Darwin Initiative Annual Report

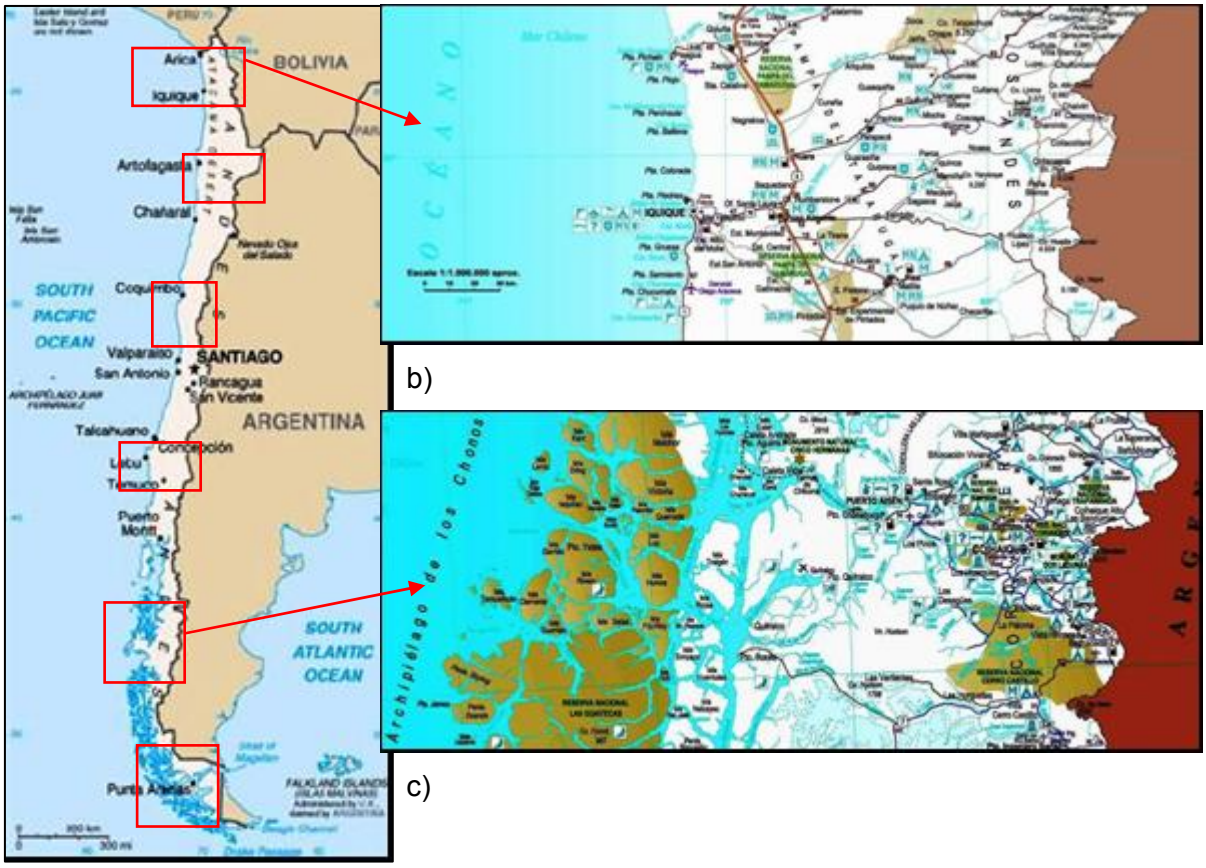
Darwin Project Information

Project Ref Number	15/004
Project Title	Conserving and using entomopathogenic fungi and nematodes within Chile
Country(ies)	UK and 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 2006 to 31 March 2007. Number 1
Project Leader Name	Dave Moore
Project website	
Author(s), date	Dave Moore, Andres France, Loreto Merino, Steve Edgington. 26th April 2007

1. Project Background

This is a study on the entomopathogenic fungal (epf) and nematode (epn) biodiversity of Chile and is a collaboration 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 ultimate goal is to use this information to help select suitable isolates as microbial biological pesticides (biopesticides), offering an alternative to chemical pesticides in Chile. The Government of Chile is dedicated to reducing the use of chemical pesticides on agricultural systems and epf and epn could form key components of new, sustainable strategies.

Six sites have been chosen in Chile by the project participants, each of which will be surveyed for epf and epn. The sites cover the main climatic, vegetation and soil types present in Chile (Figure 1. a). The sites, stretching east to west from the Andes to the Pacific coast will include desert land in the north, near Antarctic conditions in the south and rich, fertile areas in the centre.



