOR: CARBON SEQUESTRATION IN AFORESTATION AND SUSTAINABLE FOREST MANAGEMENT: PRESENTATION OF A GENERAL EVALUATION TOOL AND GENERIC CASE STUDIES

Co-ordinator: Instituut voor Bos- en Natuuronderzoek (DLO), Wageningen, The Netherlands (Frits Mohren)

Objectives

- The formulation of a general model of the carbon budget of forest ecosystems as a standard for the quantification of carbon sequestration in aforestation projects and sustainable forest management.
- The dissemination of this model and the required input data through the World Wide Web.

Activities

- Documentation of the model CO2FIX, development of a user-interface, and establishment of a test page on the Internet for use of the model by a selected user-feedback group;
- Review of selective, low-impact logging and sustainable forest management systems in tropical forests, development of a sub-model for inclusion of low-impact logging in the model CO2FIX;
- Development of a summary model of soil organic matter dynamics in forest ecosystems, accounting for climate and soil influences, and inclusion of this as a sub-model in CO2FIX;
- Development of a summary model of wood product life cycle, accounting for conversion of stem volume into various woody products, differences in life-span, and possible re-use for energy purposes, and inclusion of this as a sub-model in CO2FIX;
- Integration of the sub-models and user-interface;
- Testing of the model using field data from Mexico and Costa Rica;
- Dissemination of the final model and its results by means of opening the Internet site, providing access to model, input data, and background documentation.

Expected Outcome

The project will deliver a user-friendly tool for quantification of the potential role of forest ecosystems in global carbon relations, in carbon sequestration, and in carbon emission offsets as part of the policy evaluation of the role of forests in the greenhouse effect. It contributes basic data for evaluation of Joint Implementation projects and it aims at including management regimes such as selective logging in this tool. The model can be used by both forest managers and policy supportive research projects, and will be made freely available for a wide audience with support for users in the tropics through the local participants. For the DC regions involved, the model will be very important for evaluation of Joint Implementation forestry projects and for analyses of different management regimes in primary and secondary tropical forests by providing basic data on carbon budgets and possibilities for sustainable

carbon sequestration in tropical forest ecosystems. Together with the database on forest types for which the model has been used in the past, it effectively bridges site conditions from wet peatlands with very limited primary production and high organic soil carbon, to high production sites with high biomass and relatively small amounts of soil carbon.

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INDEX OF PROJECTS BY SUBJECTS

International Scientific and Technological Co-operation with Developing Countries (INCO-DC) 1994 – 1998
Projects by subjects (in numerical order of contracts)

Agriculture and Natural Resources

IC18*CT950002	Control of *taenia saginata* and *taenia solium* cysticercosis through specific diagnosis; systematic epidemiology and development of recombinant vaccine candidate
IC18*CT960033	Magnesium sulphate for treatment of pre-eclampsia: a trial to evaluate the effects on women and their babies (the magpie trial)
IC18*CT960037	A new approach for developing a sustainable disease management system for bean, based on healthy leaf area duration and photosynthetic efficiency
IC18*CT960038	Non-timber forest plant resource assessment in NW Amazonia
IC18*CT960044	Control of citrus virus diseases important in the Mediterranean area and South America: development of molecular probes for quick detection of severe strains of citrus tristeza virus (CTV) and psorosis-ringspot
IC18*CT960061	Detection and characterization of pathogenic entamoeba histolytica
IC18*CT960063	Fitting maize into cropping systems on acid soils of the tropics
IC18*CT960067	Sustainable production of natural resources and management of ecosystems: the potential of South American camelid breeding in the Andean region
IC18*CT960068	Environmental law and local management of natural resources. Comparative research in Brazil and Costa Rica
IC18*CT960069	Climate impact on water resources and dryland agriculture (CLIWARDA)
IC18*CT960073	Geo-environmental dynamics of pantanal-chaco: multitemporal study and previsional modelling
IC18*CT960076	Development of environmentally friendly photoactivatable compounds for treatment of microbially polluted water
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IC18*CT960086	Tropical medicine on trial: producing reliable reviews, designing better intervention studies, and using systematic reviews to inform practice
IC18*CT960087	Ecological bases for the sustainable management of flooded tropical ecosystems: case studies in the llanos (Venezuela) and the pantanal (Brazil)

IC18*CT960089	Genetic analysis and engineering of aluminium tolerance in maize and in model plants
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IC18*CT960126	Development of transgenic potato cultivars with combined protection against virus and fungal pathogens
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IC18*CT970146	Sustainable use, conservation and restoration of native forests in southern Mexico and South-Central Chile (sucre)
IC18*CT970147	Effects of changes in land use use and land management practices on land degradation in forest and grazing ecosystems
IC18*CT970148	Policies for sustaining environments and livelihoods in mountain areas
IC18*CT970149	Assessment of levels and dynamics of intra-specific genetic diversity of tropical trees for conservation and sustainable management
IC18*CT970150	Fluxes of energy, water and carbon over disturbed savanna ecosystems and their application as indicators of sustainability and carbon sequestration (Savaflux)
IC18*CT970156	Unification of indicator quality for assessment of impact of multidisciplinary systems
IC18*CT970157	Aquaculture management and ecological interaction of noxious phytoplankton in southern Latin America
IC18*CT970164	Transmission and adpatation of environment knowledge in indigenous and mixed-blood communities
IC18*CT970175	Placing fisheries resources in their ecosystem context: cooperation, comparisons and human impact
IC18*CT970180	Reduction of the chemical inputs in a vegetable crop by the use of beneficial rhizospheric micro-organisms
IC18*CT970181	Sustainable improvement of nematode resistance in coffee cultivars (*Coffea arabica*) of Central America: enhanced use of genetic resources by the development of marker-facilitated selection programmes

