Protecting small-scale herbarium collections from attack by insects and moulds

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The work of non-professional mycologists is an important global resource for documenting fungi. This is particularly so in areas other than Europe and North America where private collections inevitably contain many undescribed species. I have been collecting fungi for around 30 years and in that time amassed a private herbarium of around 6,000 specimens, over 3000 of them in the last decade since migrating from the UK to New Zealand. Keeping these collections at home provides necessary reference material, which is especially valuable in New Zealand where so few macro-fungi are documented. In addition, the need for a private reference collection is exacerbated in New Zealand because the loan of material held in institutional collections has become impossible as a consequence of restrictive biosecurity containment regulations.

All my specimens will eventually be deposited in a national collection but in the meantime it remains my responsibility to ensure they are maintained appropriately. Over the years my mycological interests have wandered across just about every group of fungi except yeasts. Of all the groups the maintenance of macro-fungi collections is most onerous as they are easily destroyed by infestations of insects and moulds. In this paper I describe a method for increasing the chances of their long term preservation, especially where ambient conditions of temperature and humidity are not easily controlled.

In the early days of collecting in the UK I did not possess a good drier to ensure collections were thoroughly dried. The collections were subsequently stored in paper packets in shoe boxes in my study. On returning to examine some collections after several years I would find evidence of extensive damage, especially by mites, psocids (booklice) and/or xerophilic fungi such as *Penicillium* and *Aspergillus*. At the time I experimented by packing some collections in zip-lock plastic bags with added silica-gel to remove moisture. Whilst this technique did stop insect damage it did not discourage attack by moulds. Many collections were lost during subsequent years.

These initial experiments with sealed plastic bags including silica-gel discouraged me from this approach. More recently, and subsequent to evidence of psocids present in my New Zealand collections, I have supplemented the technique in a way that does appear to be effective and should allow material to be preserved at home for long-periods under most ambient conditions.

In a professional herbarium infestation by insects and moulds is supressed by carefully monitoring and controlling both the humidity and temperature. The relative humidity should be kept under 50% and the temperature under 20C (Foreman, L.; Bridson, O.; 1998; The Herbarium Handbook, 3'" Edition. Kew, RBG.). These conditions can be difficult to achieve for the non-professional working at home, especially in warmer/humid climates. It is possible to buy relatively cheap de-humidifiers and air-conditioning units but their use can become both expensive and noisy in a small scale private herbarium.

In preparing fungal collections for storage it is critical to ensure the material is initially dried thoroughly. Like most collectors I now use a fruit-drier for drying material. Subsequent to drying I also place my collections in the freezer compartment of my refrigerator for several days. This ensures that any resistant insect eggs are killed. Following any subsequent examination of material retrieved from my collection I again re-freeze it. These basic procedures will minimise the chances of infestation but are rarely sufficient to ensure adequate long term preservation.

These days I still keep my collections in paper packets, although now I use archival quality acid-free thick paper, and initially wrap specimens in acid-free tissue paper. These packets are filed in standard herbarium boxes (in numerical sequence of accession number). The boxes are essentially just larger and sturdier versions of my original shoe boxes. The difference now is way I prepare and store these boxes. I place each box in an open plastic bag and then place the bag within a home-made vacuum chamber. The air is pumped out of the chamber using a vacuum pump and kept under high vacuum for at least one day. Suitable inexpensive two-stage rotary vacuum pumps are used most commonly by service engineers on air-conditioner or refrigeration units (e.g. Minivac 2). Atmospheric pressure is generally around 760mm mercury whilst the vapour pressure of water at 20C is around 17mm Hg which means that once the air pressure is reduced below this level then water boils. Keeping specimens under high vacuum for a period removes remaining traces of moisture, as well as despatching any live insects. The moisture evaporates rapidly rather than boiling and there is no risk of creating mushroom soup! Once the box has been kept under vacuum for at least a day then the chamber and bag containing the herbarium box are back-filled with inert argon gas until the pressure is equalized. Small cylinders of argon (size D) are readily available from welding suppliers and sufficient for filling around 25 boxes containing 2,000 collections. Argon cylinders can be refilled relatively cheaply. The cylinder will be supplied with a shut-off valve and requires a regulator suitable for welding applications. Please familiarise yourself with the safety requirements before purchasing and using pressurised cylinders and regulators. The back-filled argon gas penetrates deeply into the evacuated dried material. Finally the bag containing the herbarium box is sealed with PVC tape.