(a)

Figure 1. (a) Six sample sites in Chile; (b) Site 1, far north, sampled Q4 Yr1; (c) Site 5, south, sampled Q2 Yr 1.

2. 2. Project Partnerships

Project partnerships: 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 Y1 of the project. The younger scientists in the collaboration are working together extremely well and a natural division of activity is occurring, with INIA leading on the fungal work and CABI being more prominent with the nematode aspect. This division is not complete, nor is it planned to be, but is very convenient at this stage in the project and should be seen as a strength of the collaboration. 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. To date, the capital expenditure is providing support for an effective culture collection (INIA collaborators will be receiving training of relevance to CBD in April/May 2007).

It is expected that links with other groups in biodiversity conservation will increase in Y2, now that the initial surveys have resulted in a significant number of fungal and nematode isolates being found. INIA, CABI and organisations in Bolivia and

Argentina are expecting to collaborate in response to an EU expression of interest on microbial biodiversity in the Andes, in 2008.

3. Project progress

3.1 Progress in carrying out project activities

Isolates of epf and epn obtained from Chile by collaborators

A work plan for the epf and epn surveys was agreed by INIA and CABI staff in Q1 of Y1. Six survey sites in Chile were chosen (see Section 1 and Figure 1). The sites were numbered in descending order from north to south.

Site 1 in the far north (19 – 21 ^{o}S, 68 – 71 ^{o}W) and site 5 in the south (45 – 50 ^{o}S, 71 - 74 ^{o}W) were surveyed during Y1. Site 1 is just north of the Tropic of Capricorn and is on the periphery of the Atacama desert (one of the driest areas in the world). This site is the only part of Chile containing a section of the Andean plateau or Altiplano. Rainfall in the Altiplano is relatively high and forms a number of saline lakes. Water can feed from these high altitudes down the Andes. A great deal of water is lost by evaporation, however some does form oases or accumulates in subterranean aquifers. Oases permit the cultivation of tropical fruits and a large variety of vegetables in this region. Forests of the native tamarugos tree have also established in some of the driest regions as these trees are adapted to collect moisture from deep aquifers. The 201 soil samples collected throughout this region are presently being processed at INIA. These samples were collected during Q4 and require a further 1-2 months to fully process. However, a number of potential epf and epn isolates have already been found.

Figure 4. Site 1; National Park Volcán Isluga (alt. 3776 m asl.).

Figure 5. Site 1; National Reserve Pampa del Tamaruga (alt. 1100 asl.).

Site 5 is a mountainous region and one of the rainiest parts of Chile, possibly one of the rainiest in South America. Puerto Aisén, a base for the survey in this site, receives around 120″ of rain per year, spread evenly from Jan - Dec. The climate is also relatively cold, average summer temperatures at sea level are generally below 15 ^{o}C. Forests and livestock pastures predominate within this area. During the survey carried out in Q2, 184 soil samples were collected by the project team. The samples have been processed at INIA and revealed 67 isolates of epf (*Metarhizium* and *Beauveria* spp.) and 12 isolates of epn (*Steinernema* spp.).

Figure 6. Site 5; sampling in Alerce forest.

Figure 7. Nematode infected wax moth from survey.

Biological and molecular studies of isolates achieved

Biological and molecular characterisation of the epf and epn isolates is scheduled to cover Y1 to Y3. During Y1 the collaborators agreed on the methodologies for characterisation and started the work on the isolates found in Site 5. INIA have already characterised around half the current epf collection by phenotype, with approximately 10 % presently under genetic characterisation. CABI are principally involved with epn characterisation and, as well as phenotype studies, are examining ecological aspects such as temperature tolerance, moisture requirements, host searching behaviour and persistence. CABI has 1 PhD student focusing on the epn isolates collected during the Darwin project.

Institutional capacity increased in Chile

Capital equipment for cryopreservation has been purchased and the preservation of project isolates is underway. Two scientists from INIA will be visiting CABI in April/May 2007 for a course on culture collections, including cryopreservation techniques, culture maintenance and IPR/ABS.

Culture collection of epf and epn established

Culture collections of epf and epn obtained from the survey sites have been established at INIA. Scientists have produced single spore cultures of each epf isolate from Site 5 and cryopreserved them in liquid nitrogen. Each of the epf isolates are also being maintained as short-term cultures on suitable growth media under oil. The epn isolates have been purified and cultured through waxmoth larvae (*Galleria mellonella*), establishing a short-term collection in water (shelf-life approximately 12 months) for immediate access. The epn isolates have also been prepared as permanent slides and cryopreservation is presently underway at INIA.

