The Rufford Small Grants Foundation

Final Report

Congratulations on the completion of your project that was supported by The Rufford Small Grants Foundation.

We ask all grant recipients to complete a Final Report Form that helps us to gauge the success of our grant giving. We understand that projects often do not follow the predicted course but knowledge of your experiences is valuable to us and others who may be undertaking similar work. Please be as honest as you can in answering the questions – remember that negative experiences are just as valuable as positive ones if they help others to learn from them.

Please complete the form in English and be as clear and concise as you can. We will ask for further information if required. If you have any other materials produced by the project, particularly a few relevant photographs, please send these to us separately.

Please submit your final report to jane@rufford.org.

Thank you for your help.

Josh Cole, Grants Director

Your name	Seonju Marincowitz
Project title	Assessment of the health of native trees in the Pondoland Centre of the Maputaland-Pondoland-Albany biodiversity hotspot, with a specific emphasis on fungal diseases
RSG reference	43.12.07
Reporting period	27.03.2008 (release of fund) – 30.07.09
Amount of grant	£ 5000
Your email address	seonju.marincowitz@fabi.up.ac.za
Date of this report	30.07.09

1. Please indicate the level of achievement of the project's original objectives and include any relevant comments on factors affecting this.

Objective	Not achieved	Partially achieved	Fully achieved	Comments
To compile the list of pathogenic fungi on plant genera *Eugenia*, *Syzygium*, *Rhynchocalyx*		*		A catalogue of potential pathogenic fungi on 22 plant species (16 genera) is partially completed. More molecular and morphological investigations and pathogenicity tests have to be done to confirm the identity of fungi and their virulence.
To develop a fungal biodiversity database in the Pondoland			*	A database for both potential pathogenic and endophytic fungal isolates is compiled. It requires regular updates with on-going research and additional data with more collections.
To preserve fungal isolates in-vitro			*	A total of 60 % of the fungi collected are preserved at the culture collection (CMW) of FABI. Each of the isolates were preserved in two bottles of water and paraffin-oil and kept in a 4°C cold room.
To share the resultant information and knowledge and to bridge between specialists at FABI and local people			*	Scientific articles and a catalogue of microfungi in the Umtamvuna Nature Reserve (UNR) are in preparation, which will be available to the public and used for the conservation practice. The communication line was built between local scientists, Mr. Abbott, Mr. Scott-Shaw, Mr. Uys at Ezemvelo KZN Wildlife and researchers at FABI.

2. Please explain any unforeseen difficulties that arose during the project and how these were tackled (if relevant).

- Field trip
  - Two Nature Reserves (Umtamvuna and Oribi Gorge) were aimed to be visited twice in a year. However, a few days of excursion to the UNR produced enough materials to be studied for a year. A geographical feature of the UNR (very steep from 100 m to 1800 m altitude) also left us very tired after the UNR visit. Thus, the trip to Oribi Gorge was cancelled. During the project term only one field trip to the Pondoland was made.
  - The tree species were very randomly distributed unlike that of temperate forests. The inside of the forest was often inaccessible. Trees in accessible distance were either very young or too tall to approach. The trees of three initially aimed genera were too randomly distributed throughout the forest. Then the decision was made for the search to be expanded to other indigenous trees growing near the path, which resulted in a total of 24 genera and 31 tree species.

- Laboratory works
    - Genomic DNA of some fungal isolates gave difficulties in PCR and/or in sequencing. Molecular works for those isolates have been repeated until satisfactory results are obtained.
    - DNA fingerprinting of each isolates were planned for the only one gene region of the nuclear rDNA (ITS), however the necessity was raised to expand it to other regions (LSU) and other genes (β-tubulin, elongation factor 1-alpha) for the confirmation of species identity. DNA sequencing for the isolates in question is still on-going.

3. Briefly describe the three most important outcomes of your project.

- This is the first-time survey of fungal disease occurrences in the Pondoland vegetation focusing on the UNR. The study discovered some foliar diseases and die-back symptoms, the presence of very rare and new fungal species and variety of lichens, and some insect-damaged plants.
- The database of microfungi including potential pathogens and endophytes is established which can be a steppingstone to a comprehensive dataset of micro-organisms in the Pondoland.
- The live cultures are secured *in vitro* preservation system for future use and for conservation purpose in the case of habitat loss.

