Darwin Initiative Annual Report

Important note:

To be completed with reference to the Reporting Guidance Notes for Project Leaders – it is expected that this report will be about 10 pages in length, excluding annexes

Submission deadline 30 April 2008

Darwin Project Information

Project Ref Number	15/004
Project Title	Conserving and using entomopathogenic fungi and nematodes within Chile
Country(ies)	UK, Chile
UK Contract Holder Institution	CABI Europe - UK
UK Partner Institution(s)	CABI Europe-UK
Host country Partner Institution(s)	Instituto de Investigaciones Agropecuarias (INIA), Chillán, Chile
Darwin Grant Value	£299,372
Start/End dates of Project	May 2006/May 2009
Reporting period (1 Apr 200x to 31 Mar 200y) and annual report number (1,2,3..)	1 April 2007 to 31 March 2008. Number 2
Project Leader Name	Dave Moore
Project website	www.controlbiologicochile.cl (live approx. June 2008)
Author(s), date	Dave Moore, Andres France, Loreto Merino, Steve Edgington. 24th April 2008

1. Project Background

This is the annual report for Year 2 of a study on the entomopathogenic fungal (epf) and nematode (epn) biodiversity of Chile, a collaborative Darwin Project between CABI UK and the Instituto de Investigaciones Agropecuarias (INIA), Chile. The aim is to survey a range of habitats, to profile the epf and epn obtained and to identify characteristics that link the organisms to their habitats. The collaborators will then use this information to identify possible biological control agents for insect pests in Chile. Such organisms can offer alternatives to chemical pesticides and are key components of the Chilean Governments drive to reduce the use of chemical pesticides on agricultural systems.

As reported in the annual report for Year 1, six areas were chosen in Chile by the project participants, each of which has now been surveyed for epf and epn. The areas were selected on the basis that they represent the main climatic, vegetation and soil types present in Chile (Figure 1. a), the range of which is unique within just one country, including desert land in the north, near Antarctic conditions in the south, the high altitude Altiplano (at >4000 m) and temperate rainforests.

Figure 1. Survey areas, from Area 1 in the far north to Area 6 in the far south. Areas 2, 3, 4 and 6 were surveyed in Year 2.

2. Project Partnerships

Project partnerships and Other Collaboration:

The Year 1 Annual Report noted that the partner Institutions have been trying to collaborate for many years: CABI had made three visits to Chile before the Darwin project. Consequently, the project builds on a foundation of mutual respect and this has been reinforced in years 1 and 2 of the project, with INIA leading on the fungal work and CABI being more prominent with the nematode aspect. This is occurring, at least in part, because the CABI scientist is exploiting the opportunities provided within CABI to work with Dr David Hunt, a world authority in nematode taxonomy. No significant problems in relation to the collaboration have occurred.

The project is beneficial in terms of CBD, providing support for an effective culture collection and providing training to INIA collaborators in May 2007. It is expected that links with other groups in biodiversity conservation will increase in Y3, now that the initial surveys have resulted in a significant number of fungal isolates and nematode species being found. INIA, CABI and organisations in Bolivia and Argentina are listed collaborators in a project proposal on microbial biodiversity in the Andes to EU submitted in February 2008.

3. Project progress

3.1 Progress in carrying out project activities

Six survey areas in Chile were chosen (see Figure 1), numbered from north to south. Areas 1 and 5 were surveyed in Year 1, the remaining areas were surveyed in Year 2.

Area 2: the Atacama Desert in the north (approx. 30 °S) stretching to the foothill of the Andes. The coastal cordillera traps incoming moisture from the ocean, preventing rainfall over this survey area. Some regions receive little if any rainfall. Sparse vegetation relies on natural oases, subterranean aquifers or a number of narrow rivers running from the Andes.

Area 3: Valparaíso to the Andes (approx. 33 °S), with areas of wetland, dry areas of semi-desert and areas of typical Mediterranean vegetation. The climate, in general, is Mediterranean, with hot dry summers and cold wet winters.

Area 4: Concepción to Laguna del Laja (approx. 37 °S), representing a transition from dryland to wetland, with Mediterranean scrub, isolated ancient forests (predominantly in the pre-Andes highlands) and natural grassland. The climate is cooler and wetter than Area 3.

Area 4 (2) : Tirúa – Lago Galletue: (approx. 39 'S) Area with volcanic soils and relict forests of araucaria (Araucaria araucana).

Area 6: Patagonia and Tierra del Fuego in the far south (approx. 53 °S). Most months receive around 3 cm of rain. The coldest region of Chile, average summer temperatures around 11 °C, with winters around 2 °C. The near-constant wind from the South Pacific Ocean makes this area seem much colder. Land is principally used for pasture.

The results of the surveys, including the two areas sampled in Year 1 can be seen in Table 1. The soil samples from Area 2 were collected in April 2008 and are presently being processed at INIA (these samples will take approximately 1-2 months to process and will be reported on at the mid year stage of Year 3).

CABI continue to be principally involved with identifying and characterising the epn isolates, including the molecular descriptions. During Year 2, techniques for molecular examination were developed for preliminary identifications and a number of isolates of epn from Areas 1, 4 and 5 underwent morphological and molecular examination, revealing the two principle families of epn (Steinernematidae and Heterorhabditidae) and including a number of possible new species. The Heterorhabditidae have so far been the only family found in the northern (desert-like) regions, including the very recent epn from Area 2. In general the Heterorhabditidae have a strong association with sandy soils, so this is no surprise. The techniques can identify

isolates which do not match previously described isolates; these then require more detailed examination (below).