Information dissemination and conservation plans

A number of reports have been published in Chile regarding the Darwin project (see links below), including several in regional newspapers (incl. Figure 8) and one in INIA press. A reporter and film crew from a national television network will be joining the collaborators for the third survey with the intention of producing a short film on the Darwin project. There is a link to the project on the CABI website http://www.cabi.org/ProjectsDetail.asp?ProjectID=307

and further links:

http://www.inia.cl/noticias2/index.php?id=3&tx_ttnews[pointer]=1&tx_ttnews[tt_news]=133&tx_ttnews[backPid]=1&cHash=6603b8861b

http://www.diarioelsur.cl/edicion_hoy/secciones/articulo.php?id=88023&dia=1174536000

Las ventajas del control biológico

Existen varias ventajas asociadas al uso de controladores ecológicos de plagas agrícolas. Entre ellos, destaca que los organismos usados como insecticidas microbianos no son tóxicos ni patogénicos para mamíferos y otros organismos.

La acción es generalmente específica para un único grupo o especie de insectos y, por lo tanto, los efectos sobre insectos benéficos son mínimos.

De ser necesario, la mayoría de los entomopatógenos puede ser utilizado en mezclas con insecticidas sintéticos.

Los residuos son inocuos para humanos, permitiendo que los enemigos naturales puedan ser aplicados poco antes de la cosecha.

En algunos casos, los entomopatógenos pueden establecerse en un área tratada, provocando un control duradero del insecto objetivo por varias generaciones o estaciones.

En Chile existen varios ejemplos exitosos de control biológico: como el caso de la Conchuela acanalada de los cítricos, Icerya purchasi, plaga que logró controlarse gracias a la importación de Rodolia cardinalis desde Australia; o el caso del pulgón ruso del trigo, Diuraphis noxia, que fue combatido masticando los mismos enemigos naturales presentes en el medio.

Para capturar organismos que sirven para el control biológico, próximamente los investigadores repetirán la ruta que Charles Darwin realizó hace más de 150 años entre Valparaíso y la cordillera de Los Andes.

Investigadores recorrerán la "senda de Darwin"

Investigadores chilenos del Centro Regional INIA Quilamapu e ingleses del Centro Internacional CABI, han recorrido lugares tan disímiles como la patagonia chilena en la XI Región y el altiplano en la I Región en el marco del proyecto sobre uso y conservación de hongos y nematodos entomopatógenos en Chile, que apoya la Fundación Darwin.

Este proyecto tiene como objetivo recolectar, seleccionar e identificar organismos de control biológico de plagas agrícolas que se encuentran a lo largo del país, como son los hongos y nemátodos entomopatógenos, microorganismos que al liberar enzimas y toxinas son capaces de dar muerte a insectos además de diseminarse con facilidad.

Andrés France, investigador de INIA a cargo del proyecto, señaló que como fruto de las prospecciones que se realizaron en la XI Región se lograron realizar 60 aislamientos de hongos y 12 de nemátodos. "Chile es un país con gran biodiversidad por lo que lo hemos dividido en seis zonas de prospección buscando rescatar la gran variedad de hongos y nemátodos existentes, la idea es aislar nuevos organismos y poder usarlos como controladores biológicos de plagas en agricultura", señaló.

Próximamente los investigadores realizarán la "senda de Darwin" replicando la ruta que este científico realizó hace más de 150 años entre Valparaíso y la cordillera de los Andes.

Actualmente INIA Quilamapu trabaja en la multiplicación de hongos entomopatógenos nativos, que ya están siendo aplicados con éxito para combatir diversas plagas de insectos en viñedos, frambuesas, arándanos y otros frutales.

Figure 8. Investigadores recorrerán la “senda de Darwin”. La Discusíon, 28/03/2007, p. 13.

3.2 Progress towards Project Outputs

The project is on course to meet its outputs. In Y1, two of the planned six sites have been surveyed and processing from these two sites will be complete in Q1 of Y2. The collaborators are scheduled to survey two sites every year. The original Measurable Indicator of at least 300 epf and 100 epn isolates from the surveys remain (67 epf and 12 epn isolates were collected from the first survey). INIA staff connected with the Darwin project are still in place and a new member, Loreto Merino, has been taken on full-time to work solely on project activities. Year 2 will be an exciting year as the characterisation studies will generate new information on the isolates being found in Chile.

3.3 Standard Output Measures

Table 1

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)	2				
6B	Number of training weeks to be provided	2				
8	Number weeks spent by UK project staff in host country	10				
12A	Number of computer based databases to be established	2				
13A	Number of species reference collections to be established	2				
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				

Table 2 Publications

Type * (eg journals, manual, CDs)	Detail (title, author, year)	Publishers (name, city)	Available from (eg contact address, website)	Cost £
Only media articles (see links in 3.2.)				

