# Darwin Initiative – Final Report

## Darwin project information

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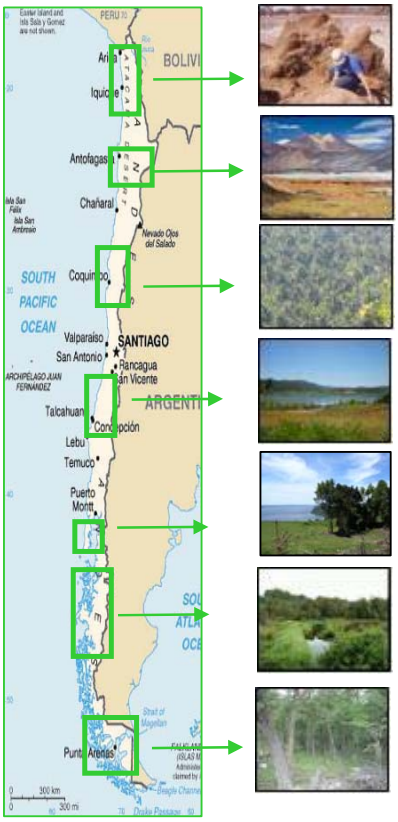
|---|---|
| Project Reference | 15/004 |
| Project Title | Conserving and using entomopathogenic fungi and nematodes within Chile |
| Host country(ies) | Chile |
| UK Contract Holder Institution | CABI Europe-UK |
| UK Partner Institution(s) | |
| Host Country Partner Institution(s) | Instituto de Investigaciones Agropecuarias (INIA), Chillán, Chile |
| Darwin Grant Value | £299,372 |
| Start/End dates of Project | May 2006-May 2009. Permission granted to extend this to accommodate Workshop in Chile in May 2009 |
| Project Leader Name | Dave Moore |
| Project Website | www.controlbiologicochile.cl |
| Report Author(s) and date | Dave Moore, Andrés France, Loreto Merino, Steve Edgington. July 2009 |

## 1 Project Background

The project examined the biodiversity of entomopathogenic fungi (EPF) and entomopathogenic nematodes (EPN) in Chile, providing a foundation for sustainable pest management through biological control using these indigenous organisms. The project collected many isolates from vastly differing ecosystems, carried out morphological and molecular identification and increased the capacity to conserve and characterise them. The longer-term aim is to enhance livelihoods by reducing dependence on chemical pesticides. Highlights included the 520 EPF and 101 EPN isolates obtained, including three, perhaps four new EPN species, training of young scientists and a high dissemination output for project activities and the Darwin Initiative.






1. Dry, desert periphery. Altiplano at 4700+ m above sea level



2. Atacama Desert. Hot, arid, saline, small pockets of vegetation



3. Valparaíso to the Andes. Semi desert, wet and dry regions



4. Mediterranean climate. Principal agricultural region in Chile



5. Chiloe archipelago. Temperate cloud forest, coastal pasture



6. The Lake Region. Mountainous, high rainfall, low temp.



7. Patagonia and Tierra del Fuego. Very low temp., high winds

Figure 1. Survey areas, from Area 1 in the far north to Area 7 in the far south, ranging between the Atacama Desert and sub-Antarctic tundra.

## 2 Project support to the Convention on Biological Diversity (CBD)

The project focussed on fundamental but little known biodiversity. Entomopathogenic fungi (EPF) and nematodes (EPN) are indicator species in that they reflect general soil health and arthropod activity in soils; their populations can only thrive when they have arthropods to attack. The project demonstrated wide spread occurrence of EPF and EPN in Chile (Table 1a and b), even at some extreme conditions (including the Atacama Desert, one of the driest places on the planet). Molecular identification of both the EPN and EPF have shown that at least three, and possibly four, new species of EPN have been found (these are in the process of being described). The remainder of the EPN are providing challenges to our understanding of the species concept as it appears that a large number of apparently inseparable species are occurring in vastly different ecosystems. This is even more apparent with EPF where two species, *Metarhizium anisopliae* and *Beauveria bassiana* dominated. Molecular characterisation cannot separate them at species level, but biological characterisation shows some remarkable differences – see Annex (4) with a poster of various EPF isolates. Studies on micro-organisms associated with the EPN have also revealed fungi, yeast and a number of

species of entomopathogenic bacteria. One of the bacterium found inside a new species of EPN is itself a new species and is being fully characterised; the bacterium belongs to the *Xenorhabdus* group, a group known to be closely associated with EPN and of commercial interest due to its production of insecticidal and anti-microbial compounds.

The project is largely directed at the sustainable use of biodiversity and access and benefit sharing. The collections of EPF and EPN are significant resources which can be exploited in the development of biological control products. Both EPF and EPN are used as the basis of commercial biological insecticides, and although at present only occupy 3% of the overall pesticide market, they are growing rapidly in market share. These, being environmentally benign, will help prevent damage to vulnerable non-target species. The replacement of chemical pesticides with biological pesticides based upon these agents will be an excellent example of the sustainable use of biodiversity. Reduced chemical pesticide use will itself be beneficial for biodiversity, reducing damage done to non-target organisms and their environments by the chemicals, whilst also reducing harmful effects on the local farming communities, including growers and consumers. However, these positive attributes would be meaningless if the biological pesticides were not effective at controlling pests. Finding suitable isolates is one component in ensuring that biological pesticides will be at least as effective as chemical insecticides.

A training course conducted at CABI for INIA staff included aspects on access and benefit sharing, to assist in a fair and equitable utilisation of resources during the transition in Chile from research to product development.

Chile is very serious about conserving and using the biodiversity that results from this project. As anticipated, involvement in an international project has enabled INIA to lever more resources from the Government of Chile. There has been significant additional support to the project, including commissioning a laboratory, dedicating a vehicle to the project, organising a Darwin Initiative session during the 2009 Biological Control Symposium in Chile and producing a film on the project. In addition a Centre for Biological Control (www.controlbiologicochile.cl) has been established due, in part at least, to the results of the Darwin Initiative project and the resources obtained. Capital equipment for storage, bought through the project, is being used to preserve the isolates. There has been much research carried out, including undergraduate and postgraduate students, resulting in a number of peer-reviewed publications. Project results have been presented at national and international meetings. In addition a number of training and educational workshops have been run – including to primary school children, growers and scientists. Consequently, the project has greatly increased the capacity within Chile to carry out similar work and also provided great stimulus to appreciate biodiversity amongst the local communities. The project scientists in Chile have liaised with The National Commission on the Environment (CONOMA) on a regular basis.

The work does not directly relate to CMS or CITES.

1a

| Transect | EPN species (no. of isolates) |
|---|---|
| 1 | *Heterorhabditis* n.sp. (4) |
| 2 | *Heterorhabditis* n.sp. (1) |
| 3 | *Steinernema* n.sp. A (8) |
| | *S. feltiae* (1) |
| | *Heterorhabditis* n.sp. (1) |
| 4 | *Steinernema* n.sp. A (20) |
| | *S. feltiae* (17) |
| | *Steinernema* n.sp. B (1) |
| 5 | *Steinernema* n.sp. A (2) |
| 6 | *Steinernema* n.sp. B (1) |
| | *Steinernema* n.sp. A (10) |
| 7 | *Steinernema* n.sp. A (15) |
| | *S. feltiae* (19) |

1b

| Transect | EPF species (no. of isolates) |
|---|---|
| 1 | *M. anisopliae* spp. (45) |
| | *B. bassiana* spp. (53) |
| | Other EPF spp. (3) |
| 2 | *M. anisopliae* spp. (38) |
| | *B. bassiana* spp. (4) |
| 3 | *M. anisopliae* spp. (18) |
| | *B. bassiana* spp. (52) |
| | Other EPF spp. (1) |
| 4 | *M. anisopliae* spp. (64) |
| | *B. bassiana* spp. (69) |
| | Other EPF spp. (28) |
| 5 | *M. anisopliae* spp. (24) |
| | *B. bassiana* spp. (3) |
| | Other EPF spp. (1) |
| 6 | *M. anisopliae* spp. (24) |
| | *B. bassiana* spp. (11) |
| | Other EPF spp. (6) |
| 7 | *M. anisopliae* spp. (47) |
| | *B. bassiana* spp. (31) |

Tables 1a and b. Summary of EPN and EPF found during project surveys. 'Other' EPF include *Fusarium* spp., *Paecilomyces* spp. and *Verticillium* spp.. Although all *Metarhizium* spp. came out as *M. anisopliae* and all *Beauveria* spp. as *B. bassiana,* doubt remains over a very small number of recalcitrant isolates. These issues will be resolved shortly and presented in a paper by Becerra, V. *et al*.