A full description of one previously unrecorded (thus new) species of epn is scheduled for completion by July 2008. The full ITS sequence from the 16S rRNA gene has been obtained for this nematode, together with a partial D2/D3 sequence, enabling the project team, with expert assistance from nematologists at CABI and the University of Florida, who are doing scanning electron microscopy (SEM) work, to confirm that the epn is indeed a new species.

In year 3, all epn isolates will undergo the ITS molecular assessments developed. This will enable us to determine which of the 100 or so isolates match with previously described species. It is likely that there will remain a number which do not match; these are likely to be new species, but it will not be possible to determine this without the full molecular characterisation and SEM work, which may be beyond the scope of the project.

INIA has been responsible for processing and identifying the epf isolates. General phenotypic studies have so far revealed abundant epf isolates throughout each survey area, dominated by *Beauveria* and *Metarhizium*, two common epf families. Molecular specialists at INIA have decided to carry out the molecular identification of the whole epf collection at one time; to date they have extracted DNA from all fungal isolates with the exception of those from survey 2 (April 2008) which are still being processed. Molecular characterisation of the full collection from the surveys is scheduled for Q2 of Year 3.

The variety of 'positive-hit' habitats (those that yielded epf and/or epn) is extraordinary. Within each survey area alone there were significant variations in soil type, salinity, altitude, plant-type and climate, providing Chile with a collection of isolates with, in all probability, a wide range of environmental adaptations. Biological and molecular characterisation to examine such links and to assess how adaptations in the collection may be of value for biological control is scheduled to cover Year 1 to Year 3, with CABI and INIA both involved. A number of characterisation studies have already been carried out (see following section), however Year 3, with all areas now surveyed, will enable the project team to focus in greater detail on this theme.

1. Area 3. National Park La Campana

2. Area 4. Natural pasture, Lleo lleo lake

3. Area 6. National Park Torres del Paine, Patagonia.

Project staff arrived back from Area 2 (the final survey, in the Atacama Desert) during the writing of this report.

Figure 2. Areas surveyed during Year 2.

Survey area	Survey year	Total samples	epf isolates	epn isolates
1	1	204	48	4
2	2	128	28*	1*
3	2	200	72	10
4 (I)**	2	208	83	27
4 (II)**	2	194	58	20
5	1	184	66	11
6	2	189	34	33

* Samples are still being processed

** Two sampling visits were made to Area 4, collecting from different areas. The second visit resulted from a last minute change of plan due to an earthquake in another survey area.

Table 1. Numbers of isolates of epf and epn collected during Years 1 and 2.

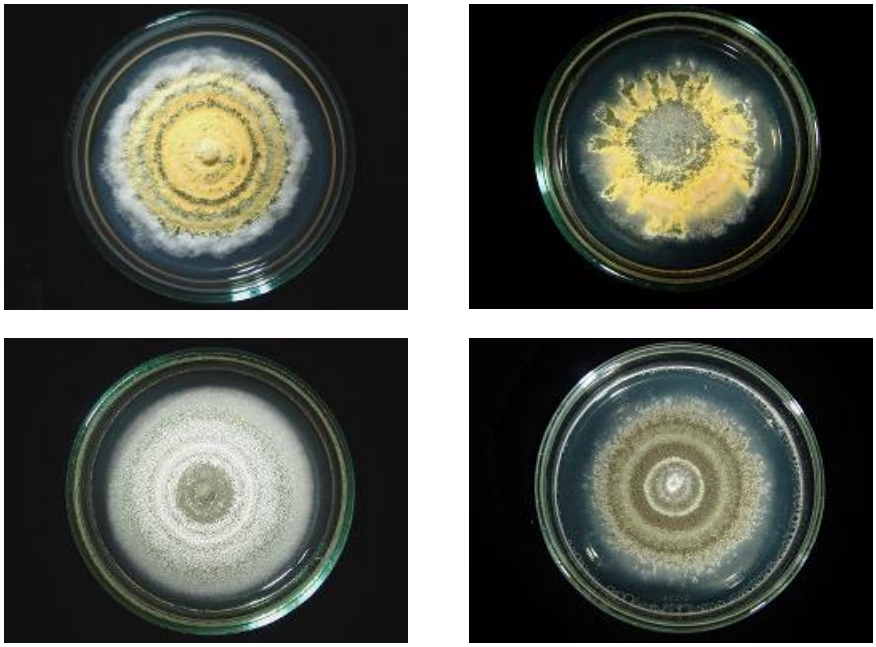


Figure 3. Examples of fungal isolates (*Metarhizium* species) obtained from survey areas

Biological and molecular studies of isolates achieved

These studies have included temperature tolerances, moisture requirements, host searching behaviour and the target-range for pests of economic importance in Chile. The biological characterisation studies have been principally carried out by students, including a PhD student at CABI and a number of undergraduate and postgraduate students from several universities in Chile. Student projects over the last year are listed in Table 2. Copies of the project abstracts (in Spanish) can be obtained from Darwin project staff at INIA. Figures 4 and 5 are examples of the screening studies carried out in Year 2, with additional information in posters of Annex 3. The objective is to develop sufficient information for a logical progression from lab-based screening to more specific pathogen-host studies (possibly semi-field).

Additionally, epn carry symbiotic bacteria which are critical for insect mortality and epn development. As part of a PhD study a number of bacteria isolates were obtained from the epn collection during Year 2. Most of the isolates obtained were of the typical *Xenorhabdus* and

Photorhabdus species associated with epn, however a species of *Paenibaccillus* was found in the 'new' species from Area 5 and its association with the epn will be examined during Year 3.

