Darwin Initiative: Half Year Report

(due 31 October 2007)

Project Ref. No.	15/004
Project Title	Conserving and using entomopathogenic fungi and nematodes within Chile
Country(ies)	UK, Chile
UK Organisation	CABI, Silwood Park, Ascot, Berks SL5 7TA
Collaborator(s)	Instituto de Investigaciones Agropecuarias (INIA) Avenida Vicente Méndez 515, Chillán, VIII Región, Chile
Project Leader	Dr. Dave Moore
Report date	October, 2007
Report No. (HYR 1/2/3/4)	HYR 2
Project website	

1. Outline progress over the last 6 months (April – September) against the agreed baseline timetable for the project (if your project has started less than 6 months ago, please report on the period since start up).

Surveys

At the start of the project six transects along Chile (see HYR 1) were selected as survey sites for entomopathogenic fungi (EPF) and nematodes (EPN). Transects 1 and 5 were sampled during Yr 1 and Transects 3 and 4 sampled during this present reporting period:

- **Transect 3:** 32 - 33 °S (Valparaiso to Los Vilos). A region traversed on horseback by Charles Darwin during his Beagle expedition in the 19th Century. Includes areas of wetland (with the 'Humedal' RAMSAR site), dry areas of semi-desert further inland and areas of typical Mediterranean vegetation. An extensive canal system supports a booming agricultural sector.
- **Transect 4:** 37 – 40 °S (Concepción to Laguna del Laja). A transition from dryland to wetland, The dominant vegetation type includes Mediterranean scrub, isolated forest areas, with a large number of endemic plant species and large extensions of natural grassland.

Sampling within each transect is systematic in that samples are taken from as many diverse habitats as possible. Within habitats, samples are taken on a relatively selective basis, for example in a very arid area samples would be taken under vegetation. Agricultural land is generally omitted. In June 2007, the first of two surveys in Transect 4 was carried out. One hundred and eight soil samples were collected and processed back at INIA. A second survey in Transect 4 is planned for Nov 2007. In September a total of 200 soil samples were collected from Transect 3 and processed at INIA.

Identification and Profiling

As outlined in HYR 1, soil samples were examined for EPF and EPN, with identities of any *positive* isolates subsequently confirmed. Surveys in Yr 1 revealed 164 and 15 isolates of EPF and EPN respectively. During the first half of Yr 2 these isolates were purified and preserved in collections at INIA. Transect 4 surveys have so far revealed 47 EPF and 9 EPN isolates. Samples from Transect 3 are still being processed, however 9 EPN isolates have already been found.

Biological and molecular profiling of EPF and EPN isolates obtained in Yr 1 has been carried out at both INIA and CABI during HY2. One aim of the profiling is to screen for characteristics useful for biological control. Studies have included temperature and UV tolerance assays, the effect of photoprotective formulations and target-pest assays. Also, beyond the timetable of the project but as an extension to the work, scientists from CABI and the University of Reading have been studying the symbiotic bacteria found in the guts of the EPN. The EPN-invertebrate infection process will often rely on both the EPN and bacteria. The studies have included one undergraduate project on bacteria-phenotyping from Transect 5 EPN. Results on the EPF, EPN and bacteria profiling will be presented in the Yr 2 Annual Report.

As part of the isolate profiling, and particularly with reference to target assays, INIA has a number of undergraduate students presently carrying out projects on the EPF and EPN isolates, including:

- Native entomopathogenic fungi for control of goldenhaired bark beetle *Hylurgus ligniperda* (Coleoptera: Scolytidae). Karen Parra, University Arsis, Chile.
- Evaluation of entomopathogenic fungi for larvae control of *Otiorhynchus sulcatus* Fab. (Coleoptera: Curculionidae). Claudia Inostroza, University of Concepción, Chile.
- Screening of entomopathogenic nematode isolates for control of Peach root weevil *Aegorhinus phaleratus* Ericsson (Coleoptera: Curculionidae). Ingrid Rozales, University of Concepción, Chile.
- Biological control of the grassland black cuncunilla *Dalaca pallens* (Lepidoptera: Hepialidae) with entomopathogenic nematode isolates. Alexis Maldonado, University of Concepción, Chile.

