Biodiversity Challenge Funds Projects
Darwin Initiative, Illegal Wildlife Trade Challenge Fund, and Darwin Plus
Half Year Report

Note: If there is any confidential information within the report that you do not wish to be shared on our website, please ensure you clearly highlight this.

Submission Deadline: 31st October 2022

Project reference	DPLUS157
Project title	Managing the pathogens threatening St Helena's biodiversity and food security
Country(ies)/territory(ies)	St Helena
Lead partner	CABI
Partner(s)	St Helena Research Institute (SHRI), Environment, Natural Resources and Planning Directorate (ENRP)
Project leader	*Rob Reeder*
Report date and number (e.g. HYR1)	*25/10/22; HYR2*
Project website/blog/social media	*https://blog.cabi.org/2021/09/27/cabi-to-work-in-partnership-to-help-protect-st-helenas-biodiversity-and-enhance-its-agriculture/*

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

The first project field site visit took only place right at the end of the last financial year in February/March 2022. Since then activities have focused mainly on these areas:

- Processing of pathogen samples from the first field survey
- Continued sampling and surveying by the project team based on S Helena
- Preparation of the next field site survey (October/November 2022) including setting up a training programme and developing tailored training material)
- Developing the experimental setup for pathogenicity testing of isolated pathogens from the endemic tree species

<u>Progress on specific activities to be covered in Q1-Q2 of Year 2:</u>

1.2 Cataloguing of pathogens and associated vectors including the ones recorded during the project. This activity will be based on excel and is an ongoing process. The catalogue will remain open for further additions beyond the termination of the project.

A first draft of the database including the results from the literature search and records collected during the first visit has already been provided as an annex to the first annual report. This included pathogens historically recorded from St Helena and those collected during the first field site visit. However, adding to this database is an ongoing process and more specimens will be added during the life time of the project.

2.1.2 Second on site survey for tree pathogens by BIFoR Phd student

This was originally scheduled for Q2 of year 2. However, due to the slow growth of seedlings from the endemic trees we are planning to delay this visit into the next project year. For further details see under box 2.

The assessment of marked endemic trees and the collection of abiotic parameters are still ongoing. This summer and winter have been unusually wet on the island leading to a strong and unusual flush of fresh growth on many endemic trees, particularly the cabbage trees. The wet weather has caused problems for cuttings of the endemic dogwood which have failed to establish leading to an even more precarious position for this species.

2.1.3 & 2.2.3 Processing of samples and development of assessment report

A wide range of plant pathogens collected during the first site survey have already been isolated and partially identified during the first half of this project year. In addition, a number of isolates are now preserved in oil and await further morphological and molecular identification.

Amongst the more interesting results was the isolation of a *Phytophthora* species (*P. kelmanii*) from the endemic Whitewood **(***Petrobium arboretum*). Two *Pythium* spp. (tentatively identified as *Pythium lutarium* and *Pythium diclinum*) were also isolated from soils associated with a black cabbage tree (*Melanodendron integrifolium*) displaying tree leaf loss in the high peaks and which was initially thought to be suffering from waterlogging. In addition, two potential pathogens infecting silky oak have been provisionally identified. The first belongs to the *Neofusicoccum parvum/ribis* species complex, which is reported as a causal agent of canker dieback in silky oak (*Grevillea robusta*) (especially in Africa). The second and most widely isolated species from the samples was *Xenoacremonium recifei*. This fungus is reported to be pathogenic on woody hosts and is of particular concern as it has been associated with wood boring beetles as vectors. On St Helena the false powder-post beetle *Xylopsocus capucinus* (Banyan beetle) has been attacking the diseased silky oaks and may also pose a threat to endemic trees such as whitewood by acting as a disease vector. It was suggested to be vigilant on the island and look out for further swarming of the beetle (most likely next January/February) to collect fresh material and to try to isolate potential diseases directly from the beetles in the lab on St Helena, remotely supervised by the CABI pathologists.

Only a few entomopathogenic fungi (EPF) have so far been collected during the project. Some of these are spider specific and it will be important to focus on this group during the next planned survey (November 2022) as potentially a range of endemic new species could be discovered. The planned survey of EPFs is likely to coincide with the visit of a spider taxonomist visiting St Helena in November this year which will greatly help the initial morphological identifications.

A finding that has raised some concern was the identification of an EPF on the endemic gumwood leafhopper S*anctahelenia decellei.* This fungus causes the dying leafhoppers to attach themselves to the upper surface of leaves, which is an unusual behaviour. It is possible that the fungus is not native to St Helena and has potentially become established on the island through the usage of a biopesticide (Mycotal) for the control of whiteflies. However, to have the provisional identification confirmed a further molecular assessment is required.