Project title	Student and affiliation
Biological control of black moth *Dalaca pallens* (Lepidoptera; Hepialidae) with entomopathogenic nematodes	Alexis Maldonado Universidad de Concepción
Screening of native entomopathogenic nematode isolates for control of *Aegorhinus supercilliosus* (Guerin) (Coleoptera:Curculionidae)	Ingrid Rozas Universidad de Concepción
Pathogenicity of native entomopathogenic nematode isolates for control of codling moth *Cydia pomonella* (l.) (Lepidoptera: Tortricidae)	Manuel Contreras Universidad de Concepción
Screening of native entomopathogenic fungal isolates for control of *Hylurgus ligniperda*	Karen Parra Universidad ARSIS
Evaluation of entomopathogenic fungi (*Metarhizium anisopliae* and *Beauveria bassiana*) collected from the south of Chile, for control of Black vine weevil O*tiorhynchus sulcatus* Fab. (Coleoptera: Curculionidae)	Claudia Inostroza Universidad de Concepción
The effect of UV radiation on the germination of native entomopathogenic fungal isolates from northern Chile.	Dayna Grimberg Universidad Austral de Chile
Survey and characterization of entomopathogenic nematodes in Chile	Steve Edgington University of Reading, UK

Table 2. Student projects in 2007-2008 involving epf and epn isolates of the Darwin collection

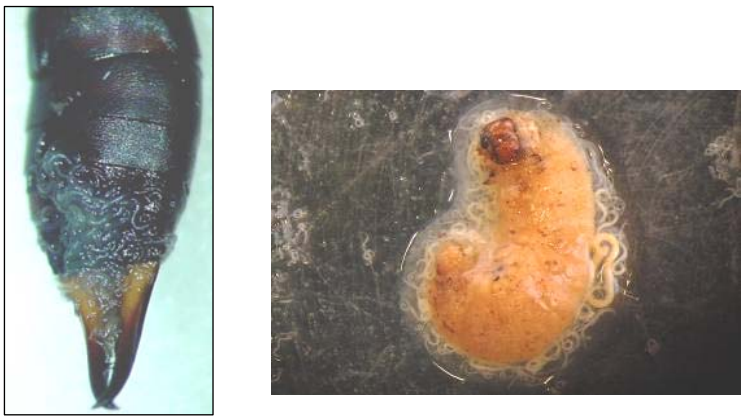


Figure 4. During Year 2 epf and epn were screened against a number of pests of economic importance from Chile (including earwig and codling moth as above). Images: L. Merino, INIA 2008, recent studies on a *Steinernema* epn collected from Area 5.

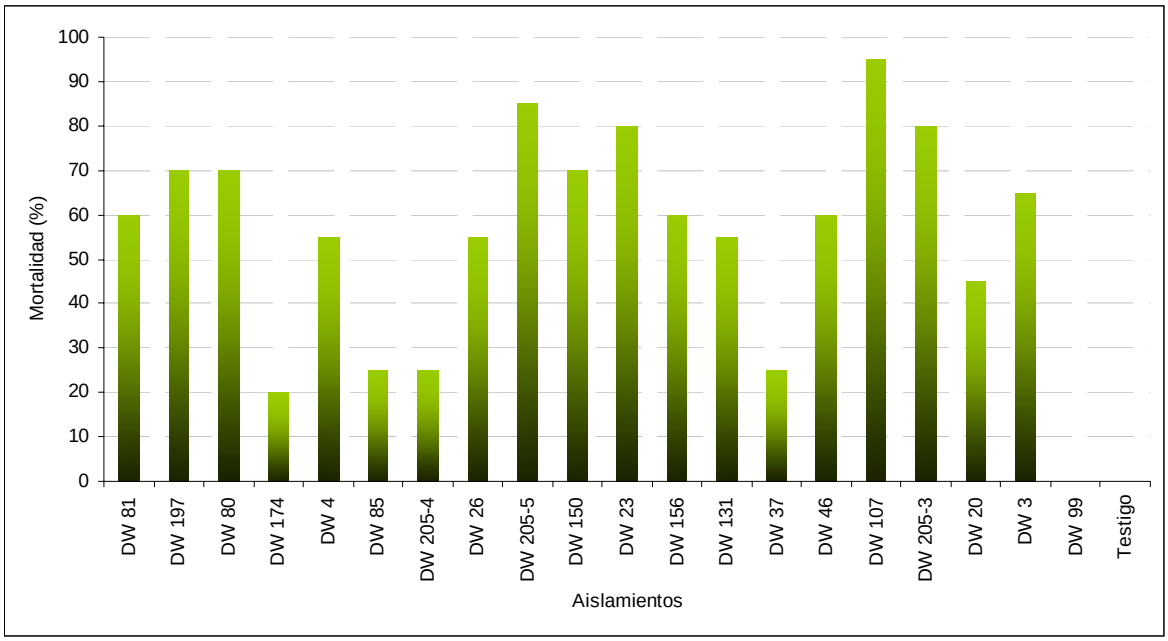


Figure 5. Results from epn screening from the Darwin collection, for pathogenicity to codling moth (*C. pomonella*). Courtesy of Manuel Contreras, Universidad de Concepcion / INIA, 2008

Culture collection of epf and epn established

Culture collections of epf and epn obtained from the survey sites were established at INIA in Year 1 and subsequently enhanced in Year 2. These collections were and still are of a short-term nature, kept on growth-media and in water for epf and epn respectively. During Year 2 however, the epf collection has been cryopreserved to increase long-term stability of isolates and the epn collection is in line for cryopreservation. Furthermore, two scientists from INIA visited CABI in May 2007 for training in a number of aspects relating to genetic resource collections, including cryopreservation techniques, culture maintenance and issues of intellectual property rights. The training was conducted by Dr. David Smith, President of the World Federation for Culture Collections and Dr Matt Ryan, Curator of CABI's Genetic Resource Collection. The INIA staff also received training on fungal and nematode identification techniques.