4. Briefly describe the involvement of local communities and how they have benefitted from the project (if relevant).

- An awareness of fungal diseases in the area was brought to their attention. For example, *Phytophthora* infestation of a ghost bush *Raspalia trigyna* is suspected and under investigation by another researcher from FABI. The plant once thrived in the area and disappeared suddenly. This incident remained a mystery and was never investigated. The plant was named as a ghost bush due to its sudden disappearance.
- Ezemvelo KZN Wildlife (regional conservation body) was contacted with the outcome of this research (a mid-term report was sent) and some practical application will be advised in their management with supplement of catalogue on microfungi in the UNR.

5. Are there any plans to continue this work?

- Yes, the identification of fungi is still on-going with further molecular and morphological studies.
- A few articles for new and rare fungal species and a more comprehensive article on the biodiversity of microfungi in the UNR will be published in peer-reviewed journals.
- Extensive field trip on other paths of the UNR and to Oribi Gorge is required to acquire a full picture of fungal diseases on the indigenous trees in the Pondoland.

6. How do you plan to share the results of your work with others?

- Potential fungal pathogens and rare and new fungi will be published in international journals to share the knowledge with scientific community.

- A catalogue of microfungi in the UNR in preparation can be used for the public awareness of the biodiversity hotspots in the micro-organism perspective.
- Herbarium specimens and live cultures are kept in accredited organizations for future users and become available to the public.

7. Timescale: Over what period was the RSG used? How does this compare to the anticipated or actual length of the project?

- From permit application to field work was planned for 2 months. However, a permit was released in a month from application so that field work could be finished in 1.5 months (27.03.08 – 09.05.08).
- From collection to publication was planned for 12 months. Laboratory works including herbarium preparation, photographing of specimens, preliminary investigation of materials, isolation of causative fungi, single spore isolation, preservation of isolates, cryofreezing of specimens for DNA extraction, and preliminary writing of articles took about 6.5 months (10.05.08 – 28.11.08).
- Both precise identification of organisms and finalizing publications were included in the previous time frame (for 12 months). However, identification process (and writing based on that result) using both molecular and morphological characteristics took much longer than the scheduled time and is still in progress. Up to now (16 months) most of the isolates were identified to generic level, producing approximately 44 fungal genera and 54 species. DNA sequencing data are also being generated to confirm the morphological identification and for the fingerprinting of each isolates. One article is at the end of its completion waiting for some molecular analysis and a catalogue with colour photoplates is partially completed (29.11.08 – present).
- Pathogenecity test to confirm the virulence of potential pathogenic fungi has not been planned for the term of this project.

8. Budget: Please provide a breakdown of budgeted versus actual expenditure and the reasons for any differences. All figures should be in £ sterling, indicating the local exchange rate used.

Item	Budgeted Amount (£)	Actual Amount	Difference	Comments
Field work	1414.29	1331.45	82.84	Consultation fee for tree identification in included which was not initially budgeted. Only one field trip was made instead of two.
Literature, documentation, information capturing, publications	285.71	273.79	11.92	Publication fees have not been considered in this amount. It will be supplemented by TPCP/CTHB program at FABI.
Laboratory work: molecular characterization, microscopy	3300	3389.50	-89.50	Molecular work is still on going so that more expenses will be incurred which will be supplemented by TPCP/CTHB program at FABI.
TOTAL	5000.00	**4994.74**	5.26	

9. Looking ahead, what do you feel are the important next steps?

The project is still on-going. The result of current project is only a fraction of what is in the greater Pondoland. More field work and collections in depth are essential to have a comprehensive figure of fungal diseases in the Pondoland.

10. Did you use the RSGF logo in any materials produced in relation to this project? Did the RSGF receive any publicity during the course of your work?

No, the RSGF logo has not been used yet. However, rticles and catalogue in preparation for publication acknowledge the RSGF. Whenever there is an opportunity to present this project, the RSGF will be acknowledged.

11. Any other comments?

The field location was far from our research base which is about 1000 km distance. During the excursion it was discussed that it would be ideal if we can stay nearby for a longer term (probably 1 month). Then we will have more easy access to the locations and thus do work more thoroughly.