3.4 Progress towards the project purpose and outcomes

Culture collections of epf and epn have now been established at INIA. The necessary capital equipment to facilitate long-term storage of isolates has been purchased and installed. Isolates of both epf and epn have already been cryopreserved, enabling INIA a storage capacity of decades rather than months. Training in culture maintenance for INIA staff is taking place in April/May 2007 at CABI, organised by Dr. David Smith, Head of the Genetic Resource Collection at CABI and President of the World Federation for Culture Collections. The characterisation of isolates found during the surveys is again in progress and is the means of identifying traits that could be useful in biological control programs.

The Government of Chile maintains support for both national biodiversity and the collaborating Institutions involved in the project. Project scientists from CABI met with Ambassador Moreno (Chilean Ambassador to UK) in Q2 who emphasised the Chilean Governments full support for the project. In May 2007 both CABI and INIA staff will be meeting again with Ambassador Moreno to discuss the future direction of this initiative, particularly beyond the project time-frame.

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

The project is on course to demonstrate significant biodiversity of epf and epn. A significant number of isolates were obtained from the first survey (and, it would appear, from the second conducted at the end of Y1). If the project progresses as anticipated, these isolates could be used to develop biological pesticides to replace chemical insecticides in Chile; this could impact on (a) reducing biodiversity loss, (b) increasing agricultural sustainability and (c) communities could receive benefits from use of the discovered biodiversity (such as reduced ill-health from a reduction in chemical insecticides or more economic pest control). Such impacts will be more predictable later in the project. From the initial findings it is clear that potentially valuable organisms are being found in a range of environments and hence demonstrating to the Government of Chile that previously unsuspected resources are present within the country.

4. Monitoring, evaluation and lessons

This is largely covered by meeting the requirements of the log-frame. The surveys are resulting in many isolates being found; these are being studied for their biological characteristics. Details, for example on numbers of isolates and scientific procedures are given in the logframe, matching the original plans. The culture collection has been established and INIA scientists are receiving training in curation techniques, intellectual property and patents etc. Dissemination is largely based around public interest – i.e. newspaper reports, news letter items. Although the project is not being managed strictly on PRINCE2 procedures, the concept of the configuration librarian is being taken on board to ensure recording and maintenance of information databases is carried out.

The second part of the project is using the biodiversity that has been found, characterised and conserved and this will become more prominent in Y2 and 3.

One significant lesson has resulted from the large numbers of isolates being found (this was hoped for, but was not inevitable), which will all be conserved, but a strategy for the characterisation needs to be developed further. Processing the isolates may turn out to be more time-consuming than allowed for in the project, if later surveys result in similar numbers. The project is determining the major aspects to be characterised and there may need to be a two stage system, with all isolates having some biological characterisation and a sub-sample having fuller characterisation.

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

NA

6. Other comments on progress not covered elsewhere

The project has been enhanced by input from Dr David Hunt (CABI Europe-UK), who is an expert on nematode taxonomy.

There have been certain dangers associated with the surveys, but two person teams assist in making them as safe as possible.

The project faces no particular risks.

7. Sustainability

The information generated on epf and epn in Chile has been publicised through a variety of outlets (see Section 3.1). Additionally, every effort has been made to explain the project to growers and the general public encountered during the survey trips, discussing and promoting the possible outputs and benefits for the local communities and the environment. The growing collection of epf and epn will be included in future research of microbial-based pest control in Chile, with the collection potentially providing a valuable source of new biological products. The epf and epn biodiversity collected/highlighted during the project will provide an important background for new proposals and initiatives in Chile, aided by an active and growing team of researchers at INIA in the area of biological control.

8. Dissemination

Activities in Chile have been disseminated locally and nationally by internet, newspaper and the INIA webpage, as previously described. Furthermore, Agronomy students from the Universidad de Chile, Pontificia Universidad Catolica de Santiago,

Universidad de Concepcion and several Technical Schools dedicated to Agriculture (Escuelas Agricolas de Cato, San Rafael and Centro de Estudios y Tecnologias) have visited INIA during Y1 and spent some time learning about the Darwin project. Two Agronomy and two Biotechnology Engineering students carried out their professional practice on the Darwin project (at INIA) during the past Chilean summer. Also two undergraduate research theses related to the epf and epn isolates are presently underway (theses available Y2). Two papers regarding the Darwin work will be presented at the Chilean Agronomy Congress in August (papers available Y2). Dissemination activities after the project will be programmed according to the output, but an interview with the Chilean Ambassador in UK is scheduled for May to discuss options for carrying out future activities and obtaining financial support.