Figure 6. Short-term epf and epn collections established at INIA. Isolates are being cryopreserved for long-term preservation.

Members of the team, from both CABI and INIA, presented project results at a number of national and international conferences during Year 2 (see Table 5 for conferences attended and Annex 3 for poster examples).

INIA staff also gave a number of educational classes to various groups (see Table 6). There is a link to the project on both the CABI and INIA websites, furthermore in June 2008 a new website developed at INIA (www.controlbiologicochile.cl) will feature more detailed Darwin information with a number of pages dedicated solely to the project.

Project scientists from CABI and INIA also met with Ambassador Moreno, the Chilean Ambassador to UK, in London during Year 2. This meeting followed an earlier meeting in Year 1 in which Ambassador Moreno visited CABI. Project progress and possible sources of additional finance to continue activities beyond the project timeframe were discussed. The Ambassador is giving his full support to this Darwin Initiative.

3.2 Progress towards Project Outputs

The project is on course to meet its outputs. In Year 2 the project team completed the surveys of the six study areas selected in Chile. This will enable the team to concentrate in Year 3 on characterisation of the isolates (identifying adaptations to habitat) and screening for pathogenicity to key insect pests, whilst also establishing the most suitable means of taking the results beyond the time frame of the Darwin Project.

It is highly likely that the original Measurable Indicator of at least 300 epf and 100 epn isolates from the surveys will be exceeded, significantly so for epf, placing pressures on project scientists. INIA staff connected with the Darwin project are still in place and INIA as a whole continues to support the Darwin project fully. Year 3 will be another exciting year as more detailed screening can reveal those isolates with serious potential as biological control agents in Chile.

3.3 Standard Measures

Table 1 Project Standard Output Measures

(See Table 6)

Code No.	Description	Year 1 Total	Year 2 Total	Year 3 Total	Year 4 Total	TOTAL
Established codes						
6A	Number of people receiving training (Chileans)	0	2			
6B	Number of training weeks to be provided	2	2			
8	Number weeks spent by UK project staff in host country	10	10			
12A	Number of computer based databases to be established	2	0			
13A	Number of species reference collections	2	0			

	to be established					
15A	Number of national press releases in host country	1				
15B	Number of local press releases in host country	2				
15C	Number of national press releases in UK	1	0			

Table 2 **Publications.** There have been a number of poster presentations at conferences, but papers have yet to be written. This will occur in year 3.

Type * (eg journals, manual, CDs)	Detail (title, author, year)	Publishers (name, city)	Available from (eg contact address, website)
Outlooks on Pest Management, Dec 2007, 260-262.	D. Moore, S. Edgington, A. France, L. Merino. 2007. *In search of Darwin's nematodes.*		Electronic copy sent to Darwin.
	Uso de enfermedades de insectos para el Control de Plagas. Loreto Merino, Andrés France y Marcos Gerding	Tierra Adentro, Chile Nº78 March – April 2008	produccion@ideograma.cl

3.4

Progress towards the project purpose and outcomes

Both short-term and long-term culture collections of the Darwin epf and epn isolates have now been established at INIA. Following four surveys during Year 2 the collection has increased significantly in numbers of both epf and epn. INIA has increased its student intake for undergraduate projects using the isolates and this is substantially increasing the project's scientific output. The standard of student work is high and a number of publications in peer reviewed journals are planned for Year 3 of the study. INIA staff completed a period of training at CABI and have subsequently worked hard to develop a fully curated, well maintained, accessible culture collection. Priorities in year 3 include identification of epf and epn isolates, production of scientific papers for peer review journals and working with local SME and Chilean organisations for practical biopesticide development, culminating in a meeting in Q4 of the year.

3.5 Progress towards impact on biodiversity, sustainable use or equitable sharing of biodiversity benefits

Impact will depend largely on practical uptake of agents for biopesticide products. The project is going extremely well in terms of exploring biodiversity and its exploitation is one aspect of year 3. If this is successful, significant benefits will be derived from biodiversity fro Chile and its economy.

4. Monitoring, evaluation and lessons

Primarily the monitoring is scientific progress, based on surveys carried out and isolates obtained, along with success in characterisation. These have been described earlier.

The importance of having good students needs emphasising. Although they cause considerable work, they have been very beneficial in accumulating significant amounts of data from routine (but necessary) studies.

5. Actions taken in response to previous reviews (if applicable)

We responded to the very helpful comments of Year 1 Annual Report, explaining the nature of our sampling. All project partners regarded the comments as helpful and positive.

6. Other comments on progress not covered elsewhere

Broadly the design remains the same, but extra work has been done (eg bacterial symbionts from epn and additional epn characterisation work has been carried out). Sustainability and exit strategies are priorities for Year 3.

Discuss any significant difficulties encountered during the year and steps taken to overcome these if not already discussed elsewhere.

