Mar 22 Technology of the month – March 2022

Fig. 1. a) Dried herbarium specimen of Myrtle rust b) Sealing a glass ampoule c) The microbank bead system

Specimen preservation

Australia has several herbaria and culture collections dedicated to plant pathology specimens. In this months TOTM we are discussing the techniques used by the NSW DPI Plant Pathology Herbarium to ensure the quality of your specimens for decades and centuries to come.

Dried specimens

Compared to the preservation of other biological specimens, plant pathology has it easy. Diseased plant material is simply pressed and dried using a piece of technology that hasn't changed much in 500 years, the herbarium press.

We use a simple design of two wooden boards in a grid style to facilitate airflow. Layers of corrugated cardboard and newspaper are placed between the samples to dry them quickly, preventing mould growth and preserving colour. The boards are strapped together, and bricks/books placed on top for extra weight. Some designs use screws to tighten the press, but this limits the number of samples that can be stacked. We also use a drying oven for particularly damp samples. They are dried low and slow, around 40ºC-50ºC, as we do not want to cook them and their DNA.

Living cultures

What's cooler than dead plant pathology specimens? Living specimens! I think you will agree that the coolest thing about working with fungi and bacteria is that we can grow them in the lab. The next step is ensuring all your hard work collecting, isolating, purifying, and identifying your pathogen and all the information you have found during your research, are preserved long term.

We store cultures in three different ways. We often use all three, ensuring redundancies, but not all groups survive all methods so this is species dependent.

Agar slants

This is a longer-term agar-based storage method where the media is set in the tubes/bottle on an angle to allow more surface area for growth. Once colonised, the media is covered with mineral oil and capped, which prevents the media drying and helps maintain the dormant culture state. The cultures are kept at 15ºC. Cultures can be stored for months to years in this method but need to be re-cultured. This method is labour intensive and re-culturing also increases the chance of changes to morphology and pathogenicity over time.

Ultra-low temperature storage

We use MicroBank™ storage tubes, similar to a glycerol stock method. These 2ml tubes come prefilled with chemically treated porous beads and specially formulated preservative. You scrape up your culture, put it in the tube and shake it about. These are stored in a - 80ºC freezer, equipped with a 24hr monitored alarm system. While this method is low effort to set-up, it has ongoing costs and risks due to the storage temperature.

Freeze Drying

By far the highest effort method to set-up. This involves drying cultures in glass ampoules using a vacuum freeze dryer. Ampoules are sealed with a butane torch to ensure the vacuum is held. Bacteria can be put into ampoules directly, suspended in a sucrose peptone broth, while fungi are first grown on a water agar plate containing sterilised carnation leaf (gamma sterilised). The fungi colonise the carnation leaf and it is these leaves that are placed in the ampoule for freeze drying. This collection is stored at 15ºC for fungi, in a dedicated cool room, and 4ºC for bacteria in dedicated fridges. As the glass ampoules make this collection fragile, we glue the ampoules to index cards for easier filing and access.

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