Since the first visit, the PhD student has been processing the samples brought back from St Helena, at CABI. In total there are 180 samples from Black Cabbage trees, Dogwood, He Cabbage, Whitewood and She Cabbage, including leaf tissue, soil and stem tissue. In addition, isolations from plant material have been undertaken and approximately 10 fungal isolates and 48 bacterial isolates have been obtained. The bacteria colonies have been preliminarily identified using the MALDI-TOF at CABI, and DNA has been extracted from the bacteria as well as lyophilised fungi for identification via Sangar sequencing which is currently underway.

Nanopore technology will be used for sequencing of the tissue and soil samples. To prepare for this, the student has been testing a protocol for tough eDNA samples using a pilot study of UK tree species. This work has revealed a large gap in the literature relating to plant microbiome studies conducted with the minION. The results of this project should help to fill this gap and are intended to be published in a peer reviewed journal. In addition, host specific peptide nucleic acid (PNA) clamps are being designed to prevent co-amplification of host DNA during 16s sequencing to increase sequencing depth and coverage. As the genome of the Black Cabbage tree has not yet been sequenced, the chloroplast and mitochondrial DNA from the St

Helena samples will be sequenced using the long read 16s primers 27F and 1492R, and used as a template for the clamp design. A pathogen database has also been created by the student to test the clamps for non-specific binding. A bioinformatic pipeline is being developed by the student to process the sequencing results. Due to the various troubleshooting issues experienced, it is estimated the first sequencing runs will be complete late December and the first results of the tree's microbiome assembly will be produced late January.

The next steps for the student are planning for the next research visit to the island, which has been postponed until October 2023. An experimental protocol has been written for artificial inoculations of healthy endemic saplings to look at symptom development caused by pathogens isolated from surveys. As well as pathogenicity studies, the student will be resampling tissue and soil for sequencing. This is necessary to capture the microbiome during an alternative season to when the first samples were taken (February/March). If a candidate pathogen is identified, the student may also carry out digital droplet PCR (ddPCR) or qPCR to observe absolute quantification of the pathogen, to assess changes in abundance across space. This will help inform the spread and severity of disease and can be incorporated into management decisions. By postponing the visit until October 2023, there will also be an opportunity to train a CABI member of staff in the minION protocol to assist in future sequencing of diseased crops.

2.2.2 Second onsite survey of crop and forestry pathogens as well as EPFs

This was originally scheduled for Q2 but will now take place in Q3. The reason for this change is to cover a different seasonal aspect to the first survey period (February/March 2022), when already a large number of pathogens (higher than initially anticipated) were collected. A survey of pathogens including a special survey of entomopathogenic fungi will continue until mid-November.

3.*1 Action plan to mitigate identified threats in all assessed sectors developed jointly with and made available to all stakeholders*

The development of the action plan is intertwined with the training activities to prepare stakeholders for a more sustainable crop management. In a first step we have developed targeted training modules initially focusing on diagnostics. A first round of workshops and training sessions is planned for Q3 of this project year.

Plans for the second field survey and onsite training activities have been drawn up and the next visit by the CABI team is scheduled for the period 13 October to 20th of November. As part of this, two project team members will conduct a series of training events over a two-week period in October (see training plan below).

OCTOBER 2022

Mon	Tue	Wed	Thu	Fri	Sat	Sun
					1	2
3	4	5	6	7	8	9
10	11	12	13	14	15 Arrival of CABI Team	16
17	18 Training with Farmers	19 Training with Forestry/ Conservation staff—provisional	20	21 11.40-1310 Training PAS Students	22	23
24 11.40-1310 Training PAS Students Laboratory work with Martina, Rosie, Freddy & Zac	25	26 Training with Agricultural staff	27 Training with Agricultural staff	28 Summing up	29 CABI Team Depart	30
31						

4.2 Improvement of laboratory facilities for diagnostic of pathogens and/or preparation of samples for shipment for external identification. Final review of material and equipment list in Q3 Y1 immediately after project approval; order of new equipment in Q3 Y1 to allow equipment to arrive prior to first site visit; shipment and instalment until Q2 Y2

and

4.3 First onsite training of at least 6 staff in using improved diagnostic facilities & online tools; established Plantwise test applied before and after training to measure the increase in knowledge by an increase in the score on the two tests in Q4 Y1; further onsite supervision of trained staff during follow on CABI team visits Q2 Y2, Q4 Y2 and Q1 Y4

Setting up of the lab at ENRD has now been completed and isolation and culturing of pathogens is an ongoing activity in the lab. Following the March visit, some initial problems isolating fungi were encountered by ENRD. To address this, direct supervision in the form of Zoom meetings, were given to guide staff through the inoculation and isolation procedures directly on screen (in July). This approach has helped to address the problem and further lab work has become more successful.