The key to this technique is the evacuation of the material prior to filling with argon. Simply flushing air from a container with argon will not remove either moisture or oxygen pockets embedded within dried fleshy fungi. So far I haven’t come across any organisms capable of surviving near zero moisture with most oxygen replaced by argon, regardless of the ambient storage conditions.

The technique does have the disadvantage that the bag must be opened to retrieve inidvidual packets but it doesn't take long to re-evacuate it and refill with argon. On subsequent fills it is not necessary to maintain a prolonged vacuum.

In addition to this procedure I also place a packet of silica-gel and solid insecticide in each box – just in case the sealed bag ruptures or the argon diffuses from the bag. I use thick gas-impermeable bags to minimise gas diffusion (commercial bag suppliers have this data). In addition to silica gel it is also possible to include oxygen absorber packets. These packets will be familiar and are used extensively in the packaging of food and electronics.

Constructing a robust vacuum chamber and associated vacuum/gas supply lines required some trial and error and it is worth passing on the experience I gained. The vacuum chamber must serve a number of functions. It must be capable of keeping a high vacuum when sealed. It must be possible to open and close the container and have a large enough opening to insert the herbarium box and its packets. It must have a gas-tight supply line, both for evacuating the chamber and introducing argon. It must be physically very strong; atmospheric pressure pushing against a vacuum is equivalent to a column of water 10m high pushing in every direction! Please test your box before placing valuable collections inside it.

My solution to these requirements was to build a sturdy vacuum chamber of 12mm thick chipboard with double walls such that every edge was supported by a full length of board (figure 1). The joints were both glued and screwed together. This chamber was then placed inside a large vacuum bag. These are available for storage of duvets or clothes. Air can be sucked out of the bag with a vacuum cleaner so reducing the storage size. These bags are resealable and strong enough to withstand a full vacuum. The inner chipboard chamber must not have any sharp edges which could pierce the plastic film of the outer vacuum bag when it is sucked onto the chamber during evacuation. Initially I tried using the vacuum-cleaner valve already on the bag as the evacuation point but it did not prove to be sufficiently robust. I therefore left this valve sealed and created a separate assembly for the vacuum/argon connection. I experimented with several designs which failed to maintain a vacuum seal before arriving at a design which has now survived many cycles of use. This assembly uses a hosepipe connector with a screw thread and plastic nut. These are available from garden supply centres. This is pushed through a hole made in a lid from a plastic Tupperware container which provides a flat base against which the plastic vacuum bag seals. The lid has raised edges allowing it to be screwed to the wooden box with the screw heads recessed. The vacuum joints are sealed with a thin layer of silicone gasket sealant. The arrangement of the various components is shown in Figure 2. All the piping used to connect to the vacuum pump and argon supply was thick-walled flexible PVC tubing available for DIY stores. A valve capable of sealing a high vacuum is necessary between the chamber and the vacuum pump. I found that plumber's ball-valves were most effective. The completed evacuated assembly is shown in figure 3 and a close-up of the bag vacuum connection in figure 4.

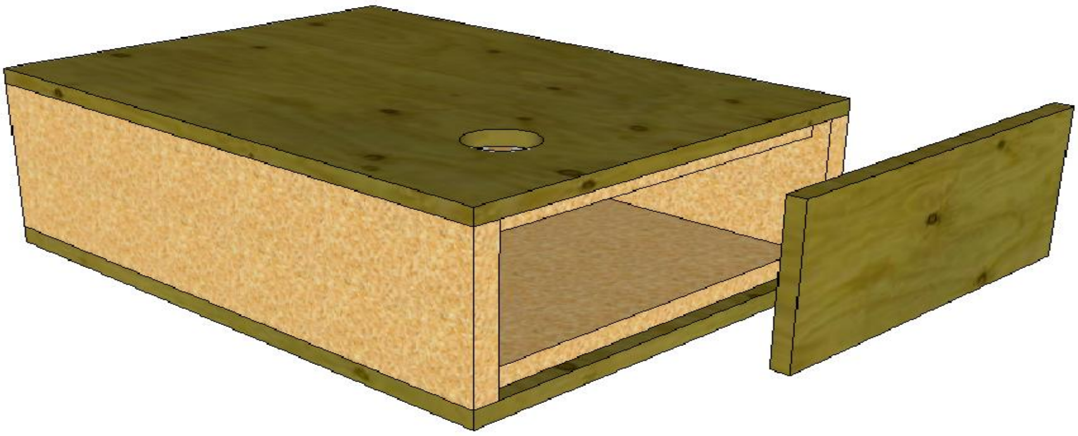

Figure 1: Construction of the wooden vacuum chamber. Note the arrangement of the double-layer wall of the box and the recessed end-lid, thus ensuring the force of the vacuum is distributed across the length of the walls.