## 3 Project Partnerships

Although CABI and INIA have been in contact for twelve years, the Darwin Initiative project enabled physical collaboration to take place and enabled contact between Dr. Andrés France and Dr. Dave Moore to turn into practical activity. The principle project scientists, Loreto Merino (INIA) and Steve Edgington (CABI), have developed an extremely good and productive working relationship, this has also enabled wider activity through the supervision of a number of student projects, mainly students from the Universidad de Concepcion, but also from three other universities in Chile. INIA, in Chillán, has collaborated with other INIA scientists, university contacts and with biological control companies. As a progression of activities, INIA has opened the Centre for Biological Control which CABI plans to continue working with. This facility is one of the most sophisticated biocontrol research facilities in Latin America.

No MoU has been established, but INIA and CABI are developing an agreement on joint exploitation of agents resulting from the Darwin Initiative project. The original project was demand-led from INIA, reflecting a longer-term desire to work with CABI, reflected in earlier visits made by Dave Moore to Chile. During the project, all plans and decisions were jointly made. In practical aspects, for example surveys logistics, INIA were the responsible party, while overall strategy was decided jointly. INIA carried out the major part of the scientific work in Chile.

Strengths of the collaboration include extensive support for the project in terms of Government resources (this was always likely as external funding is a major factor in levering funding from the Government), access to students interested in agricultural projects and a complement of interests; INIA focussed largely on EPF while CABI worked on EPN. In addition the project has fostered a great mutual respect between the collaborators. A significant lesson is that the collaborators worked extremely well as a team building a great mutual respect and are keen to continue working together.

CABI has provided additional support to the project, internally funding molecular characterisation. Along with this there has been extensive input from Dr David Hunt, a world authority on the taxonomy of EPN and in addition, the University of Reading has collaborated in various areas, for example studies on the symbiotic bacteria found inside EPN and scanning electron microscopy (SEM) to assist in describing new species of EPN. The success of the project has resulted in the Linnean Society funding a small piece of work on the first new EPN species. The Biotechnology Department at INIA provided approximately half the cost for the molecular analysis of the EPF.

# 4 Project Achievements

## 4.1 Impact: achievement of positive impact on biodiversity, sustainable use or equitable sharing of biodiversity benefits

There has been great progress in obtaining large numbers of new isolates of EPF and EPN from a range of ecosystems. These had been a relatively neglected group of organisms in Chile, until this project. They now offer scientists and producers a new strategy to combat pest problems in agriculture. A fully curated, long-term collection is in place in Chile, providing future scientists and biopesticide producers an invaluable resource of indigenous biodiversity. Studies have demonstrated great physiological diversity amongst the isolates, including traits desirable as biological control agents, such as cold tolerance and host specificity for example. At least three (possibly four) new EPN species have been discovered, one description of which has been accepted and is in-press in the journal *Nematology* (see Annex (4)), and another has recently been submitted for peer-review to *Journal of Nematode Morphology and Systematics*.

The move from neglected, underutilised organisms to developing biological controls based on these organisms is an excellent example of creative and sustainable use of biodiversity. Most activities towards this aim will, naturally, occur after the Darwin Initiative project has finished, but the accumulation and safe storage of these isolates provides a vital genetic resource. Taxonomic characterisation and efficacy studies will be invaluable for registration requirements of biological pesticides. It will also achieve a basic objective of the project, to demonstrate financial benefits from biodiversity, hence aiding livelihoods; beneficiaries will include biocontrol producers together with growers using safer control strategies and technologies and producing crops that will not be rejected because of pesticides residues. Local communities would benefit from a reduction in potentially harmful pesticide-use on neighbouring farmland and from a reduction in the chemical residues on produce, and in turn consumers will benefit from 'safer' products. Thus the project is contributing to the transition from unsustainable-use to sustainable-use, humans will have negative impacts reduced and benefits increased by sustainable exploitation of previously unknown biodiversity. However, success depends on the degree of exploitation of the organisms as biological pesticides. As such, this project is more directly increasing awareness and institutional capacity, by demonstrating the wide variety of EPF and EPN that exists. The genetic resource vastly increases Chile's institutional capacity in biopesticides and the scientific research accompanying the collections also enhances capacity.

Members of project staff from INIA visited CABI for training on access and benefit sharing and the development of intellectual property. INIA is now working closely with a number of new biocontrol production companies, in Chile, to move into a new phase of development using isolates from the Darwin project.

The project has worked tirelessly to increase awareness of biodiversity, focussing primarily on the poorly understood EPF and EPN organisms and approaching the subject in an applied nature, i.e. conservation and utilisation (for examples of dissemination see Tables 3 and 4 in Annex 4 and the CD that accompanies this report). Numerous presentations and workshops have been given by project staff, to a range of audiences, including primary school children, scientists and growers. Social impacts are difficult to gauge, but the presentations have generated considerable interest.

### 4.2 Outcomes: achievement of the project purpose and outcomes

The Darwin Initiative project has resulted in a major physical resource of many hundreds of isolates of EPF and 101 isolates of EPN from a wide range of ecosystems, including climatic and topographic extremes. The isolates have been conserved in a controlled and effective storage centre in Chile and will be available for commercial exploitation as biological control organisms for insect pests. There has been a significant rise in awareness of biodiversity within Chile along with a substantial increase in scientific capacity within the host country. Eight undergraduate students from a number of Universities in Chile have undertaken scientific projects on the Darwin Initiative isolates (see Annex (4), Table 2 and the accompanying CD for student project details and abstracts).

Interest and active collaboration with biological control companies has begun and it is hoped that this will continue.

### 4.3 Outputs (and activities)

The project achieved its major objective of obtaining isolates of EPF and EPN from Chile. Five hundred and twenty EPF and 101 EPN isolates were recovered, greatly exceeding the estimate of the former and just reaching the target for the latter as suggested in the project application. The EPF isolates included species of *Metarhizium*, *Beauveria* and *Paecilomyces,* all well-known insect pathogens (Table 1b). The isolates of EPN included species of the two principle genera, *Steinernema* and *Heterorhabditis* (Table 1a). Both EPF and EPN were recovered from a wide range of ecosystems, including EPN from subpolar tundra and the Atacama Desert, and EPF from the periphery of thermal geysers and at 4500m above sea level. A culture collection of these isolates has been established at INIA, maintained by staff trained in culture curation and preservation, the training was part of the Darwin project. A range of studies by project staff and students has helped in the biological and molecular characterisation of the isolates (see Table 2). These studies have included profiling for desirable traits such as environmental persistence and mass-production capability, and assays against some of the principle insect pests in Chile. These trials have been both laboratory and field based; an active field-trial is

presently testing one of the more promising EPN (a new species) on apple trees for control of the codling moth, a serious horticultural pest in Chile.

Molecular identification was carried out on all 101 EPN isolates and the *Metarhizium* and *Beauveria* EPF isolates. Experimental protocols are and will be described in full in the relevant peer-reviewed papers, with accession details available from a.buddie@cabi.org (EPN) and vbecerra@inia.cl (EPF). We will, with Darwin Initiative approval, make these available to global networks (e.g. the Global Biodiversity Information Facility) at final completion of the project, that is on final approval of this report. Morphological and molecular identification of the EPN demonstrated a new species of *Heterorhabditis* from the north of Chile and two new species of *Steinernema* from the south (all are being, or have been, described) along with *Steinernema feltiae.* Interestingly, all EPN species have been found in more than one survey transect.

Genetic analysis of 118 *Beauveria* and 121 *Metarhizium* EPF isolates, using SSR markers, was carried out to study intraspecific variation and to define a genetic structure for these populations. The SSR analysis showed genetic diversity amongst isolates of *B. bassiana* and *M. anisopliae,* with a moderate number of alleles, although this diversity was not associated with the geographical origin of these isolates.

A novel yeast:nematode association has been found. A yeast, previously only found from insect-infested trees in Chile has been obtained from a *Heterorhabditis* sp.. Work is on-going.

The higher than expected numbers of EPF isolates placed pressure on the team in terms of the biological and molecular identification and characterisation, and in some instances this has delayed the production of peer-reviewed papers. However, the extension of the project time-frame by the Darwin Initiative enables these papers to be prepared and submitted in 2009. Both CABI and INIA have provided extra resources to enable all isolates to be analysed. Molecular characterisation of EPN proved difficult as there were a number of recalcitrant isolates and these had to be sampled on a number of occasions. Following extensive morphological classification the sheer number of EPF made genetic analysis problematic.

