A study of tree disease on St Helena

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Background

St Helena is a volcanic island situated in the South Atlantic ocean (15°56'S, 5°43'W). The cloud forests are a hotspot for biodiversity and many endemic trees have become established there. Some of the most prominent tree species that can be seen, are the gumwoods, dogwoods and cabbage trees[1].
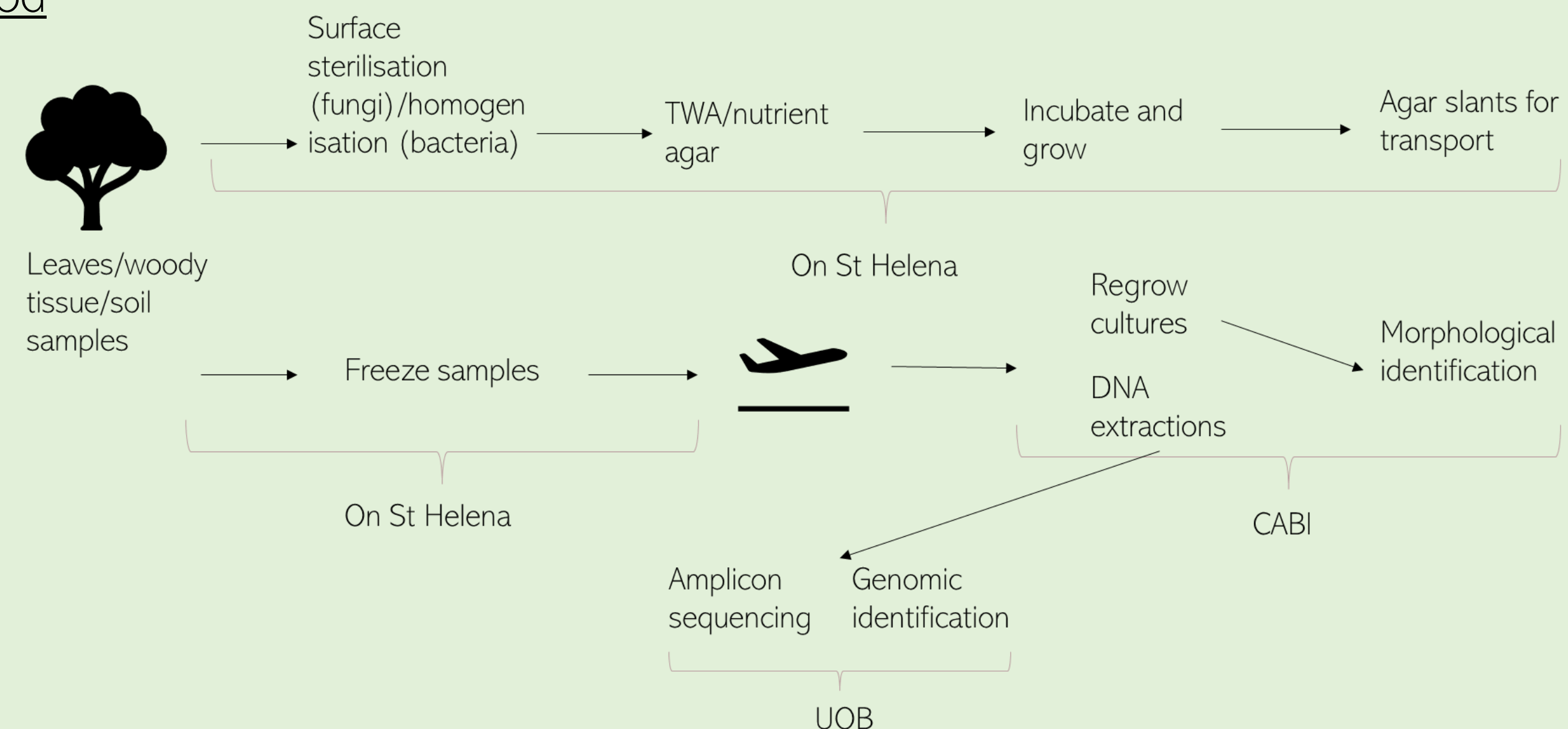
Settlement on the island as early as the 17th century, has left much of the land wiped clear or native vegetation, and the cloud forests fragmented dramatically. This loss of genetic diversity and population size has resulted in many of the endemic trees becoming threatened. Coupled with an influx of tourism, trade and climate change, the introduction of pests and diseases could result in complete devastation of these already vulnerable trees[2].