Capacity building

Two Project scientists from INIA visited CABI in Q1 of Yr 2 for training in Intellectual Property, Access and Benefit Sharing, and the identification, preservation and curation of microorganisms.

Dissemination

Scientists from both CABI and INIA have presented project findings at a number of Conferences during Yr 2 including the Annual Meeting of the Society for Invertebrate Pathology (Aug. 12 – 16th, Quebec) (www.sip2007quebec.com) and the 1st International Symposium on Nematodes as Environmental Bioindicators, a meeting of the Association of Applied Biologists (12 - 13th June, Edinburgh) (www.aab.org.uk; http://qs.aqvs.co.uk/aab/images/PROGRAMME_NAEB.pdf). Conference poster see appendix.

Project papers are in preparation and it is expected that at least one will be submitted in the next 6 month period. A short article has been accepted for *Outlooks in Pest Management.*

Project scientists from CABI and INIA met with Ambassador Moreno, the Chilean Ambassador to UK, in London during Q1 or Yr 2. This meeting followed an earlier meeting in Yr 1 in which Ambassador Moreno visited CABI. Project progress and possible sources of additional finance to continue activities beyond the project timeframe were discussed. The Ambassador is giving his full support to this Darwin Initiative.

2. No notable problems or unexpected developments have been encountered.

Have any of these issues been discussed with the Darwin Secretariat and if so, have changes been made to the original agreement?

Discussed with the DI Secretariat: **no/yes, in……. (month/yr)**

Changes to the project schedule/workplan: **no in…….(month/yr)**

3. Are there any other issues you wish to raise relating to the project or to Darwin's management, monitoring, or financial procedures? No

If you were asked to provide a response to this year's annual report review with your next half year report, please attach your response to this document.
Please note: Any planned modifications to your project schedule/workplan or budget should not be discussed in this report but raised with the Darwin Secretariat directly.
Please send your **completed form email** to Eilidh Young, Darwin Initiative M&E Programme at Darwin-Projects@ectf-ed.org.uk . The report should be between 1-2 pages maximum. **Please state your project reference number in the header of your email message eg Subject: 14-075 Darwin Half Year Report**

Appendix

Conference poster

Surveys of indigenous entomopathogenic fungi and nematodos of Chile and studies on their pathogenicity towards pests of economic importance.

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

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

Six 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 and south of the country.

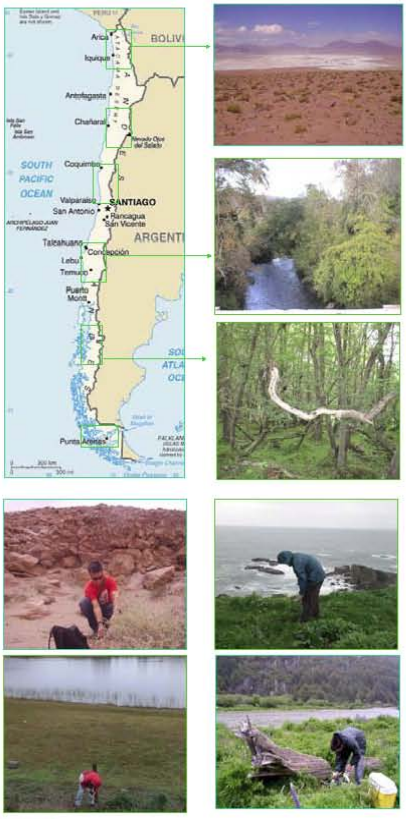

Figure 1 Designated survey sites (Year 1 surveys, arrowed)00

SAMPLING

Approximately 200 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.

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

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). Two more sites will be surveyed in Year 2, with the final two in Year 3.

ISOLATES

Processing of samples has presently revealed 15 epn isolates (*Steinernema* and *Heterorhabditis* spp.) and 140 epf (*Metarhizium* and *Beauveria* spp.) (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.