IC18*CT970182	Developing Latin America fruits using the yeast *Kluyveromyces marxianus* and its selected pectinolytic enzyme endopolygalacturonase
IC18*CT970185	Development of bioprocesses for the conservation, detoxification and valorisation of coffee pulp (Biopulca)
IC18*CT970188	Improvement of scallop production in rural areas
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IC18*CT970194	Evaluation and utilization of *Calliandra calothyrsus* microsymbiont biodiversity for optimizing forage production on small farms in humid regions
IC18*CT970199	New technology for controlling insect pests of oil palm and coconut crops: research and development in selective trapping using synthetic attractants
IC18*CT970201	PHA production from sugar cane derivatives
IC18*CT970202	Analysis and management of organic matter and nitrogen in aquacultural ponds for a minimal waste production and optimal efficiency
IC18*CT970203	Quality improvement of sugar-cane fibres for their use as raw material in the production of paper and animal feed
IC18*CT970204	Optimization of new breeding strategies for banana for local markets
IC18*CT970206	Development of new processes for the extraction of oils and active products from non conventional oilseeds and vegetables for the pharmaceutical and food industries
IC18*CT970208	Alleviating abiotic and biotic soil constraints by combining arbuscular mycorhizal fungi with banana and plantain micropropagation systems
IC18*CT970209	Characterization of immune effectors in penaeids: application to prophylaxis and selection of resistant shrimp
IC18*CT980259	Buffer zones for the sustainable use of rainforest biodiversity: the example of the eastern slope of three Andean countries
IC18*CT980262	The sustainable management of wetland resources in Mercosur
IC18*CT980263	Fertility management in the tropical Andean mountains : agroecological bases for a sustainable fallow agriculture (tropandes)
IC18*CT980264	Tropical and sub-tropical cost-effective tools for an integrated risk assessment of wetlands (TROCA)
IC18*CT980271	Intelligent management system for water and energy minimisation in Latin American food industries (Waterman)
IC18*CT980282	Evaluation of the usefulness of bacteriophages as model micro-organisms for the assessment of water treatment processes and water quality
IC18*CT980284	Innovative strategies for the preservation of water quality in the mining areas of Latin America (WAQUAMINAR)

IC18*CT980290	Quantitative indicators and indices of environmental quality; a Euro-Latin American network for environmental assessment and monitoring (ELANEM)
IC18*CT980293	Occurrence of toxic cyanobacteria waterblooms: impact on aquatic environments and potential human health risk environmental, physiological and genetic mechanisms involved in toxins production
IC18*CT980296	Measuring, monitoring and managing sustainability: the coastal dimension
IC18*CT980297	Appropriate marine resource management and conflict resolution in island ecosystems. Test case: marine invertebrates and the co-existence of conservation, tourism and fisheries interests
IC18*CT980298	Amazonia 21: Operational features for managing sustainable development in Amazonia
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IC18*CT980317	Study of the genetic variability of the pathosystem common bean: anthracnose and identification of durable resistance sources to reduce bean yield losses in Latin America and Africa
IC18*CT980318	Enrichment of potato breeding programmes in Latin America and Europe with resistance to late blight (*Phytophthora infestans*) Ecopapa
IC18*CT980320	Exploitation of the genetic biodiversity of wild relatives for breeding potatoes with sustainable resistance to late blight (*Phytophthora infestans*)
IC18*CT980321	Exploiting the biodiversity of rhizobia for the sustainable improvement of common bean crops in South America
IC18*CT980323	Tree resources outside the forest: development of methods for assessment and monitoring of natural resources to support regional planning, with study areas in Central America
IC18*CT980324	CASFOR: Carbon sequestration in aforestation & sustainable forest management : presentation of a general evaluation tool and generic case studies

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IC18*CT950017	Isolation, characterisation and immunological evaluation of recombinant vaccines for filarial parasites
IC18*CT950020	A concerted European approach towards the development of malaria vaccines

IC18*CT950021	Pre-clinical study of the immunogenicity of MSP3 and GLURP, two *P. falciparum* antigens targeted by protective antibodies
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IC18*CT960027	Early events in rotavirus infection: role of viral proteins on particle internalization and membrane permeability
IC18*CT960028	A network approach to research on *leishmaniasis* in Central America, with emphasis on drug sensitivity in the field
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IC18*CT960058	Developments reached by the health system in El Salvador and Nicaragua in the post-war period (1990-1995), focusing on the efforts of civil society
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IC18*CT960084	Antileishmanial and antitrypanosomal activities of alkyllysophospholipids
IC18*CT960115	Measuring and monitoring the performance of reforming health systems
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