One tree facing major dieback due to an unknown diseases causing agent is the black cabbage tree *(Melanodendron integrifolium)*. Symptoms such as wilt, leaf spotting and sudden dieback can be seen across the peaks. In addition, nurseries are facing similar issues, limiting the ability to increase endemic stocks. These issues are abundant across not only black cabbages but most of the remaining endemics[3].

Objectives

- Use amplicon sequencing and isolation techniques to investigate a candidate pathogen for disease.

- Make an assembly of the forests microbiome

- Establish a management plan for the conservation of threatened endemic trees in this unique ecosystem
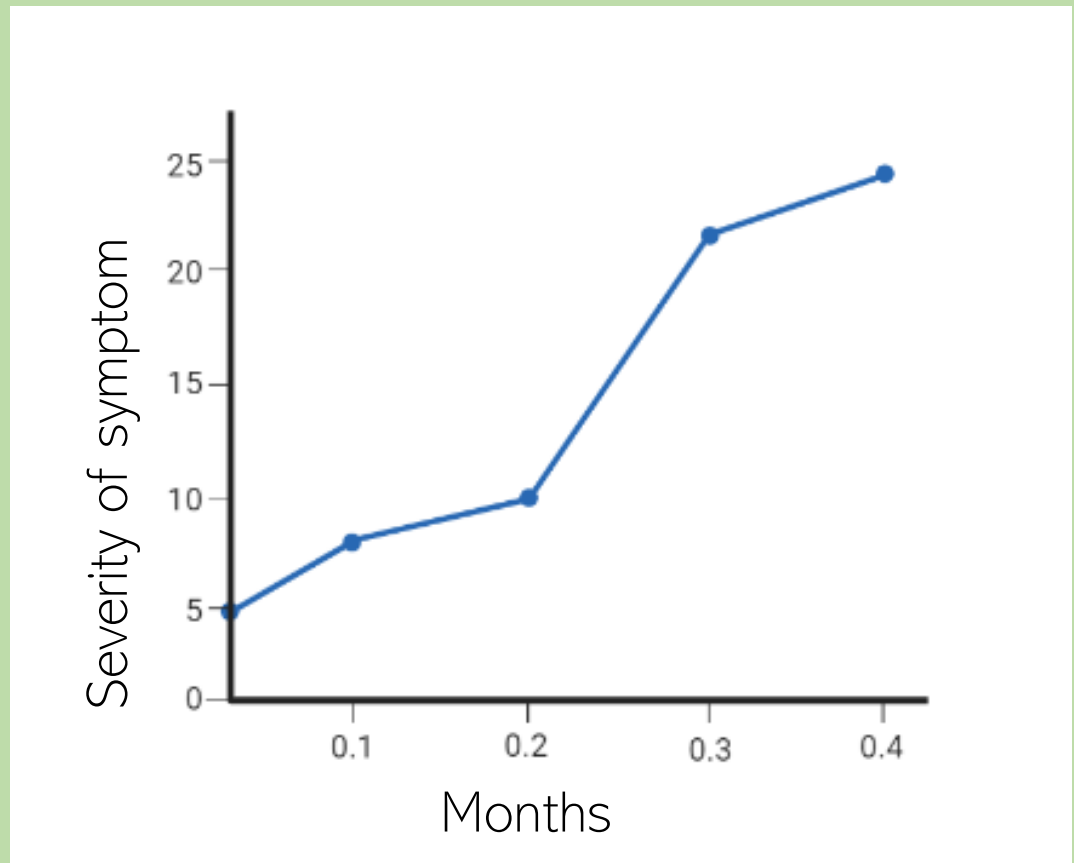
Method

Leaves/woody tissue/soil samples → Surface sterilisation (fungi)/homogenisation (bacteria) → TWA/nutrient agar → Incubate and grow → Agar slants for transport

On St Helena

Freeze samples → ✈ → Regrow cultures → Morphological identification
DNA extractions
On St Helena   CABI
Amplicon sequencing   Genomic identification
UOB

Leaf spotting on nursery sapling (left) galls on mature whitewood (right)

Collecting samples   Estimation of crown size and tree height
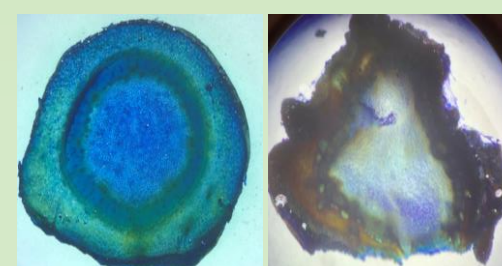
Stained healthy and diseased cross section of black cabbage branches