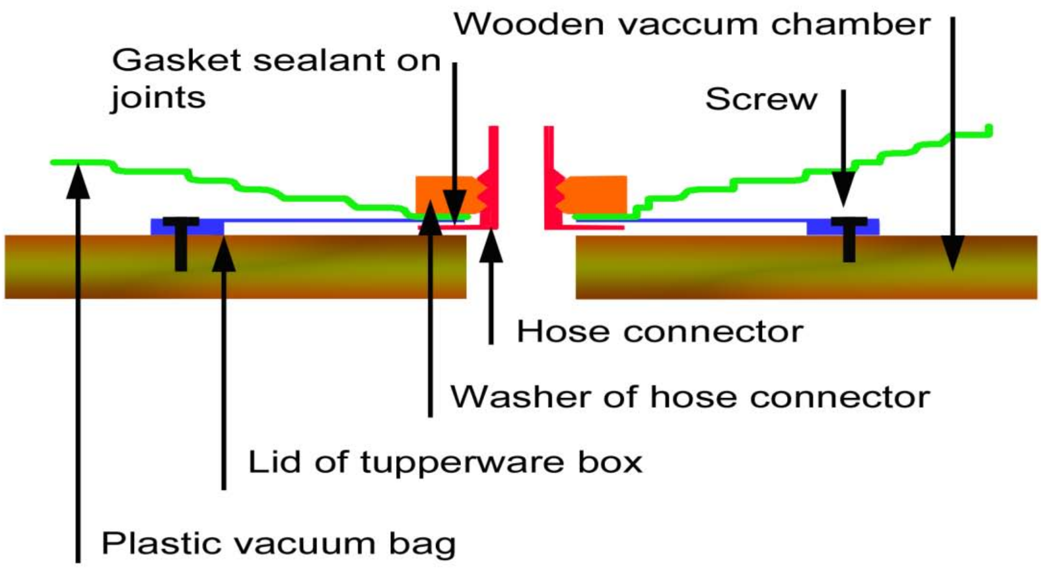

Figure 2: Details of the vacuum-tight joint between an external hose-connector, the plastic vacuum bag, and the internal wooden chamber.