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Annex 1 Report of progress and achievements against Logical Framework for Financial Year: 2006/07

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 1.1. Work plan for survey sites and survey methodologies		Six survey sites identified, representing major ecological habitats within Chile. Two sites to be sampled each year by combined INIA and CABI team. Two groups at each site to cover greater area. Survey methods agreed, collecting soil samples from a variety of locations (including	

		different soil types, land-uses, altitudes and micro/macro-climate). Soils analysed for epf and epn back at INIA laboratories, together with analysis of pH and electro-conductivity.
Activity 1.2. Surveys 1 and 2		First and second surveys completed by INIA and CABI scientists, site 5 in the south and site 1 in the far north. A total of 385 soil samples collected, each processed twice at 20-22 ^{o}C and once at 15 ^{o}C with waxmoth (*Galleria mellonella*) bait. Sixty seven potential epf isolates and twelve potential epn isolates obtained from first survey (mountainous region characterised by heavy rainfall and areas of dense, cold forest). Samples from second survey presently being processed at INIA.
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 2.1. Protocols for molecular and biological characterisation standardised		Profiling activities: Phenotype characterisation. Nematodes: 1) temperature effects on infectivity and reproduction, 2) storage characteristics, 3) foraging strategy and 4) effect of soil moisture on infectivity. Fungi: 1) temperature effects on vegetative growth and on persistency in soil, 2) UV tolerance and 3) water demands of spores
Activity 2.2. Characterisation		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.
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	

Activity 3.1. Capacity building		Cryopreservation equipment purchased and installed at INIA. INIA staff presently at CABI for courses on culture collections and IPR/ABS. To complete by Q1 Y2.
Output 4. Culture collection of epf and epn established		
Activity 4.1. Culture collection established		Fungal isolates from first survey purified and placed under long-term storage (cryopreservation). Nematode isolates fixed and prepared as long term reference slides, with identification underway. One species of epn with provisional ident. *Steinernema glaseri* (previously undescribed from Chile). A number of epn isolates cryopreserved. Second survey completed Q4 Y1, samples to be processed April/May 2007.
Output 5. Simple isolate collection, curation and characterisation protocols developed	Protocols established	Y2/3
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	
Activity 6.1. Dissemination		A number of articles have appeared in local Chile newspapers and INIA press regarding the project (see Section 3). Journalist from national Chilean television network to accompany scientists on next survey. Project web-site to be established with links on www.INIA.cl and www.CABI.org (Y2), will include annual reports.

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. 3. Institutional capacity increased in Chile. 4. Culture collection of epf and epn established.	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. 3. INIA staff trained in a) epf and epn characteristics and culture curation and b) IPR/ABS of microbial biodiversity. 4. Presence of viable epf and epn collections in	1. Field survey reports, species inventories, scientific publications. 2. Study reports, scientific publications. 3. Training programme records. 4. Collection records.	**For all:** trained staff will remain in Institute and Universities and have positions to use skills acquired.

	Chile.		
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.

8.1.1 Activities	*8.1.2 Activity Milestones*	*8.1.3 Assumptions*
Work plan.	Yr 1: Survey-sites, methodologies and procedures for each ecosystem (months 1 – 4).	***For all:*** *trained staff will remain in Institute and Universities and have positions to use skills acquired and Government provides continuing support for activities and biodiversity.*
Protocols standardised.	Yr 1: Molecular and biological characterisation methodologies agreed (CABI / INIA).	
Surveys.	Yr 1 - 3: Chile, regular surveys, 2 – 3 with CABI (2 wk).	
Characterisation.	Yr 1 – 3: Characterisation. Chile, biochemical, temperature, growth, nutritional (NB. Bioassays will be carried out as part of INIA's biopesticide work). UK, UV-tolerance studies.	
Capacity Building.	Yr 2: INIA, training in culture collection in UK. Yr 2: INIA, training in IPR/ABS.	
Culture Collection Established.	Yr 1: Protocols confirmed. Yr 1 onwards: Epf and epn collections maintained in Chile.	
Protocols for Characterisation.	Yr 3: Collation of data and production of protocol. This will be prepared as a journal paper, manual and/or for internet as appropriate.	*Outputs reach stakeholders.*
Dissemination.	Annual reports supplied to Darwin (and placed on web-sites). INIA extension literature Journal papers prepared end Yr 2 and Yr 3 (via CABI Publishing to access wider audience), radio or TV presentations as appropriate. Review paper for Biocontrol News and information (Yr 3).	