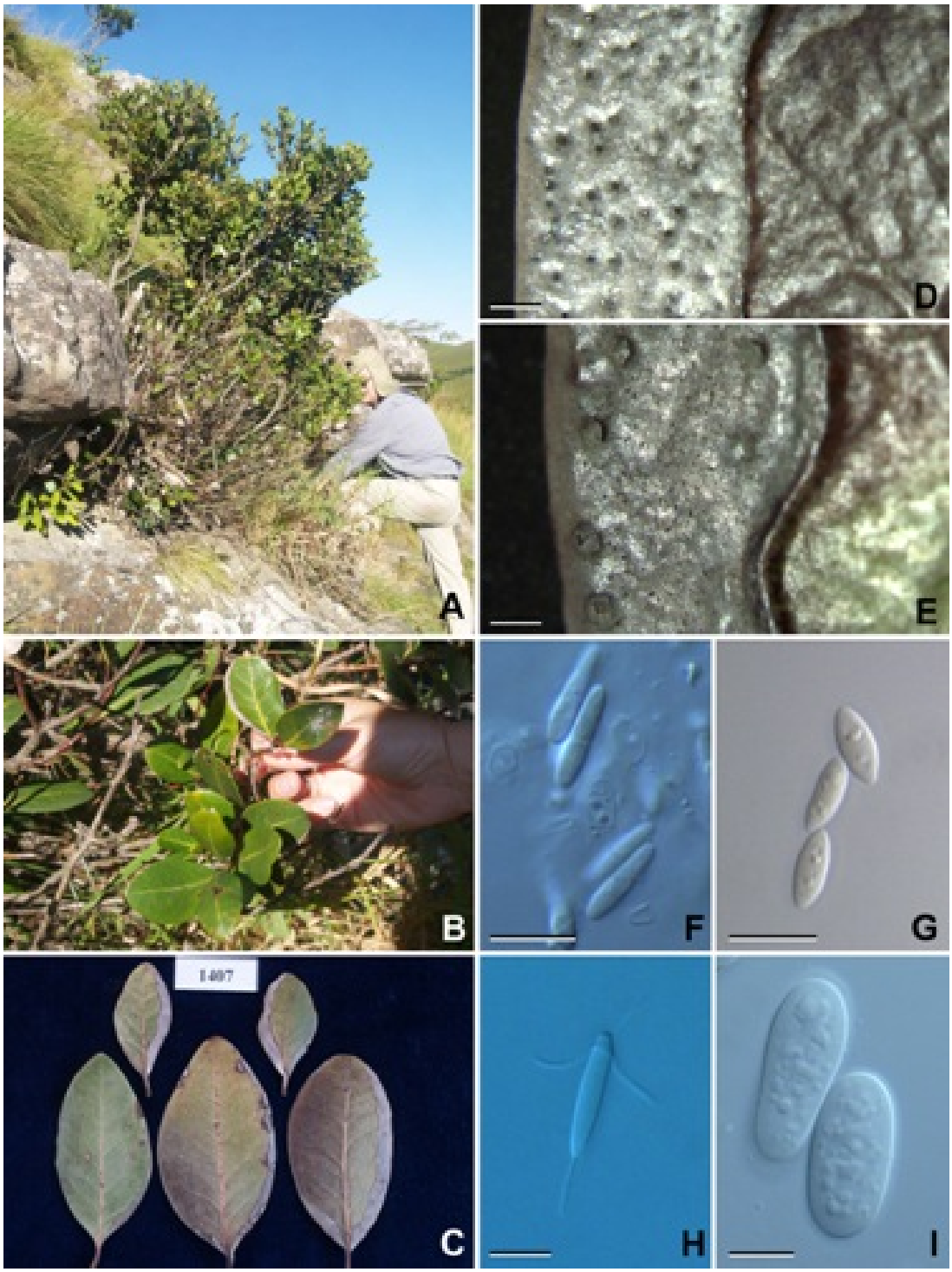

Fig. 1. Microfungi on *Apodytes abbottii* (Icacinaceae) at Porcupine trail, Umtamvuna Nature Reserve. A. A sampled tree. B, C. Leaves with necrotic lesions. D, E. Fungal structure in the lesions. F–I. Fungal spores isolated from the lesions (F. *Mycosphaerella*, S.L.1407A; G. *Discosporium*, S.L.1407C; H. *Mycotribulus*, S.L.1407D; I. *Botryosphaeria*, S.L.1407E). Scale bars: D, E = 500 µm; F, G, I = 10 µm; H = 5 µm

Fig. 4. Sooty molds on *Eugenia natalitia* (Myrtaceae) at Fish Eagle trail, Umtamvuna Nature Reserve (S.L.1445B, S.L.1447). A, D. Leaves with symptoms. B, E. Close-up of sooty molds on leaf surface. C, F. Microscopic view of hyphae and mucronate hyphopodia of sooty molds. Scale bars: B = 500 µm; E = 250 µm; C, F = 50 µm.