There have been many scientific presentations and posters at conferences, including an afternoon dedicated to Darwin activities at the 2nd Chilean Symposium of Biological Control, 12th – 15th May, 2009, Chillán, Chile (Table 4, Figure 2 and Annex (4)), together with numerous media articles (newspaper, radio and TV), a photo-diary on www.youtube.com (*Following in Darwin's footsteps: unearthing Chile's hidden world*; http://www.youtube.com/watch?v=-3viXZSvd84) and a 16-minute film on the project (*Conserving and utilising entomopathogenic fungi and nematodes in Chile*), in both Spanish and English, which was premiered and distributed at the Symposium (a copy of which accompanies this report).

In addition to a large number of isolates being found, there has been significant training of students and many public events to publicise the Darwin initiative (see Annex 4 Project standard measures).

There were some difficulties related to the vagaries of field work. One survey had to be transferred, at 24 hours notice, from the north to the south due to an earthquake; one survey site was destroyed by volcanic activity a few weeks after our visit (the isolates obtained from this site provide one example of obtaining unique isolates before devastating habitat loss); other surveys had to be modified due to impassable routes, there were weather problems and transport difficulties. These difficulties were dealt with as they arose.

### 4.4. Project standard measures and publications

**Training measures**

1a: One scientist is to submit a PhD thesis from the University of Reading on the taxonomy and bionomics of nematodes obtained from the Darwin project in Chile. Submission is scheduled for 2010.

4a & b: Eight undergraduate students from four universities in Chile received individual training in aspects of EPF and EPN biology from Darwin project staff (from both Chile and UK), prior to undertaking undergraduate thesis on isolates from the Darwin collection. In addition, project staff also gave classes in agronomy and agroecology, based around the use of EPF and EPN, to undergraduate students at three universities in Chile (see Table 3). In total, lectures and laboratory training of undergraduates in host country amounted to approximately ten weeks.

6a & b: Two scientists from Chile visited CABI-UK for two weeks for training on aspects of EPF and EPN, including identification, preservation, intellectual property and access and benefit sharing.

**Research measures**

8: UK scientists spent a total of 32 weeks in the host country.

11a: Three peer-reviewed papers have been accepted and are in print. Details are provided in the Annex (5) and briefly on the CD. One highlight is the identification of a new nematode species (*Steinernema* n.sp.), which is in-press in the journal *Nematology* (the actual name cannot be printed prior to publication). Description of a second species has recently been submitted and a third description is being prepared and will be submitted before the end of 2009.

11b: Six review papers have been published: Full details are provided in the Annex (5).

12a: Two databases were established, one of the EPF isolates and the other of EPN isolates, collected during the project, both collections were handed over to the host country. The

databases included full details of isolate identification, molecular characterisation, ecological parameters of location sites, complemented by photos and drawings of a number of key species (including the new species of EPN).

13a: Two species reference collections were established and handed over to the host country. These collections, of EPF and EPN, included all of the live organisms (600+) collected during the project, together with permanent slide preparations of a number of key isolates (including the new species of EPN).

**Dissemination measures**

14a: Conferences/workshops/seminars organised specifically by project staff to disseminate project findings are summarised in Table 3. Workshops and presentations were given to a range of audiences throughout the project life-span; project staff were particularly pleased with the success of the classes given to school children and have been asked by school heads to continue these if possible.

**14b:** Conferences/seminars/ workshops at which project findings were disseminated are summarised below in Table 4. The list is extensive which reflects the exceptional dissemination effort made by project staff and students. The outputs included both oral and poster presentations at meetings within and outside the host country (copies of the poster presentations are provided in the annex and are on the CD that accompanies this report). The project culminated in a half-day session at the 2nd Chilean Symposium on Biological Control, 12th – 15th May, 2009, Chillán, Chile, dedicated to outputs from the projects (Figure 2). During the session, project scientists from Chile and UK gave a number of presentations and a film on the project was premiered. The Darwin Initiative project was one of two projects highlighted for CABI's Executive Council meeting in December 2008 when the project was explained to representatives of CABI's 44 member countries.

15a & b: There were five local and national press releases in the host country (for examples see Figure 3), all of which are available as pdf copies on request from Loreto Merino at INIA (lmerino@inia.cl) and are included on the accompanying CD. The circulation ranged from 10000 locally to 300000 nationally, however the articles are available to a much wider audience via the respective newspaper web-sites.

On-line project information can be found on:

http://www.controlbiologicochile.cl/content/view/32/130/

http://www.inia.cl/link.cgi/Noticias/5546


Figure 2. A session on the Darwin activities was part of the 2nd Chilean Symposium of Biological Control, 12th - 15th May 2009, in Chillán, Chile


Figure 3. Examples of host-country press releases on project activities

15d: Information on the project is available on the CABI website www.cabi.org

16a & b: There have been six newsletters on the project produced within the host country. The newsletters are distributed regionally to growers, extension workers, scientists and agronomists. The circulation is approximately 1000.

18a: In 2009 project scientists Andrés France and Loreto Merino appeared on *Tierra Ciencia,* a topical science program on the national Chilean TV channel TVN, in which they discussed the project and the Darwin Initiative.

19d: In 2009 Andrés France and Loreto Merino appeared on *Segunda Opinions*, a 30-minute question-answer show on the regional Chilean radio station Radio Alborada, to discuss and answer questions on the Darwin project.

21: The host country built a new laboratory for the Darwin project. The laboratory was for the use of project scientists and students working with project isolates. The Darwin Initiative logo appears above the laboratory and the facility will remain the *Darwin Laboratory* beyond completion of the project time-frame (see Fig 4a).

**4.5 Technical and Scientific achievements and co-operation**

One significant scientific achievement is the discovery and full description of a new species of EPN from Chile (see Annex (4) for front page). The description was recently accepted in the peer-reviewed journal *Nematology* and will be the first published record of an indigenous EPN of Chile. Descriptions for another two (possibly 3) new EPN species are in various stages of preparation; one is presently being peer-reviewed.

Project scientists from Chile have been actively encouraged to publish project findings in peer-reviewed International journals, something that they were previously unaccustomed to doing. Three peer-reviewed papers have been accepted and a number are in-preparation for both regional and international journals (see Annex (5)). As a result, the profile of project scientists in Chile will be increased, both nationally and internationally.

Table 3 clearly illustrates the considerable time spent within local communities promoting project activities and discussing means of uptake of project outputs. Project scientists in the host country have also signed agreements with two biocontrol production companies, one local to INIA in Chillán (NaturalChile) and one in Santiago (Biogram), and are working closely with these companies to develop biological control products based on project isolates. INIA and CABI are developing an agreement to reflect benefit sharing.

**4.4 4.6 Capacity building**

The project has both provided and stimulated capacity building within the host country. The project directly trained scientists from INIA in aspects of culture conservation and curation, and

supplied capital equipment so a Genetic Resource Collection for project isolates could be set up in Chile (see Figure 4a). The INIA scientists also received training from Dr David Hunt on nematode taxonomy.

There was extensive training in EPF and EPN, largely conducted in Chile, of undergraduate and postgraduate students. This included training *in-situ* at the INIA laboratories and classes given at a number of universities, including two of the principle universities in Santiago, Universidad de Catolica and Universidad de Chile (see Table 2). Training ranged from taxonomy of EPF and EPN, to use of isolates in field situations.

The project provided leverage for additional in-country support for biological control in Chile, most significantly from the Government of Chile, through INIA. This financial support included commissioning a Darwin laboratory and, at least partly catalysed by the project, building a Biological Control facility (including laboratories, production facilities and office space) to exploit the findings of the project (see Figure 4b).

4a


4b

Figures 4a and b. a) Cryogenic storage facilities in the *Darwin Laboratory* at INIA, bought through the project and b) the new Centre for Biological Control established at INIA, Chile in 2009.

The scientific collaboration between CABI and INIA enhanced the capacity of all involved, with CABI benefiting greatly from the project, with exposure to excellent scientists in Chile and an environment where biological control (and agriculture generally) is considered of importance. The collaborating scientists have gained practical experience and Steve Edgington has developed extensive EPN expertise, such that he is now becoming recognised as an international authority. CABI has also used the project to develop expertise in molecular

characterisation of EPN (at own cost). The collaborating CABI scientists have gained extensive experience of agriculture in Chile and of the requirements of a Darwin Initiative project. Consequently, CABI has improved its capacity as an effective project partner.