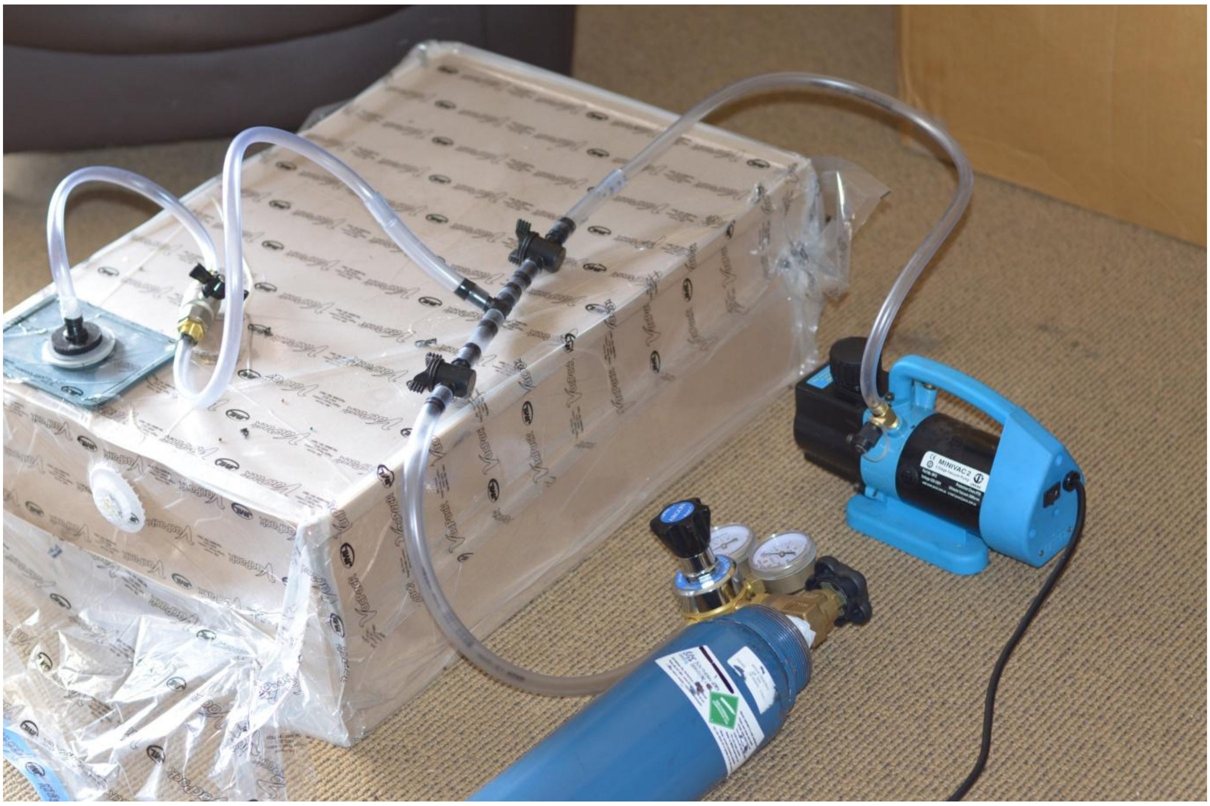

Figure 3: Complete assembly showing argon bottle, PVC tubing, T-connector and valves to switch between evacuation and back-filling, rotary vacuum pump, vacuum bag and the inner wood vacuum chamber. The vacuum chamber contains the plastic bag with a herbarium box (fig. 6) and is fully evacuated in this photograph.