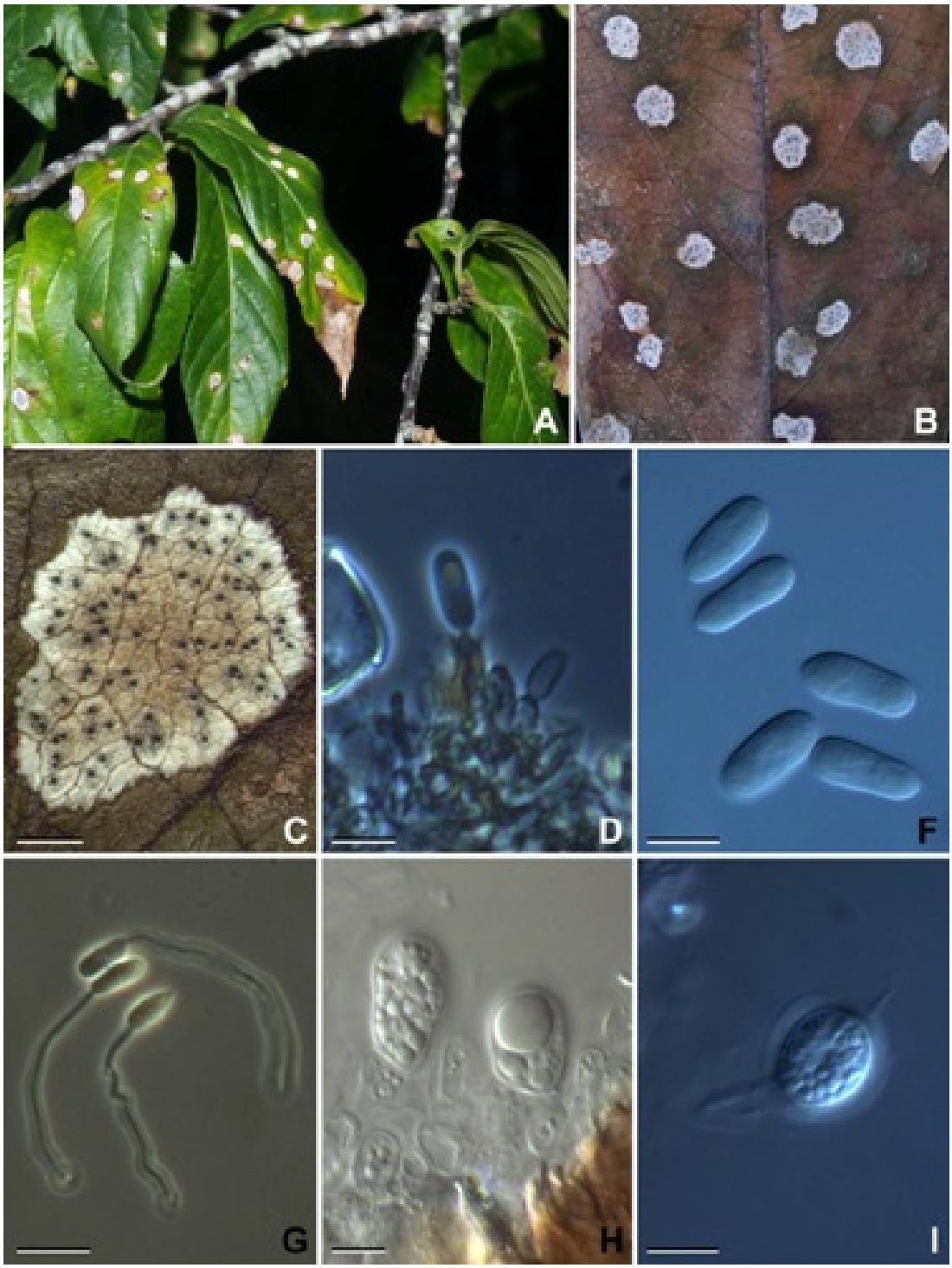

Fig. 23. *Phyllosticta* (S.L.1414C) and its synanamorph, *Leptodothiorella* c.f. (S.L.1414B) on *Turraea floribunda* (Meliaceae) at Mr. Abbott's residence, Umtamvuna Nature Reserve. A. Leaves in the field with symptoms. B. Necrotic leaf spots. C. Close-up of lesion showing fungal structures. D, H. Young spores still attached to the spore bearing structures. F, I. Spores (F. *Leptodothiorella* c.f.; I. *Phyllosticta*), G. Germinating spores *in-vitro*. Scale bars: G = 20 µm; C–F, H, I = 10 µm.