The survey of Area 2 in the Atacama Desert was originally planned for November 2007. Sadly there was an earthquake in the region 24 hours prior to starting the survey, forcing the team to postpone sampling in this region. It was decided that additional samples would be taken in Area 4 (sampled earlier in the year). Area 4 has some particularly interesting volcanic larval fields, which the group thought merited further sampling.

NB. 2 weeks later the principle volcano in Area 4 erupted, covering some of the sampled land with fresh larva – and also causing the evacuation of a number of towns and villages.

Does the project face any particular risks?

See above

6. Sustainability

Details of dissemination at various levels are given later. INIA has prioritised the project for publicity for 2008. The exit strategy depends on commercial interest, itself largely dependent on the efficacy of isolates found and Government support. This is a priority for year 3, as was anticipated from the beginning.

7. Dissemination

Members of the project team have presented project results at a number of national and international conferences (see Table 5 and Annex 3 for poster examples). Project members from INIA have also given talks, run classes and hosted meetings for a variety of groups in relation to the Darwin Initiative in general and to this specific project (see Table 6).

Conference	Location	Title of presentation and participants	Date
40th Annual Meeting of the Society for Invertebrate Pathology and the 1st International Forum on entomopathogenic nematodes and symbiotic bacteria	Quebec, Canada	Surveys of indigenous entomopathogenic fungi and nematodos of Chile and studies on their pathogenicity towards pests of economic importance. Loreto Merino, Andrés France, Dave Moore, Steve Edgington. (One in Fungal session, another in Nematode session)	August 2007
National Congress of Entomology	Universidad. Metropolitana de Ciencias de la Educación, Santiago Chile	Evaluation of native entomopathogenic fungi for control of *Otiorhynchus sulcatus* Fab. (Coleoptera: Curculionidae). XXIX. Loreto Merino, Andrés France.	November 2007
National Congress of Entomology	Universidad Metropolitana de Ciencias de la Educación, Santiago Chile	Biological control of Hylurgus ligniperda (Fabricius) with native entomopathogenic fungi Loreto Merino, Marcos Gerding, Andrés France.	November 2007
II International Symposium of organic agriculture.	Universidad de Las Américas. Santiago de Chile	Conservation and use of native entomopathogenic organisms in Chile. Loreto Merino	March 2008.
II International Symposium of organic agriculture.	Universidad de Las Américas. Santiago de Chile	Evaluation of entomopathogenic fungi for control of *Otiorhynchus sulcatus* fab. (Coleoptera: Curculionidae. Loreto Merino	March 2008.
1st Internacional Symposium of nematodes as biological indicators	Herriot-Watt University, Edinburgh, Scotland.	Entomopathogenic nematodes of Chile. Steve Edgington	June 2007
Nematology Congress: Association of Applied Biology	Linnean Society, London	Characterisation of entomopathogenic nematodes of Chile. Steve Edgington	December 2007

Table 5. Conferences attended by project staff during Year 2

Class/talk and location	Participants	Date
Course in Agronomy, Universidad Católica de Chile	Undergraduates	13/12/2007
Darwin, Nematodes and Fungi: basics. Colegio El Carmen	Infants (< 10 years)	07/12/2007
Course in Agronomy, Universidad	Undergraduates	08/11/2007

Santo Tomás, Chile		
Meeting of local farmers from the Pinto community.	Small-holders	07/11/2007
Program: '1000 scientists 1000 classes' Escuela Monte Blanco de la comuna de San Carlos	Infants	09/10/2007
Course in Agroecology, Instituto Santo Tomás	Undergraduates	12/07/2007
Meeting of berry producers of the Bio- Bio region	Small-holders	06/07/2007
Course in Agronomy, Universidad de Chile	Undergraduates	23/01/2007
Meeting of organic producers	Small-holders	10/01/2007

Table 6. Educational classes and talks given by project staff regarding the Darwin project and the use of the epf and epn isolates

8. Project Expenditure

Table 3 Project expenditure <u>during the reporting period</u> (Defra Financial Year 01 April to 31 March)

Item	Budget (Letter of offer of 18 April 2006 from Eilidh Young)	Expenditure	Balance
Rent, rates, heating, overheads etc			
Office costs (eg postage, telephone, stationery)			
Travel and subsistence			
Printing			
Conferences, seminars, etc			
Capital items/equipment			
Others			
Salaries (specify)			
TOTAL			

Highlight any agreed changes to the budget and explain any variation in expenditure where this is +/- 10% of the budget.

9. OPTIONAL: Outstanding achievements of your project during the reporting period (300-400 words maximum). This section may be used for publicity purposes

I agree for ECTF and the Darwin Secretariat to publish the content of this section. The major achievements have been completion of the surveys, isolation of significant numbers of epf and epn isolates, development of the molecular techniques to sequence the epn and extraction of the DNA of the epf isolates. The culmination of the epn work is that we are now in the position to formally describe the first new species to derive from the project; this process should take place in Q1 of year 3.

Overall, year 2 has positioned the project such that the characterisation work of both epf and epn can be achieved in Y 3.