### 4.7 Sustainability and Legacy

The genetic resource collections of EPF and EPN in INIA, significant scientific capacity, scientific outputs and specialised teams working in the field of EPF and EPN for biological control will persist in Chile. INIA is carrying out research and development of biological control for the foreseeable future. INIA project staff are already working on a number of new biological control projects funded by the Government of Chile, using Darwin isolates. The *Darwin Laboratory* will remain as such and will be incorporated into the new biological control facility at INIA.

The partners will remain in touch; to date there have been five proposals for funding to facilitate further collaboration and more will be produced as the outputs of the collaboration have been both excellent and significant. Steve Edgington and Loreto Merino are working closely together to publish results from project activities, and will continue to do so over the next 12 months. CABI staff are working with Chilean students to help translate papers into English, for submission to international peer-reviewed journals and Dave Moore is now an editor for *The Chilean Journal of Agriculture.*

## 5 Lessons learned, dissemination and communication

Key lessons.

Scientifically, a key lesson was the extent of the biodiversity found of these critical indicator species; despite the pivotal role of EPF and EPN in soil and plant health, these organisms have been largely neglected in biodiversity research.

Collaborative relationships are key to achieving a successful project. Part of the strength of the collaboration was that CABI and INIA had been communicating over a number of years prior to the project, developing strong personal ties which have manifested into an excellent working relationship. Both parties have been using PRINCE2 project management guidelines throughout the project, creating a clear and structured system of evaluation, reporting and decision-making. In addition, the clear links being drawn between biodiversity and improving livelihoods in this particular project ensured that there was significant additional support for the project from the Chilean Government and from private industry.

Dissemination. This has ranged from peer-review papers through to reports, posters and conference presentations aimed at scientists. A photo diary was placed on www.youtube.com, articles and newsletters have been written for interested professionals in related areas and in newspapers for the public. There have also been presentations at events such as farmers' shows and for school children. A film of the project has also been produced. The means of dissemination are described in greater detail in Section 4 and the Annex (4). Dissemination of the project outputs will continue after the project, as many of the scientific papers to be peer-reviewed need to be completed and at that point a review paper on the project is planned.

### 5.1 Darwin identity

Darwin Initiative awareness. The name Darwin is widely mentioned/noted in Chile, more so than in the UK (in Chile there are hotels, guest houses, restaurants etc named after Charles Darwin). However, although there have been a number of Darwin Initiative projects in Chile, awareness amongst the wider public of the Initiative is still limited. Agricultural and environmental scientists, civil servants and politicians do have an appreciation of the Darwin Initiative, which has been greatly enhanced by the project.

The Darwin logo has been used on as many dissemination outputs as possible. This has included all presentations, posters, a project film, and the www.youtube.com photo diary, promotional and accompanying literature for the 2nd Chilean Biological Control Symposium, on all newsletters and at every workshop/seminar organised by project scientists including a regional agricultural fair. The Darwin logo appears above the new Darwin Laboratory in the host country and on the vehicle used for the surveys (Figure 5). The CD attached with the hard copy carries dissemination outputs clearly displaying the Darwin Logo

Figure 5. INIA provided a vehicle for surveys and other experimental procedures for the Darwin Initiative project.

# 6 Monitoring and evaluation

There were no major changes to the project design (other than a far higher number of EPF isolates found, resulting in a greater amount of work, which, in turn delayed final dissemination outputs). The logframe, with their original indicators and PRINCE2 planning, monitoring and evaluation proved useful management tools. Actions taken in response to annual report reviews clarified the sampling procedures and emphasised the internal planning systems between collaborators.

6.1

The Annual Report Review process was excellent; we had a thoughtful and supportive referee(s) who made relatively few, but highly pertinent, comments which caused us to clarify a few points to ourselves. We responded to observations made on the sampling protocol and gained encouragement from positive comments. A slightly wry comment was made on health and safety, reflecting earthquakes and volcanic eruptions; we continued with surveys even when rapid changes were needed as, for example, when an earthquake in the north caused us to change to sampling in the south.

We believe that we addressed the issues raised and would like to acknowledge the referee with thanks. The annual reports were written jointly between all collaborating scientists and the reviews fully discussed.

# 7 Finance and administration

## 7.1 Project expenditure

**Darwin Chile PS03086**

**Project Expenditure Statement July 2009**

| | Original Budget | Expenditure Claimed | Explanation for Variance +/- 10% |
|---|---|---|---|
| **Staff Costs** | | | |
| **Rent Rates Overheads** | | | |
| **Postage Stationery** | | | INIA covered many costs<br>More electronic dissemination |
| **Travel & Subsistence** | | | |
| **Conferences/Seminars** | | | INIA covered costs plus gained sponsorship |
| **Printing** | | | Collaborators covered costs |
| **Capital** | | | |
| **Consumables/Other** | | | |
| **Total** | | | |

## 7.2 Additional funds or in-kind contributions secured

INIA made available considerable scientific and technical support as necessary, in addition to infrastructure. A small laboratory was renovated, equipped and maintained for Darwin Initiative work and the INIA insectary provided pest insects as necessary for experimentation. A vehicle was provided for the more local surveys (within 1000 km) and INIA supported the activities of a number of undergraduate research projects. Support was given to the project scientists to attend national and international conferences. Molecular characterisation of the EPF required additional support from INIA. Matching resources supplied in Chile are estimated conservatively at £100K.

CABI carried out molecular identification of 101 EPN isolates (commercial value £15000) and have contributed Steve Edgington's and David Hunt's time respectively to the project. These can be added to the sacrifice of 120% overheads.

## 7.3 Value of DI funding

The project would not have happened without the Darwin Initiative funding. In turn it would not have been possible to leverage the additional resources for either INIA or CABI, as described above.

# 1. Report of progress and achievements against final project logframe for the life of the project

| Project summary | Measurable Indicators | Progress and Achievements April 2006 - March 2009 | 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<br>• The conservation of biological diversity,<br>• The sustainable use of its components, and<br>• The fair and equitable sharing of the benefits arising out of the utilisation of genetic resources | | (Established a culture collection to be used for developing biological pesticides. Described new species and biological diversity of many isolates. Trained young scientists and establishing a sustainable use and equitable benefits agreement. Encouraged the establishment of a Biological Control Centre). | (do not fill not applicable) |
| **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<br>Protocols on conservation and IPR/ABS drafted by Yr 1 and finalised Yr 3 | Outputs were achieved as per original planning, or exceeded. Two important assumptions, those of continued government support and maintaining personnel, were borne out. | |
| **Output 1**.<br>1. Isolates of EPF and EPN obtained from Chile by collaborators. | 1. At least 300 isolates of EPF and 100 of EPN from Chile, distributed across all ecosystems. | 520 isolates of EPF and 101 of EPN were obtained | |
| Activity 1.1 Surveys in various regions of Chile | | All planned surveys completed plus an additional small survey to Chiloe to obtain the final EPN required. Sampling process resulted in great diversity of isolates | |

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|---|---|---|
| Activity 1.2, Isolation through wax moth baiting | | Wax moth baiting was effective in terms of numbers obtained. Concerns must remain as to whether this method is optimal for diversity of species found |
| **Output 2**. 2. Biological and molecular studies of isolates achieved. | Biological profiles established, e.g. temperature, RH and UV tolerance. indicators)<br>Molecular and biochemical data generated for EPF and EPN isolates. | Indicators should have limited the number of isolates to be examined. It was possible to carry out molecular identification and characterisation (due to extra investment by CABI and INIA), but full biological characterisation of all isolates proved impossible. |
| **Activity 2.1.** | | Molecular identification of all isolates of *Metarhizium* and *Beauveria* and all EPN isolates has been completed.<br>(E-mail afrance@inia.cl for access to EPF databases and A.Buddie@cabi.org for EPN databases) |
| **Activity 2.2.** | | Biological characterisation for a limited number of isolates has been carried out. |
| **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. | Original indicators satisfactory |
| **Activity 3.1.** | Culture collection maintenance and IPR and ABS training was given. | Training successful |
| **Activity 3.2.** | EPF and EPN characterisation training | Training given largely to students and more junior staff at INIA |
| **Output 4** Culture collection of EPF and EPN established. | Presence of viable EPF and EPN collections in Chile. | The culture collection has been established and is being maintained. |
| **Activity 4.1** | Capital equipment purchased in year 1. | Original indicators met |
| **Activity 4.2** | Isolates prepared and put into isolate bank | Activities satisfactorily carried out |
| **Output 5.** Simple isolate collection, curation and characterisation | Protocols published. | In hand, but delayed because of the high number of isolates obtained |

| | | |
|---|---|---|
| protocols developed | | |
| **Activity 5.1.** | Experimental techniques developed | As per laboratory records. |
| **Activity 5.2. etc** | Protocols published. | These will be published via all the major peer reviewed papers that are currently being prepared/have already been submitted. |
| **Output 6** Information dissemination and conservation plans | Scientific papers for international journals by Yr 3. Extension literature Yr 1-3. Report from Comite de Biodiversidad by Yr 3.<br>Project data CD Yr 3.<br>Radio & TV as appropriate | Satisfactory indicators, largely met extensively. Peer reviewed papers delayed as explained previously, but expected all submitted by end 2009<br>These will include all protocols and data. |
| **Activity 6.1.** Dissemination outputs prepared and disseminated | | CDs attached to the hard copy submitted give all written outputs. Delays to some peer-reviewed papers. |