Black cabbage tree skeleton
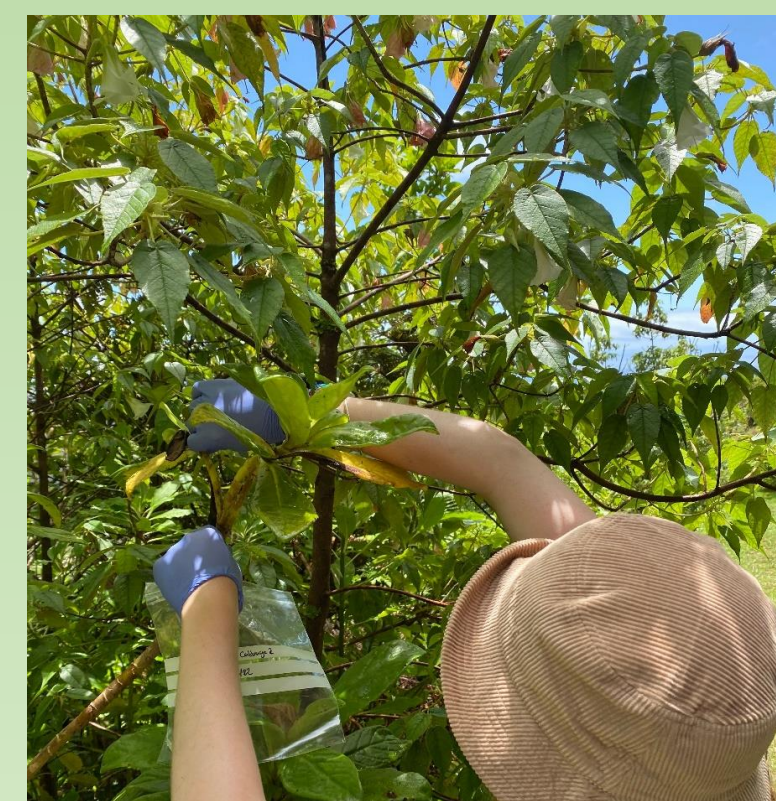
Healthy vs diseased tips on the same branch

Monitoring of tree health

- Tree morphology - approximate height, age crown cover

- Health status – percentage leaf drop, leaf wilt, discolouration and branch dieback

- Symptoms – spots, water soaking, lesions, bleeding, insect damage etc.

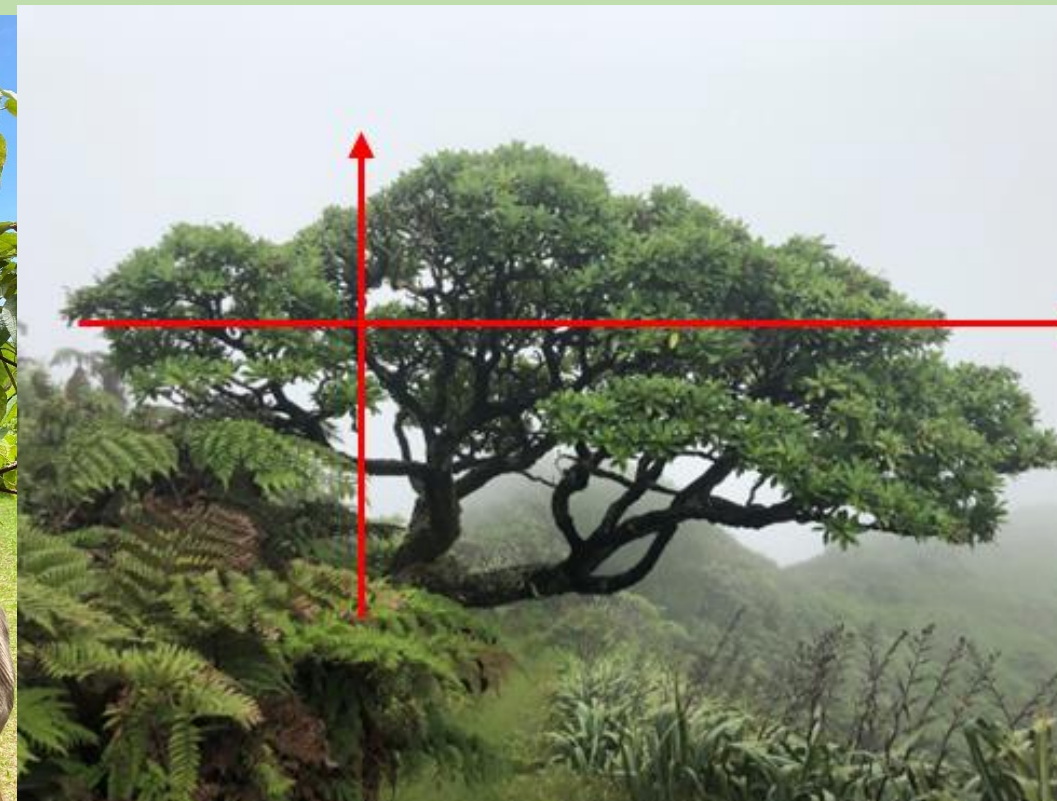- Environmental conditions - humidity, wind speed and direction, soil type, elevation