<u>Other activities:</u>

A larger project team meeting took place in July during which ongoing activities were reviewed and plans put in place for the next team visit to the island. We also discussed the two reviewers' comments to the annual report and action points were put in place to respond to them in time for the next annual report.

I addition the reviewers asked to for the below query to be addressed in the half year report

"there is not a clear methodology to address the underlying drivers of the risk of pathogen introduction, so it is not clear how the project will reduce the risk in the long term"

In response to the above: This project cannot address all of the underlying drivers of pathogen introductions; however certain elements of the project are helping to strengthen the biosecurity within the island. The training given to the biosecurity team with regard to recognising the symptoms associated with pests and diseases and the strengthening of the diagnostic capacity of the lab to isolate potential pathogens, will help in the detection and interception of potential pathogens. CABI thorough its member country scheme will continue to backstop the laboratory

and undertake investigations of problems that require more sophisticated molecular investigations. Training has also been given in symptoms/diagnostics to farmers, conservationists and members of the public. This will raise the awareness of the potential threats of bringing disease onto the island by the public and will provided greater surveillance by the biosecurity team. The early detection and management, to minimise the spread of pathogens, is at the core of this project and the long-term preservation of the endemic trees. The project team is in ongoing discussions with conservationists on the island to ensure that surveys and monitoring work can continue in a safe way.

A further comment relating to improving the output indicators so that they provide a better measure of the change or quality expected is currently being addressed and the team will contact the DI with an improved set of indictors shortly.

2. Give details of any notable problems or unexpected developments/lessons learnt that the project has encountered over the last 6 months. Explain what impact these could have on the project and whether the changes will affect the budget and timetable of project activities.

An essential part of the project is confirming the pathogenicity of fungi isolated from endemic trees by re-infecting seedlings with these pathogens and fulfilling Koch's postulates. To do this a large number of seedlings have first to be produced. Although this was set in motion at the earliest possible point in the project, seedling production turned out to take longer than initially anticipated. To address this, we would like to shift the visit of one member of the CABI team and the PhD student Amy Webster, initially planned for the current financial year, into the next year (most likely October 2023). This will allow sufficient time to produce seedlings of the size required for the planned experiments. We are planning to submit a change request shifting some of the staff time and travel budget from this into he next financial year as soon as possible. Otherwise the project is on track to achieve its objectives and no other significant problems have been encountered.

3. Have any of these issues been discussed with NIRAS-LTS International and if so, have changes been made to the original agreement?

Discussed with NIRAS-LTS:	~~Yes~~/No
Formal Change Request submitted:	~~Yes~~/No yet
Received confirmation of change acceptance	~~Yes/~~No
Change request reference if known:	

4a. Do you currently expect to have any significant (e.g. more than £5,000) underspend in your budget for this year?

Yes x☐ **No** ☐ Estimated underspend: [redacted]

This is only due to the planned change request asking for a delayed field site visit in the next year.

4b. If yes, then you need to consider your project budget needs carefully. Please remember that any funds agreed for this financial year are only available to the project in this financial year.

If you anticipate a significant underspend because of justifiable changes within the project, please submit a re-budget Change Request as soon as possible. There is no guarantee that Defra will agree a re-budget so please ensure you have enough time to make appropriate changes if necessary. Please DO NOT send these in the same email as your report.

5. Are there any other issues you wish to raise relating to the project or to BCF management, monitoring, or financial procedures?
Not at this stage

If you are a new project and you received feedback comments that requested a response (including the submission of your risk register), or if your Annual Report Review asked you to provide a response with your next half year report, please attach your response to this document.

Please note: Any planned modifications to your project schedule/workplan can be discussed in this report but should also be raised with NIRAS-LTS International through a Change Request. Please DO NOT send these in the same email.

Please send your **completed report by email** to BCF-Reports@niras.com. The report should be between 2-3 pages maximum. **Please state your project reference number, followed by the specific fund in the header of your email message e.g. Subject: 29-001 Darwin Initiative Half Year Report**