# 2. Project's final logframe, including criteria and indicators

| Project summary | Measurable Indicators | Means of verification | Important Assumptions |
|---|---|---|---|
| **Goal:**<br>**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**<br>• **the conservation of biological diversity,**<br>• **the sustainable use of its components, and**<br>• **the fair and equitable sharing of benefits arising out of the utilisation of genetic resources** | | | |
| **Purpose**<br>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<br>Databased by Yr 3<br>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**<br>1. Isolates of EPF and EPN obtained from Chile by collaborators. | 1. At least 300 isolates of EPF and 100 of EPN (in the event 520 and 101 isolates respectively) from Chile, distributed across all ecosystems. | 1. Field survey reports, species inventories, scientific publications. | **For all:** trained staff will remain in Institute and Universities and have positions to use skills acquired. |
| 2. Biological and molecular studies of isolates achieved. | 2. Biological profiles established, e.g. temperature, RH and UV tolerance. Molecular and biochemical data generated for EPF and EPN isolates. | 2. Study reports, scientific publications. | |
| 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 | 5. Protocols published. | 5. Copies sent to Darwin. | |

| | | | |
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| protocols developed.<br><br>6. Information dissemination and conservation plans. | <br><br>6. Scientific papers for international journals by Yr 3. Extension literature Yr 1-3.<br>Report from Comite de Biodiversidad by Yr 3.<br>Project data CD Yr 3.<br>Radio & TV as appropriate | <br><br>6. Copies of all sent to Darwin. | <br><br>6. Information reaches stakeholders and is put to positive use. |

## 3. Project contribution to Articles under the CBD

**Project Contribution to Articles under the Convention on Biological Diversity**

| Article No./Title | Project % | Article Description |
|---|---|---|
| 7. Identification and Monitoring | 35 | Identification of EPF and EPN isolates. Relate to ecosystems. |
| 12. Research and Training | 35 | Biological and molecular identification and characterisation; extensive training of students and dissemination of research findings |
| 13. Public Education and Awareness | 20 | Extensive dissemination to public |
| Other Contribution | 10 | Activities with biocontrol companies for sustainable use to benefit livelihoods. Conservation via collections established. |
| Total % | 100% | Check % = total 100 |

## 4. Standard Measures

| Code | Description | Totals (plus additional detail as required) |
|---|---|---|
| **Training Measures** | | |
| 1a | Number of people to submit PhD thesis | 1 (*EPN of Chile*; UK-based) |
| 1b | Number of PhD qualifications obtained | |
| 2 | Number of Masters qualifications obtained | |
| 3 | Number of other qualifications obtained | |
| 4a | Number of undergraduate students receiving training | approximately 70 (all Chile-based), including student lectures |
| 4b | Number of training weeks provided to undergraduate students | 10 (at INIA and at universities) |
| 4c | Number of postgraduate students receiving training (not 1-3 above) | |
| 4d | Number of training weeks for postgraduate students | 10 |
| 5 | Number of people receiving other forms of long-term (>1yr) training not leading to formal qualification( ie not categories 1-4 above) | |
| 6a | Number of people receiving other forms of short-term education/training (ie not categories 1-5 above) | 2 (INIA staff in the UK) |
| 6b | Number of training weeks not leading to formal qualification | 4 (UK-based) |
| 7 | Number of types of training materials produced for use by host country(s) | |
| **Research Measures** | | |
| 8 | Number of weeks spent by UK project staff on project work in host country(s) | 32 |
| 9 | Number of species/habitat management plans (or action plans) produced for Governments, public authorities or other implementing agencies in the host country (s) | |
| 10 | Number of formal documents produced to assist work related to species identification, classification and recording. | |
| 11a | Number of papers published or accepted for publication in peer reviewed journals | 3<br>(7 in preparation) |
| 11b | Number of papers published or accepted for publication elsewhere | 6 review articles |
| 12a | Number of computer-based databases established (containing species/generic information) and handed over to host country | 2: EPF and EPN. |
| 12b | Number of computer-based databases enhanced (containing species/genetic | |

| Code | Description | Totals (plus additional detail as required) |
|---|---|---|
| | information) and handed over to host country | |
| 13a | Number of species reference collections established and handed over to host country(s) | 2: EPF & EPN collections at INIA. |
| 13b | Number of species reference collections enhanced and handed over to host country(s) | |
| **Dissemination Measures** | | |
| 14a | Number of conferences/seminars/workshops organised to present/disseminate findings from Darwin project work | 17 (see Table 3) |
| 14b | Number of conferences/seminars/ workshops attended at which findings from Darwin project work will be presented/ disseminated. | 27 (see Table 4) |
| 15a | Number of national press releases or publicity articles in host country(s) | 2 |
| 15b | Number of local press releases or publicity articles in host country(s) | 3 |
| 15c | Number of national press releases or publicity articles in UK | |
| 15d | Number of local press releases or publicity articles in UK | |
| 16a | Number of issues of newsletters produced in the host country(s) | 6 |
| 16b | Estimated circulation of each newsletter in the host country(s) | 1000 |
| 16c | Estimated circulation of each newsletter in the UK | |
| 17a | Number of dissemination networks established | |
| 17b | Number of dissemination networks enhanced or extended | |
| 18a | Number of national TV programmes/features in host country(s) | 1 |
| 18b | Number of national TV programme/features in the UK | |
| 18c | Number of local TV programme/features in host country | |
| 18d | Number of local TV programme features in the UK | |
| 19a | Number of national radio interviews/features in host country(s) | |
| 19b | Number of national radio interviews/features in the UK | |
| 19c | Number of local radio interviews/features in host country (s) | 1 |
| 19d | Number of local radio interviews/features in the | |

| Code | Description | Totals (plus additional detail as required) |
|---|---|---|
| | UK | |
| **Physical Measures** | | |
| 20 | Estimated value (£s) of physical assets handed over to host country(s) | Culture collection preservation equipment from year 1. £30k |
| 21 | Number of permanent educational/training/research facilities or organisation established | 1: The Biological Control Centre was stimulated by the Darwin Initiative project and has liaised closely throughout. |
| 22 | Number of permanent field plots established | |
| 23 | Value of additional resources raised for project | See above – laboratory, vehicle, staff, molecular characterisation, dissemination etc. Minimum value £100K |
| **Other Measures used by the project and not currently including in DI standard measures** | | |
| 24 | http://www.youtube.com/watch?v=-3viXZSvd84&feature=channel_page | 1: www.youtube.com photo diary of the project. |
| 25 | http://www.inia.cl/link.cgi/Noticias/5546 | On line information on Darwin Initiative session at Biocontrol Symposium |
| 26 | http://www.controlbiologicochile.cl/content/view/ | On line information on Darwin Initiative. |
| 27 | "Conserving and using EPF and EPN within Chile" | 1: 16-min project movie in English and Spanish |

Table 2. Undergraduate projects in host country, involving EPF and EPN isolates collected during the Darwin project. An example of a student abstract is printed after the table and full details are on the CD that accompanies this report.