Annex 1 Report of progress and achievements against Logical Framework for Financial Year: 2007/08

Project summary	Measurable Indicators	Progress and Achievements April 2006 - March 2007	Actions required/planned for next period
Goal: *To draw on expertise relevant to biodiversity from within the United Kingdom to work with local partners in countries rich in biodiversity but constrained in resources to achieve* *The conservation of biological diversity,* *The sustainable use of its components, and* *The fair and equitable sharing of the benefits arising out of the utilisation of genetic resources*		*(report on any contribution towards positive impact on biodiversity or positive changes in the conditions of human communities associated with biodiversity eg steps towards sustainable use or equitable sharing of costs or benefits)*	*(do not fill not applicable)*
Purpose To enhance conservation and sustainable use of the entomopathogenic fungal (epf) and nematode (epn) biodiversity in Chile, through increased capacity in collection, curation and characterisation	Isolate collections established by Y 1 with additions until Y 3. Isolate characterisation databased by Y 3. Protocols on conservation and IPR/ABS drafted by Y 1 and finalised Y 3.	Isolates of epf and epn collected from distinct ecological habitats; placed under long-term storage in newly established culture collections in Chile. Capacity building through training courses undertaken by INIA staff at CABI UK.	(Highlight key actions planning for next period)
Output 1. Isolates of epf and epn obtained from Chile by collaborators	At least 300 isolates of epf and 100 of epn from Chile, distributed across all ecosystems.		

Activity 1.2. Surveys 1 and 2		The remaining 4 areas of the planned 6 were surveyed in Year 2. Between 128 and 402 soil samples were collected from each area, with each sample processed twice at 20 °C with waxmoth (*Galleria mellonella*) bait. From Year 2 a total of 91 epn and 275 epf have been isolated (with soil still being processed). Including both *Steinernema* and *Heterorhabditis* species of epn and both *Beauveria* and *Metarhizium* species of epf.
Output 2. Biological and molecular studies of isolates achieved	Biological profiles established, eg. temperature, RH and UV tolerance. Molecular and biochemical data generated for epf and epn isolates	
Activity 2.2. Characterisation		Characterisation activities: Nematodes: 1) temperature effects on survival, infectivity and reproduction, 2) storage characteristics, 3) foraging strategy, 4) effect of soil moisture and 5) insect-host range Fungi: 1) temperature effects on vegetative growth and on persistency in soil, 2) UV tolerance, 3) water demands of spores and 4) insect-host range. Temperature profiling of epn from site 5 presently underway, with 4 isolates already profiled for infectivity and reproduction at a range of temperatures. Data on profiling to accompany six-month report for Y2. Molecular characterisation of epf and epn will be carried out in Q1 &2 of year 3.
Output 3. Institutional capacity increased in Chile	INIA staff trained in a) epf and epn characteristics and culture curation and b) IPR/ABS of microbial biodiversity	Two members of the INIA project team visited CABI for intensive training in key aspects of genetic resource collections and epf/epn identification. Year 3 will see further improvements in storage of isolates.

Output 4. Culture collection of epf and epn established	Reports in regional newspapers and INIA press.	
Activity 4.1. Culture collection established		Fungal isolates purified and placed under long-term storage (cryopreservation). Nematode isolates being fixed and prepared as long term reference slides, with molecular and morphological identification underway. A number of possible new species of epn provisionally identified.
Output 5. Simple isolate collection, curation and characterisation protocols developed	Protocols established	Continued additions to collection continue through Year 3.
Output 6. Information dissemination and conservation plans	Scientific papers for international journals by Y3. Extension literature Y1-3. Report from Comite de Biodiversidad by Y3. Project data CD Y3. Radio and TV as appropriate	A number of peer reviewed papers will be submitted through year 3 and a workshop held in Q4.
Activity 6.1. Dissemination		