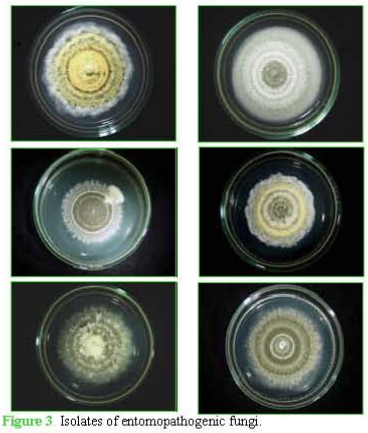

Figure 3 Isolates of entomopathogenic fungi.

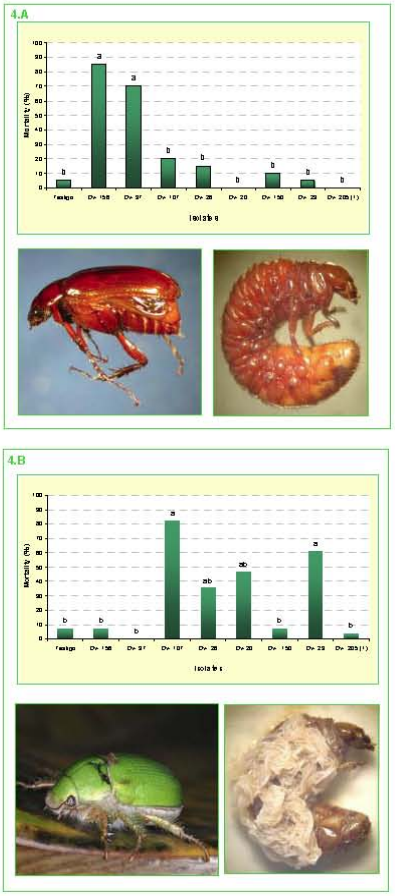

Figure 4. Example of NEP screening on important target pest show the variability of different isolates, like the parasitism in *Sericoides virides* (Figure 4.A) and *Hylamorpha elegans* (Figure 4.B).

INIA will be working in Chile to establish a "use" plan for epf and epn as biological control agents and to publicise both the project and the value of indigenous microorganisms). The new collection, together with the profiling information will be valuable in Chile's search for alternatives to chemical pesticides.

ACKNOWLEDGEMENTS: This project is funded by the Darwin Initiative (www.darwin.gov.uk), a grants programme administered by the UK Department for Environment, Food and Rural Affairs (DEFRA).

Response to comments on the Annual Report of 2007.

The reviewer questioned whether the project had a sampling strategy or whether the sampling was targeted or random. A number of factors need to be considered including:-

- The project aims to obtain isolates of EPF and EPN with characteristics that may be useful for practical biological control using biopesticides. The development of such biopesticides will follow as separate activities.

- It is believed that the different ecosystems sampled will have greater significance to isolate characteristics than potential arthropod sources.

- Extensive surveying in agricultural or forestry areas would result in isolates with reduced diversity of characteristics, reflecting the land management practices.

- Isolates are most likely to be found where there is some vegetation; totally barren areas are unlikely to carry many isolates.

Consequently the surveys were designed to systematically cover different ecosystems. Six broad areas have been selected in Chile, from North to South, covering climatic ranges from hot, dry desert to near Antarctic conditions. Within each of these regions the surveys covered areas incorporating high altitude, down to sea level, broadly East to West. With perhaps 20 or more sites sampled in a day, each sample may be separated by 10-20 Km. Agricultural land is not sampled, although some terrain is pastoral, so occasional grazing by animals can occur. Natural forest is also sampled, however man-managed forested areas would not be unless there were particular reasons, such as areas devastated by periodic outbreaks of a range of insects (these samples are very much the minority). There is also an element of spontaneous sampling from any area that is clearly different, such as an unusual patch of vegetation or an uncharacteristic soil-type.

The referee suggested that the ultimate aim of producing a biopesticide might mitigate against random sampling. We hope that this will be the case and so have adopted a sampling process which we believe will both maximise our knowledge of the biodiversity and result in isolates which will be effective against agricultural pests. Partly this reflects the nature of much of Chile's agriculture which is of a perennial nature, being best protected by persistent, long-term isolates, rather than quick acting, but transient, isolates. However, sampling designed to maximise our knowledge of biodiversity is the primary objective, with the biopesticide component as the secondary objective.