| Project title | Student and affiliation | Activity |
|---|---|---|
| Biological control of black moth *Dalaca pallens* (Lepidoptera; Hepialidae) with entomopathogenic nematodes | Alexis Maldonado<br>Universidad de Concepción | Submitted to University |
| Screening of native entomopathogenic nematode isolates for control of *Aegorhinus supercilliosus* (Guerin) (Coleoptera:Curculionidae) | Ingrid Rozas<br>Universidad de Concepción | Writing-up |
| Pathogenicity of native entomopathogenic nematode isolates for control of codling moth *Cydia pomonella* (l.) (Lepidoptera: Tortricidae) | Manuel Contreras<br>Universidad de Concepción | Writing-up |
| Screening of native entomopathogenic fungal isolates for control of *Hylurgus ligniperda* | Karen Parra<br>Universidad ARSIS | Writing-up |
| Evaluation of entomopathogenic fungi (*Metarhizium anisopliae and Beauveria bassiana*) collected from the south of Chile, for control of Black vine weevil *Otiorhynchus* | Claudia Hinostroza<br>Universidad de Concepción | Submitted to University |

| | | |
|---|---|---|
| *sulcatus* Fab. (Coleoptera: Curculionidae | | |
| The effect of UV radiation on the germination of native entomopathogenic fungal isolates from northern Chile. | Dayna Grimberg<br>Universidad Austral de Chile | Writing-up |
| Evaluation of EPN isolates for control of *Cydia pomonella* (l.) (Lepidoptera: Tortricidae) on apples. | Daniel San Martín<br>Universidad Adventista de Chile | Field-work |
| Biological control of *Cydia pomonella* (L.) (Lepidoptera: Tortricidae) with indigenous entomopathogenic fungi in Chile | Daniela Machuca<br>Universidad de Concepción | Laboratory-work |

Example of abstract for undergraduate thesis on Darwin project isolates. More abstracts are on CD accompanying this report

**SELECCIÓN DE AISLAMIENTOS NATIVOS DE NEMÁTODOS ENTOMOPATÓGENOS PARA EL CONTROL DE LA CUNCUNILLA NEGRA DE LAS PRADERAS *Dalaca pallens***

**(SELECTION OF NATIVE ENTOMOPATHOGENIC NEMATODES FOR THE CONTROL OF BLACKMOTH (*DALACA PALLENS*) IN PASTURES)**

**[1]Alexis Maldonado**, [1]Pedro Casals, [2]Loreto Merino y [2]Andrés France
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[2]INIA Quilamapu. Chillán. Chile.

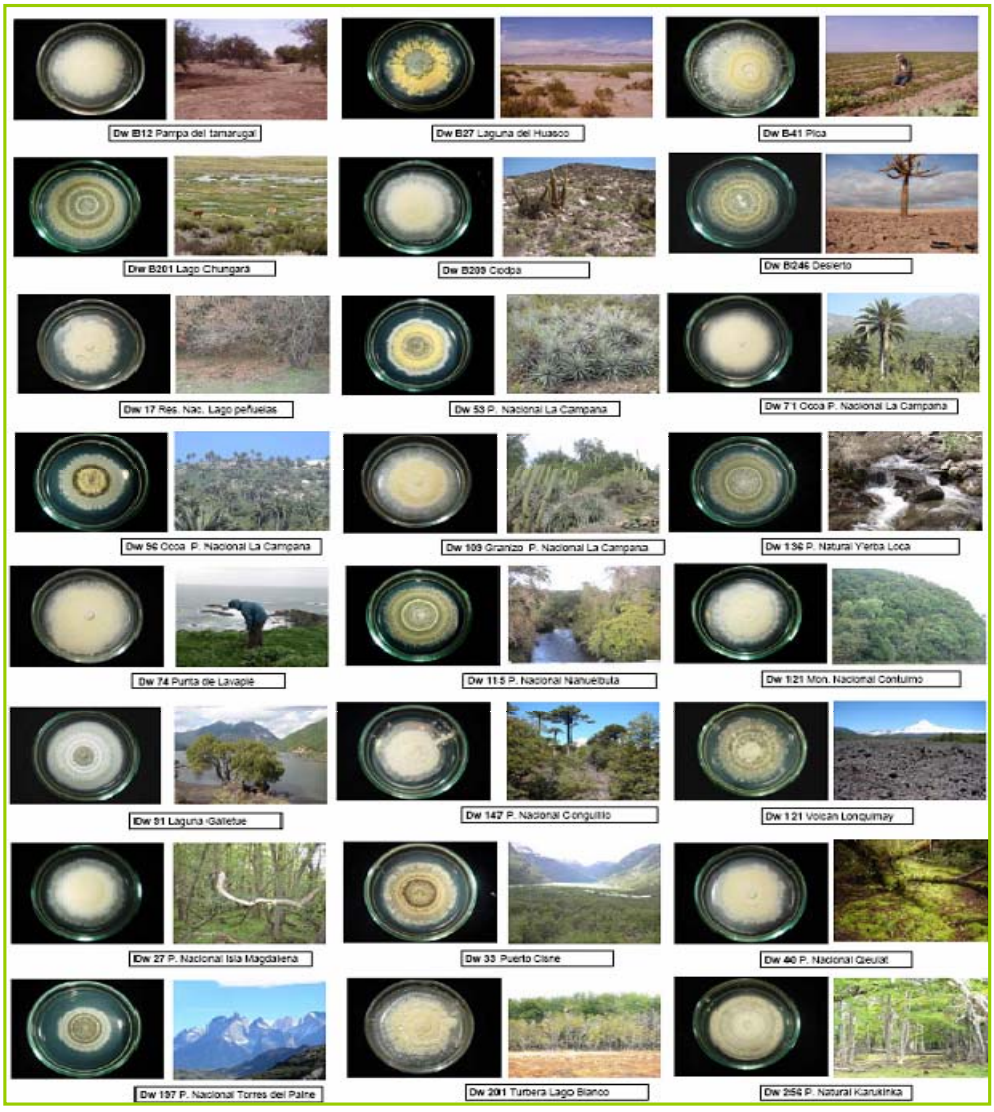
La cuncunilla negra de las praderas *Dalaca pallens* es una de las plagas de mayor incidencia en las praderas del sur de Chile. El principal control de *D. pallens* son los insecticidas químicos. El Programa de Patología de Insectos de INIA Quilamapu ha prospectado nemátodos entomopatógenos a lo largo de Chile y en este trabajo se evaluó 20 aislamientos de nemátodos para el control de la concunilla negra. La unidad experimental consistió de cinco larvas de 0,139 g de peso promedio, inoculadas con 50 nemátodos dauers $mL^{-1}$ por larva. El diseño experimental fue completamente al azar, con cuatro repeticiones por tratamiento. Diecinueve aislamientos mostraron diferencias ($P \leq 0,05$) con el testigo, pero sólo los dos aislamientos que causaron la mayor mortalidad fueron seleccionados: el aislamiento DW N3 alcanzó el 100% de mortalidad, similar ($P > 0,05$) al aislamiento DW N13 (95% de mortalidad). Ambos aislamientos pertenecen al género *Steinernema*. Para el cálculo de la concentración letal ($CL_{50}$ y $CL_{90}$) se evaluaron concentraciones de 0 (testigo), 10, 20, 30, 40 y 50 dauers $mL^{-1}$ aplicadas sobre el sustrato en un frasco cónico, colocando una larva por frasco. El diseño experimental fue completamente al azar. La evaluación de mortalidad se realizó el día 13 después de la inoculación, correspondiente al día en que el primer aislamiento produjo un 100% de mortalidad de larvas con la concentración de 50 dauers $mL^{-1}$. Las $CL_{50}$ y $CL_{90}$ fueron de 15 y 41 dauers $mL^{-1}$ respectivamente para el aislamiento DW N3. Además se estudió la estrategia de forrajeo de los aislamientos seleccionados, sobre una placa Petri que contenía agar y arena y se aplicó una concentración de 200 dauers aproximadamente. Ambos aislamientos presentaron una doble estrategia de forrajeo (*ambusher* y *cruiser*). Se pudo inferir que ambos aislamientos poseen atributos para ser considerados como una alternativa de control biológico de esta plaga.

Co-financiamiento: Darwin Initiative (Department of Environment, Food and Rural Affairs, UK).

Examples of poster presentations given by students and project staff regarding the Darwin project. Complete copies of posters and presentations are on the CD that accompanies this report

Author: Loreto Merino, INIA.