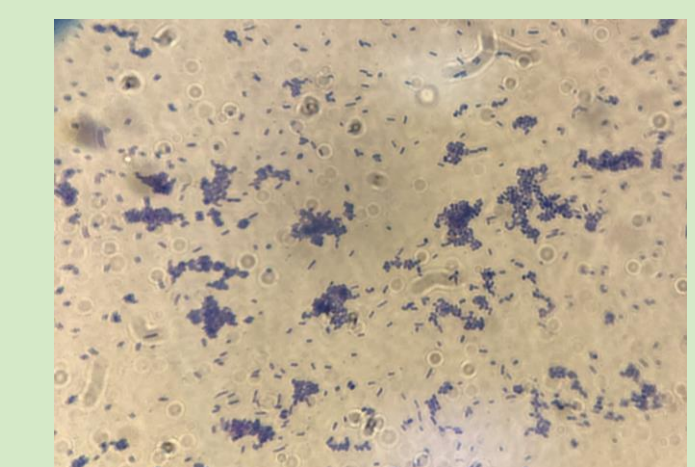All measurements are being continually recorded on a monthly basis by a colleague on the island

Look at disease progression over a year
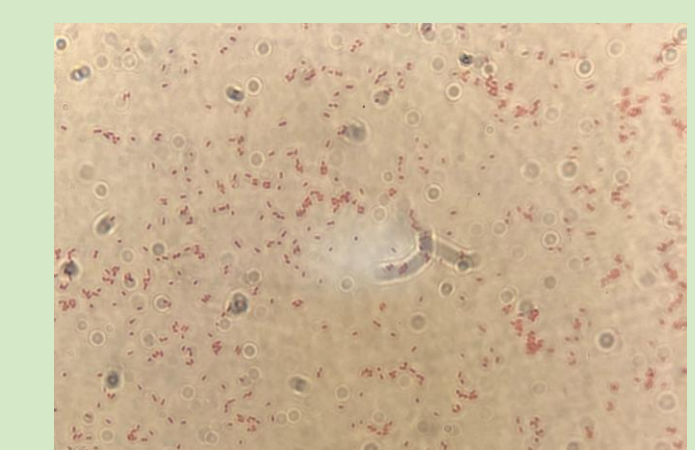
Identification of disease-causing agent(s)

- Grow cultures from material
- Fungi and bacteria on selective media
- Gram staining
- Spore structures
- Colony counts
- MALDI-TOF
- Molecular identification

- Extract DNA from samples of leaf, soil and wood from black cabbage trees, in addition to leaves from 4 other symptomatic endemic trees
- Multiplex amplification of ITS, 16S and YPH regions
- Sequencing of amplicons using the minION (long read capabilities)
- Microbiome assembly and identification of candidate pathogens

Future plans

- Inoculate healthy black cabbage saplings with potential disease causing agent(s)
- Grow saplings from seed and from cuttings
- Change soil used for growing
- Use qPCR to look at pathogen abundance across different species and distributions
- Transcriptomics to identify pathogenic pathway
- Management plan for the island

Black cabbage 2 peaks – gram positive

Black cabbage 2 nursery – gram negative

References:
1. DETHERIDGE, A. P., CHERRETT, S., CLASEN, L. A., MEDCALF, K., PIKE, S., GRIFFITH, G. W. & SCULLION, J. 2020. Depauperate soil fungal populations from the St. Helena endemic Commidendrum robustum are dominated by Capnodiales. *Fungal Ecology,* 45.
2. LAMBDON, P. & CRONK, Q. 2020. Extinction Dynamics Under Extreme Conservation Threat: The Flora of St Helena. Frontiers in Ecology and Evolution. 8.
3. GRAY, A., WILKINS, V., PRYCE, D., FOWLER, L., KEY, R.S., MENDEL, H., JERVOIS, M., HOCHKIRCH, A., CAIRNS-WICKS, R., DUTTON, A.J. AND MALAN, L. 2019. The status of the invertebrate fauna on the