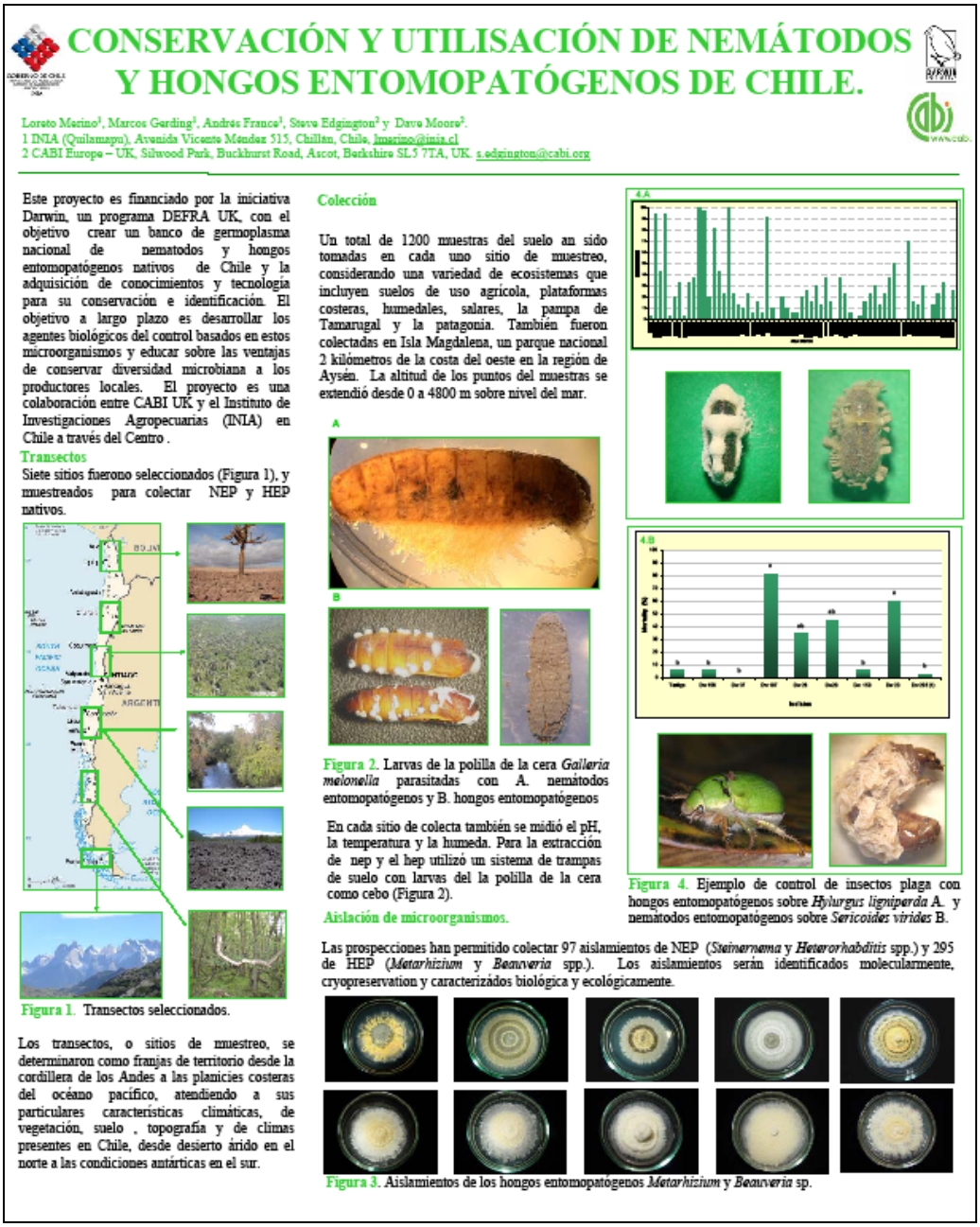
Annex 2 Project's full current logframe

Project summary	Measurable Indicators	Means of verification	Important Assumptions
Goal: **To draw on expertise relevant to biodiversity from within the United Kingdom to work with local partners in countries rich in biodiversity but poor in resources to achieve** • **the conservation of biological diversity,** • **the sustainable use of its components, and** • **the fair and equitable sharing of benefits arising out of the utilisation of genetic resources**			
Purpose *To enhance conservation and sustainable use of epf and epn in Chile through increased capacity in collection, curation & characterisation.*	*Isolate collections established by Yr 1 with additions until Yr 3. Isolate characterisation* *databased by Yr 3* *Protocols on conservation and IPR/ABS drafted by Yr 1 and finalised Yr 3*	*INIA and CABI reports.*	*Governments maintain support for biodiversity and for collaborating Institutions.*
Outputs 1. Isolates of epf and epn obtained from Chile by collaborators. 2. Biological and molecular studies of isolates achieved.	1. At least 300 isolates of epf and 100 of epn from Chile, distributed across all ecosystems. 2. Biological profiles established, e.g. temperature, RH and UV tolerance. Molecular and biochemical data generated for epf and epn isolates.	1. Field survey reports, species inventories, scientific publications. 2. Study reports, scientific publications.	**For all:** trained staff will remain in Institute and Universities and have positions to use skills acquired.

3. Institutional capacity increased in Chile.	3. INIA staff trained in a) epf and epn characteristics and culture curation and b) IPR/ABS of microbial biodiversity.	3. Training programme records.	
4. Culture collection of epf and epn established.	4. Presence of viable epf and epn collections in Chile.	4. Collection records.	
5. Simple isolate collection, curation and characterisation protocols developed.	5. Protocols published.	5. Copies sent to Darwin.	
6. Information dissemination and conservation plans.	6. Scientific papers for international journals by Yr 3. Extension literature Yr 1-3. Report from Comite de Biodiversidad by Yr 3. Project data CD Yr 3. Radio & TV as appropriate.	6. Copies of all sent to Darwin.	6. Information reaches stakeholders and is put to positive use.

Annex 3 onwards – supplementary material (optional)

Examples of posters on Darwin project results presented at International conferences in Year 2. The second poster shown won first prize for 'best poster' at' the 2nd International Symposium of Organic Agriculture (Santiago, Chile). A report of this prize can be seen following the link on http://www.inia.cl/quilamapu/



CONSERVACIÓN Y UTILISACIÓN DE NEMÁTODOS Y HONGOS ENTOMOPATÓGENOS DE CHILE.

Loreto Merino[1], Marcos Gerding[1], Andrés France[1], Steve Edgington[2] y Dave Moore[2].
1 INIA (Quilamapu), Avenida Vicente Méndez 515, Chillán, Chile, lmerino@inia.cl
2 CABI Europe – UK, Silwood Park, Buckhurst Road, Ascot, Berkshire SL5 7TA, UK. s.edgington@cabi.org

Este proyecto es financiado por la iniciativa Darwin, un programa DEFRA UK, con el objetivo crear un banco de germoplasma nacional de nematodos y hongos entomopatógenos nativos de Chile y la adquisición de conocimientos y tecnología para su conservación e identificación. El objetivo a largo plazo es desarrollar los agentes biológicos del control basados en estos microorganismos y educar sobre las ventajas de conservar diversidad microbiana a los productores locales. El proyecto es una colaboración entre CABI UK y el Instituto de Investigaciones Agropecuarias (INIA) en Chile a través del Centro .

Transectos

Siete sitios fueron seleccionados (Figura 1), y muestreados para colectar NEP y HEP nativos.

Figura 1. Transectos seleccionados.

Los transectos, o sitios de muestreo, se determinaron como franjas de territorio desde la cordillera de los Andes a las planicies costeras del océano pacífico, atendiendo a sus particulares características climáticas, de vegetación, suelo , topografía y de climas presentes en Chile, desde desierto árido en el norte a las condiciones antárticas en el sur.