# Conserving entomopathogenic fungi in Chile



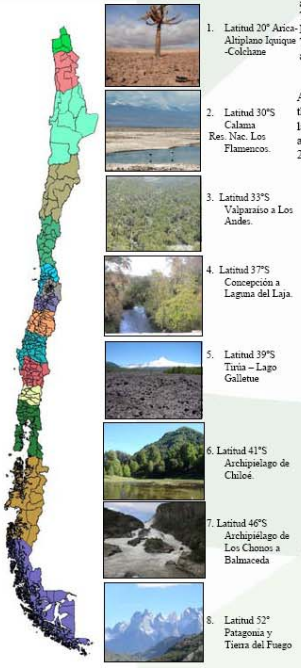
# Surveys of indigenous entomopathogenic fungi and nematodes of Chile and studies on their pathogenicity towards pests of economic importance

Steve Edgington[1], Dave Moore[1], Loreto Merino[2], Andrés France[2]
[1]CABI, UK.
[2]INIA, Chile.

**ABSTRACT**
This 3 year project is funded by the Darwin Initiative, with the aim of creating a national database of entomopathogenic fungi (epf) and nematodes (epn) found within Chile and to build on the expertise required to curate and profile them. The long-term objective is to develop biological control agents based on these microorganisms and to highlight the benefits of conserving microbial diversity to local growers. The project is a collaboration between CABI (Europe - UK) and the Instituto de Investigaciones Agropecuarias (INIA) in Chile.

## SURVEY SITES

Eight sites have been selected in Chile (Figure 1), each of which will be surveyed for epf and epn.




The sites, stretching from the Andes to the Pacific coast, cover some of the major topography, vegetation, soil types and climates present in Chile, from hot, arid desert in the north to near Antarctic conditions in the south. Two surveys were carried out in Year 1, in the north, centre and south of the country.

**Figure 2**. Waxmoth larvae infected with entomopathogenic fungi (EPF) and Entompatogenic nematodes (EPN).

## SAMPLING

Approximately 1400 soil samples were taken in each survey site, collecting from a variety of ecosystems including agricultural land, coastal platforms, salt lakes and the Tamarugal Pampa. Samples were also taken on Isla Magdalena, a national park 2 km off the west coast of Chile. The altitude of sampling points ranged from 0 to 4800 m above sea level.

At each soil sample site the pH, temperature and humidity of the soil was taken, then the samples returned to the INIA laboratories. Processing for epf and epn used waxmoth larvae as bait and was carried out twice for 4 days at 20 °C (Figure 2).

**Figure 3**. Isolates of entomopathogenic nematode.

## ISOLATES

Processing of samples has presently revealed 101 epn isolates (*Steinernema* and *Heterorhabditis* spp.) and 528 epf (Figure 3). Isolates are in process of molecular identification, cryopreservation and will then be biologically and ecologically profiled to identify links between habitat and isolate.

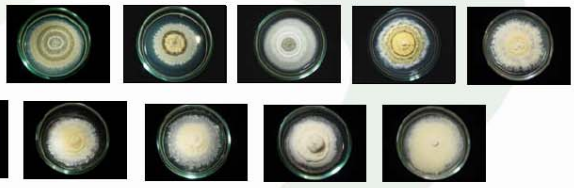


**Figure 3**. Isolates of entomopathogenic fungi.

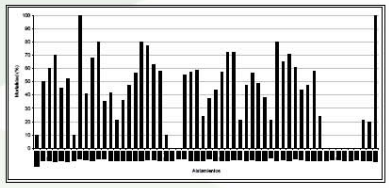


**Figure 4.** Example of NEP screening on important target pest show the variability of different isolates, like the parasitism in *Cydya pomonella*.

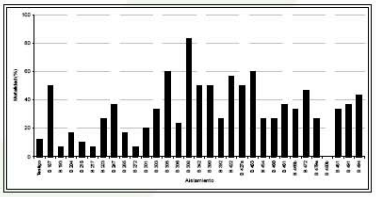


**Figure 5.** Example of EPF screening on important target pest show the variability of different isolates, like the parasitism in *Xanthogaleruca luteola*.

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Centro Tecnológico de Control Biológico

Table 3. Conferences/workshops/seminars organised to disseminate project findings in host country

| **Class/talk and location** | **Participants** | **Date** |
|---|---|---|
| School visit (Santa Juana): 'Initiativa Darwin' | Infants ( < 10 years) | 28/09/2006 |
| Program EXPLORA 2006 CONICYT | Infants ( < 12 years) | 30/10/2006 |
| Presentation to local farmers fro bún community: 'Iniciativa Darwin: Uso de nematodos entomopatógenos en agricultura.' | Small-holders | 08/11/2006 |
| Meeting of organic producers | Small-holders | 10/01/2007 |
| Course in Agronomy, Universidad de Chile | Undergraduates | 23/01/2007 |
| Presentation to berry producers of the Bio-Bio region: 'Iniciativa Darwin: Uso de Nemátodos entomopatógenos en agricultura.' | Producers | 06/07/2007 |
| Course in Agroecology, Instituto Santo Tomás | Undergraduates | 12/07/2007 |
| Program: '1000 scientists 1000 classes' uela Monte Blanco de la comuna de San Carlos | Infants | 09/10/2007 |
| Presentation to local farmers from the Pinto community: 'Iniciativa Darwin: Uso de Nemátodos entomopatógenos en agricultura.' | Small-holders | 07/11/2007 |
| Course in Agronomy, Universidad Santo Tomás, Chile | Undergraduates | 08/11/2007 |
| Darwin, Nematodes and Fungi: basics. Colegio El Carmen | Infants ( < 10 years) | 07/12/2007 |
| Course in Agronomy, Universidad Católica de Chile | Undergraduates | 13/12/2007 |
| Presentation to organic producers of the Bio-Bio region: 'Iniciativa Darwin: Uso de Nemátodos entomopatógenos en agricultura organica.' | Small-holders | 25/09/2008 |
| EXPOINIA 2008 I 27/11/2008 | Stall-Holders | 26/11/2008 |
| Course in Agronomy, Universidad de Catolica, Santiago | Undergraduates | 16/12/2008 |
| Presentation to agronomists at HORTIFRUT | Agronomists | 04/03/2009 |
| School visit (San Vicente de Paul): 'Iniciativa Darwin' | Infants ( < 10 years) | 13/04/2009 |

Table 4. Conferences/seminars/ workshops attended at which project findings were disseminated, together with the titles of the presentations.

| Conference | Location | Title of presentation and principle presenter |
|---|---|---|
| CABI Executive Council, Dec. 2008. (Representatives invited from each of CABI's 44 member countries) | London, UK | The Darwin Initiative in Chile. Moore, D.<br>(The project was one of two chosen to demonstrate CABI's work) |
| National Congress of Entomology. Nov 2007 | Universidad. Metropolitana de Ciencias de la Educación, Santiago Chile | Evaluation of native entomopathogenic fungi for control of *Otiorhynchus sulcatus* Fab. XXIX. Merino, L. |
| National Congress of Entomology. Nov 2007 | Universidad Metropolitana de Ciencias de la Educación, Santiago Chile | Biological control of *Hylurgus ligniperda* (Fabricius) with native entomopathogenic fungi. Merino, L. |
| II International Symposium of Organic Agriculture. March 2008 | Universidad de Las Américas. Santiago de Chile | Conservation and use of native entomopathogenic organisms in Chile. Merino, L. |
| II International Symposium of Organic Agriculture. March 2008 | Universidad de Las Américas. Santiago de Chile | Evaluation of entomopathogenic fungi for control of *Otiorhynchus sulcatus* Fab. Merino, L |
| 59th Congreso Agronomico de Chile. Oct 2008 | Universidad de la Serena, Chile | Darwin Initiative: manejo biologico de avispas sociales. Merino, L. |
| 59th Congreso Agronomico de Chile. Oct 2008 | Universidad de la Serena, Chile | Darwin Initiative: nemátodos entomopatógenos para el control de larvas del cabrito del duraznero *Aegorhinus superciliosus*. Merino, L. |
| 59th Congreso Agronomico de Chile. Oct 2008 | Universidad de la Serena, Chile | Darwin Initiative: colecta de hongos y nemátodos nativos de Chile y estudio de patogenicidad sobre insectos plagas. France, A. |
| 3rd International Symposium on Biological Control of Arthropods. Feb 2009, | Christchurch, New Zealand | Conserving and using entomopathogenic fungi and nematodes within Chile. France A. |
| 3rd International Symposium on Biological Control of Arthropods. Feb 2009, | Christchurch, New Zealand | Pre-lethal effect of entomopathogenic fungi *Beauveria bassiana* (Bals.) Vuill. over European paper wasp *Polistes dominulus.* Merino L. |
| 2nd Simposio Chileno de Control Biológico. 12-15 May 2009. | INIA Chillán; Chile | Hongos entomopatogenos para el control biologico de la vaquita del olmo *Xanthogaleruca luteola* (Müller). Merino, L. |
| 2nd Simposio Chileno de Control Biológico May 2009 | INIA Chillán; Chile | Colecta, conservación y uso de hongos y nematodos nativos de Chile. France, A. |