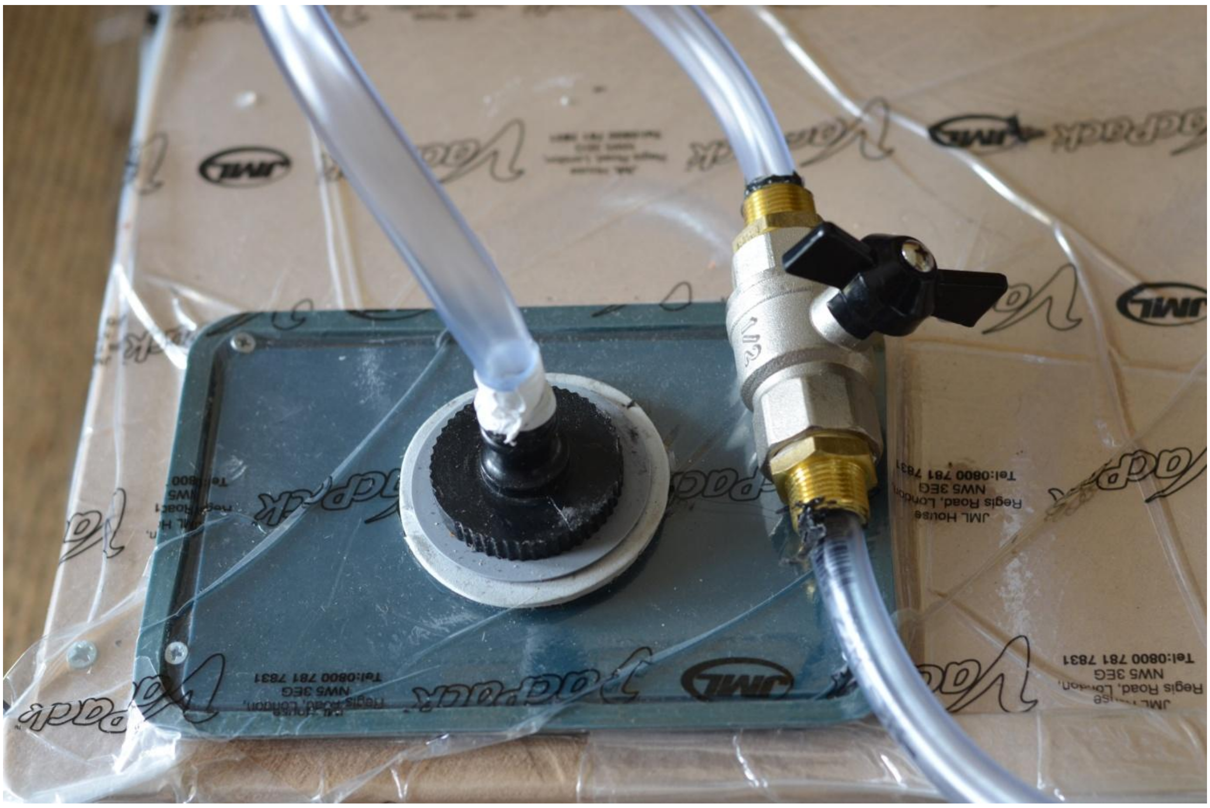

Figure 4: Connection to vacuum bag and chamber, showing vacuum-tight ball-valve.

The inner bag containing the herbarium box must remain open during the evacuation procedure and subsequent flushing with argon. A key question with this arrangement is how to ensure that air does not re-enter the bag during its removal from the chamber prior to sealing with broad PVC tape. My solution is to seal most of the inner bag before it is placed in the chamber. I leave a gap at one end and insert a plastic tube, figure 6. The tube allows air to be sucked from the bag during evacuation, and allows the argon to re-enter. Once at atmospheric pressure the bag can be removed and only little air will enter via the tube. Once removed the bag can be squeezed a little to flush out any small amount of air. The section of bag around the tube is then pressed together and the tube pulled from the bag. The remaining small gap is then sealed with tape.

This assembly has been scaled down to process a few collections at a time in individual zip-lock bags.

Figure 5: Herbarium box containing specimen packets and fail-safe packet of silica gel and solid insecticide.

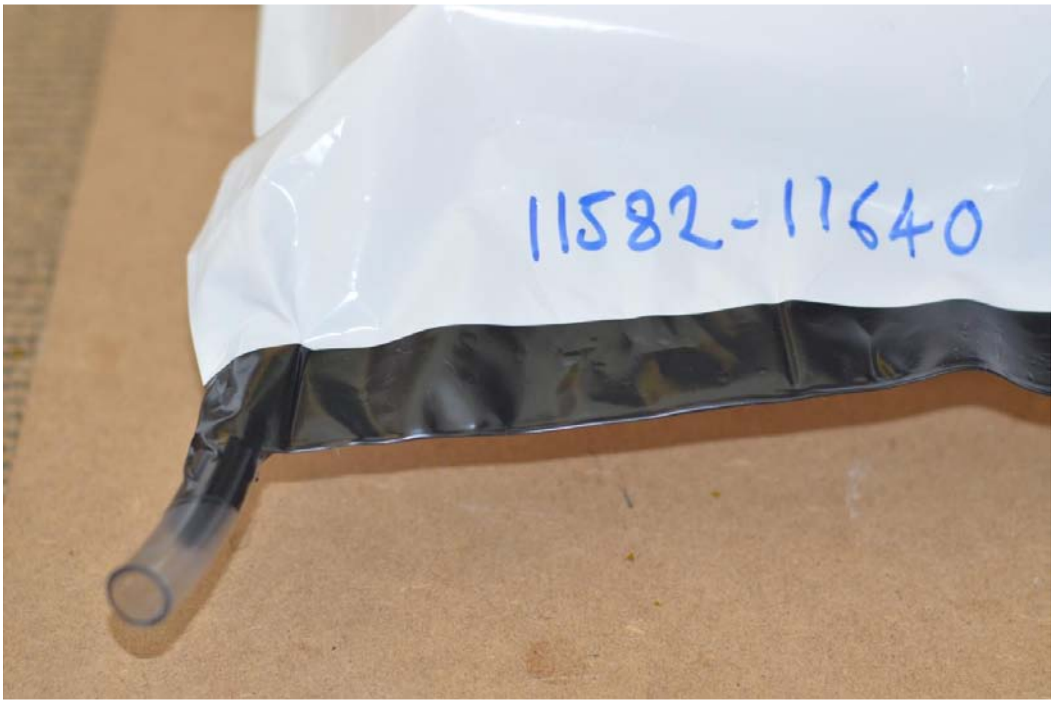

Figure 6: The herbarium box is enclosed in a gas-impermeable plastic bag which is sealed with PVC tape, with a tube inserted at one end to allow evacuation and argon back-fill. On removal from chamber the tube is pulled out and remaining gap sealed.

Figure 7: The herbarium box and enclosing bag with protruding tube is inserted into the vacuum chamber inside the vacuum bag which is then sealed.