Colección

Un total de 1200 muestras del suelo an sido tomadas en cada uno sitio de muestreo, considerando una variedad de ecosistemas que incluyen suelos de uso agrícola, plataformas costeras, humedales, salares, la pampa de Tamarugal y la patagonia. También fueron colectadas en Isla Magdalena, un parque nacional 2 kilómetros de la costa del oeste en la región de Aysén. La altitud de los puntos del muestras se extendió desde 0 a 4800 m sobre nivel del mar.

Figura 2. Larvas de la polilla de la cera *Galleria melonella* parasitadas con A. nemátodos entomopatógenos y B. hongos entomopatógenos

En cada sitio de colecta también se midió el pH, la temperatura y la humeda. Para la extracción de nep y el hep utilizó un sistema de trampas de suelo con larvas del la polilla de la cera como cebo (Figura 2).

Aislación de microorganismos.

Las prospecciones han permitido colectar 97 aislamientos de NEP (*Steinernema* y *Heterorhabditis* spp.) y 295 de HEP (*Metarhizium* y *Beauveria* spp.). Los aislamientos serán identificados molecularmente, cryopreservation y caracterizádos biológica y ecológicamente.

Figura 3. Aislamientos de los hongos entomopatogenos *Metarhizium* y *Beauveria* sp.

Figura 4. Ejemplo de control de insectos plaga con hongos entomopatógenos sobre *Hylurgus ligniperda* A. y nemátodos entomopatógenos sobre *Sericoides viridis* B.

ANNEX 3 CONTD.

EVALUACION DE CEPAS NATIVAS DE HONGOS ENTOMOPATÓGENOS SOBRE *Otiorhynchus sulcatus* Fab. (COLEOPTERA: CURCULIONIDAE)

Loreto Merino, Claudia Inostroza, Marcos Gerding y Andrés France.
INIA Quilamapu, Casilla 426, Chillán, CHILE. E-mail: lmerino@inia.cl

Foto 1. Adulto y larvas de *Otiorhynchus sulcatus*.

Foto 2. Larvas de *Otiorhynchus sulcatus* parasitadas por el HEP *Metarhizium anisopliae*.

RESULTADOS

Evaluaciones de aislamientos de HEP demuestran que diferentes aislamientos alcanzaron distintos niveles de mortalidad sobre larvas de *O. sulcatus* indicando la existencia de especificidad entre las distintas cepas, características de los hongos entomopatógenos (figura 1). los aislamientos de *Metarhizium anisopliae* alcanzaron porcentajes de mortalidad superiores a los de *B. bassiana*, siendo Dw M66 y Dw M15 los más altos con el 100% de mortalidad a los 11 y 12 días respectivamente, lo que contrasta con los testigos que no presentaron mortalidad durante el desarrollo de la evaluación.

CONCLUSION

Existen aislaciones nativas del HEP patogénicas a *Otiorhynchus sulcatus* .

Este proyecto cuenta con el financiamiento de Darwin Initiative un programa perteneciente a DEFRA UK.

INTRODUCCIÓN

El gorgojo de la frutilla *Otiorhynchus sulcatus* Fabricius (Coleoptera: Curculionidae), es considerada una plaga cuarentenaria para mercados de Europa y Norteamérica. Las larvas de este insecto consumen raicillas anillando las raices principales provocando la muerte de la planta. El control es difícil una vez que *O. sulcatus* se ha establecido en un cultivo, a causa de la baja eficacia de los métodos químicos y a problemas de contaminación ambiental, la aplicación de insecticidas químicos se ha visto limitada y han surgido medidas de control biológico, como el uso de hongo entomopatógeno. El objetivo de esta investigación fue determinar patogenicidad y esporulación de aislamientos nativos del hongos entomopatógenos *Beauveria bassiana* y *Metarhizium anisopliae* sobre larvas de *O. sulcatus*.

METODOLOGIA

Se inocularon larvas de *O. sulcatus* en forma directa con suspensiones de 10⁷ conidias por mL-1, tanto del HEP *Beauveria bassiana* como *Metarhizium anisopliae* mediante el sistema de pulverización de la torre Porter, los que fueron mantenidos en forma individual en contenedores con aserrín estéril húmedo y en oscuridad durante 30 días evaluando la mortalidad diariamente.

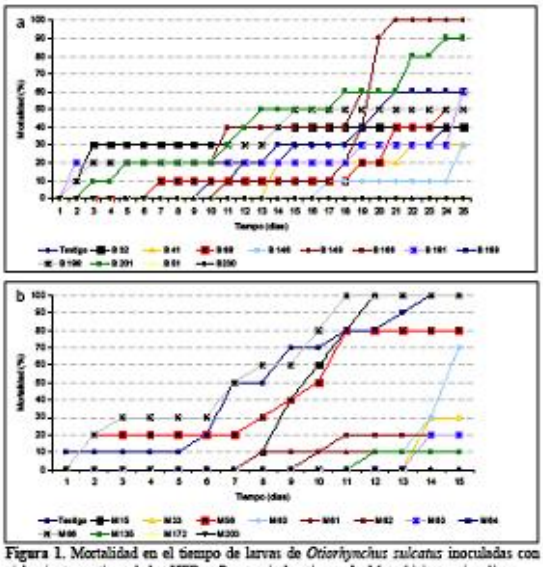


Figura 1. Mortalidad en el tiempo de larvas de *Otiorhynchus sulcatus* inoculadas con aislamientos nativos de los HEP a. *Beauveria bassiana* y b. *Metarhizium anisopliae*.

Checklist for submission

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