| | | |
|---|---|---|
| 2nd Simposio Chileno de Control Biológico. May 2009 | INIA Chillán; Chile | Hongos entomopatógenos para el control de la avispa chaqueta amarilla *Vespula germanica.* Merino, L. |
| 2nd Simposio Chileno de Control Biológico. May 2009 | INIA Chillán; Chile | Evaluacion de cepas nativas de hongos entomopatógenos sobre *Otiorhynchus sulcatus* Fab. Claudia Hinostroza. Gerding, M. |
| 2nd Simposio Chileno de Control Biológico. May 2009 | INIA Chillán; Chile | Worms under Darwin's feet: spectacular (and useful?). Edgington, S. |
| 2nd Simposio Chileno de Control Biológico. May 2009 | INIA Chillán; Chile | The Darwin Initiative: Uncovering entomopathogenic fungi along the path of Darwin. Moore, D. |
| 2nd Simposio Chileno de Control Biológico. May 2009 | INIA Chillán; Chile | The Darwin Initiative: using biodiversity for biological control. France, A. |
| 2nd Simposio Chileno de Control Biológico. May 2009 | INIA Chillán; Chile | Diversidad genética y estructura poblacional de hongos entomopatógenos (HEP) colectados en Chile mediante marcadores moleculares. Becerra, V. |
| 2nd Simposio Chileno de Control Biológico. May 2009 | INIA Chillán; Chile | Hongos entomopatógenos para el control biologico del escarabajo rojo de la corteza *Hylurgus ligniperda*. Merino, L. |
| 2nd Simposio Chileno de Control Biológico. May 2009 | INIA Chillán; Chile | Selección de aislamientos nativos de nemátodos entomopatógenos para el control de *Aegorhinus superciliosus*. Rozas, I |
| 2nd Simposio Chileno de Control Biológico. May 2009 | INIA Chillán; Chile | Selección de aislamientos nativos de nemátodos entomopatógenos para el control de la cuncunilla negra de las praderas *Dalaca pallens.* Maldonado, A. |
| 2nd Simposio Chileno de Control Biológico. May 2009 | INIA Chillán; Chile | Selección de aislamientos nativos de nemátodos entomopatógenos para el control de larvas de polilla de la manzana *Cydia pomonella* (L.). Contreras, M. |
| 40th Annual Meeting of the Society for Invertebrate Pathology, August 2007 | Quebec, Canada | To protect and enhance conservation and sustainable use of the entomopathogenic nematode biodiversity of Chile. France, A. |
| 40th Annual Meeting of the Society for Invertebrate Pathology, August 2007 | Quebec, Canada | Survey of indigenous entomopathogenic fungi and nematodes of Chile and studies on their pathogenicity towards pests of economic importance. Merino, L. |
| Meeting of the Association of Applied Biologists: Advances in Nematology, December 2007 | London, UK | Surveys of indigenous entomopathogenic fungi and nematodes of Chile and studies on their pathogenicity towards pests of economic importance. Edgington, S. |
| 1st International Symposium on Nematodes as Environmental Indicators, June 2007. | Edinburgh, Scotland | To protect and enhance conservation and sustainable use of the entomopathogenic nematode biodiversity of Chile. Edgington, S. |

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|---|---|---|
| International Congress of Entomology, July 2008 | Durban, South Africa | The conservation and use of entomopathogenic nematodes from Chile. Edgington, S. |
| Entomological Society of America, Nov 2009 | Reno, USA | Surveys for entomopathogenic nematodes and fungi in Chile. Moore, D. |

## 5. Publications

| Type * | Detail<br>(title, author, year) | Publishers<br>(name, city) | Available from | £ |
|---|---|---|---|---|
| Journal | *Steinernema* n.sp., a new species of nematode from Isla Magdalena, Chile. Edgington *et al.* 2009 | *Nematology*<br>(in press) | *Nematology*<br>S. Edgington | NA |
| Journal | Selección de aislamientos nativos de hongos patogénicos a *Vespula germanica* (Hymenoptera: Vespidae). Merino L., France A. and Gerding M. | *Chilean Journal of Agricultural research.*<br>(in press) | *Chilean Journal of Agricultural research.*<br>L. Merino | NA |
| Journal | Patogenicidad del hongo entomopatógeno *Beauveria bassiana* y su efecto sobre el comportamiento de la avispa social *Polistes dominulus* Christ. (Hymenoptera: Vespidae). Merino L., France A. and Gerding M. | *Chilean Journal of Agricultural Research.*<br>(in press) | *Chilean Journal of Agricultural Research.*<br>L. Merino | NA |
| Journal | The ecological characterization of *Steinernema* n.sp., a new entomopathogenic nematode from, Chile. Edgington, S. & Gowen, S. | Submitted to *Russian Journal of Nematology* | | NA |
| Journal | Presencia y distribucion de los hongos entomotomopatogenos *Beauveria* y *Metarhizium* en ecosistemas de Chile. Merino L., France A., Edgington S. and Moore D. | To be submitted to<br>*Biological Control* 2009 | | NA |
| Journal | *Steinernema* n.sp., a new species of nematode from Tierra del Fuego, Chile. Edgington, S. *et al*. | Submitted to *Journal of Nematode Morphology and Systematics* 2009. | | NA |
| Journal | *Heterorhabditis* n.sp., a new species of nematode from the Atacama Desert, Chile. Edgington, S. *et al*. | In preparation – due to submit to *Nematology* 2009 | | NA |
| Journal | Survey of entomopathogenic nematodes in Chile. Edgington, S., Merino, L. , France, A. & Moore, D. | In preparation – due to submit to *Nematology* 2009 | | NA |
| Journal | Genetic diversity and population structure of entomopathogenic fungi collected in Chile, determining molecular markers. Becerra, V. & France, A. | In preparation – due to submit 2009 | | NA |
| Journal | Characterisation of the symbiotic bacteria from *Steinernema* n.sp., a new EPN from Chile. Edgington, S., Buddie, A., Tymo, L. & Moore, D. | In preparation – due to submit to *Journal of Invertebrate Pathology*2009 | | NA |
| Journal | Novel yeast association with *Heterorhabditis* n.sp. from northern Chile. Buddie, A., Tymo, L., Edgington, S. & Moore, D. | In preparation – due to submit 2009 to *Journal of Invertebrate Pathology.* | | NA |

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|---|---|---|---|---|
| Review articles | In Search of Darwin's Nematodes. Moore, D. and Edgington, S. | *Outlooks on Pest Management* | Edition: Oct 2007, 1-4. | NA |
| Review articles | Conserving and using entomopathogenic fungi and nematodes within Chile. Edgington, S. and France, A. | *Biocontrol News and Information* | 28: 58N-60N 2007 | NA |
| Review articles | Iniciativa Darwin: Uso de hongos entomopatógenos como alternativa para el control biológico de avispas sociales. Merino, L. y France, A | *Informativo Agropecuario Bioleche*. | 20: 8-10, 2007. | NA |
| Review articles | Iniciativa Darwin: Uso de enfermedades de insectos para el control de plagas. Merino, L. France, R. y Gerding, M. | Rev. Tierra Adentro | Nº 78: 44-46 Mar 2008 | NA |
| Review articles | Iniciativa Darwin: Hongos y nemátodos de Chile. L. Merino | Rev. Red Agrícola | Nº 18: 10-11 Jan 2009 | NA |
| Review articles | Nemátodos entomopatógenos Apuesta en el control biologico de plagas. Merino, L. | Informativo CTCB | No. 2: May 2009 | NA |
| CD | Proyecto Conservación y uso de nemátodos y hongos entomopatógenos nativos de Chile. | INIA (produced June 2009) | on request | NA |
| CD | Iniciativa Darwin: Colecta de hongos en Chile | INIA (produced March 2009) | on request | NA |

Publications on project activities. Full scripts of completed materials are available on the CD that accompanies this report and all publications will be available from authors at publication.

## 5. Darwin Contacts

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| **Ref No** | 15/004 |
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