

Mould growths as indicators of timelines: post-mortem intervals, food and materials spoilage, and damp in buildings

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Moulds are generally rapidly growing fungi that can cause spoilage on surfaces and materials whenever the conditions are suitable for growth. Almost everyone must have experienced circular often somewhat fluffy or powdery circular patches on stale bread, overripe apples, and citrus fruits, or damp plaster; and in shades of brown, black, green, grey, pink, white, or yellow. Individual mould species differ in the levels of humidity and temperature they require to develop, but they also grow at characteristically different rates. Some can form colonies several cm across in 1–2 days, while others may take several weeks to reach a similar size. These characteristics have led to them providing crucial intelligence on time intervals in a variety of criminal and civil investigations – but often their potential goes unrecognized by scenes of crime officers, pathologists, and insurance claims investigators.

It may seem daunting to learn that there are around 2,000 species of fungi that are commonly considered

as moulds, and that precise identification requires critical microscopic, cultural, or molecular sequence data. However, only around 100 are regularly encountered in Europe and North America, and it is not always necessary for investigators to name the fungus to obtain pertinent intelligence. Here we outline the key points of the methodology we apply, and give some examples of types of cases where this has provided information on the times of events.

Methodology

The same methodology can be applied regardless of the nature of the material on which colonies develop. We are only able to present a brief outline here, but plan to submit detailed protocols for publication in *Forensic Science International* in the next few months. The sizes of the largest discrete colonies are measured, tape lifts taken and transferred to sterile slides, and swabs taken. In the laboratory, the material on each tape-lift is stained and examined microscopically for preliminary identification, while the swabs can be

cultured on agar plates, and (or) used for molecular identification. It is critical to measure ambient temperature and relative humidity prevailing at the time of sampling as well as ascertaining how these had varied over a specific period. This is achieved by using data loggers which record values at specified intervals during the period chosen for the observation.

Growth rates of moulds at a range of temperature are regularly recorded in the mycological literature and, in some cases, are of value in species identifications. If the identity of a mould is known, and growth rate data are already available for the temperature range of concern, it is possible to use the published growth rates to determine the time period of colony growth at the crime scene. If the identity of the mould is unknown, or not readily determined, it can be inoculated onto agar plates. These experimental plates are maintained at the temperature of the material (biological or otherwise) on which it was growing, and measurements of the colony size made periodically. The growth rate of the fungus can then be measured for any pertinent temperature range. Fungal growth rate varies greatly and, depending on species and ambient conditions, the time taken for a colony to spread over a 5 cm diameter Petri dish can vary between 1-2 days to 1-2 weeks; it can even take several months. A calibration curve can then be constructed from the measurements made of colony size against time, and the age of the colony can be read directly from the growth curve.

Fungi will only grow when sufficient moisture is available, either from humid air or damp surfaces, and the ambient temperature is suited to them. As not all colonies present in a single situation necessarily started to develop at the same time, it is important to concentrate on data from the largest discrete colonies. Further, as the moisture and temperature values can vary, conditions may not have permitted continuous growth from colonization to when they were measured. For this reason, estimates of time obtained from this type of approach can only be used to indicate a minimum and not a maximum number of days since colonization commenced. Nevertheless, in some cases in which we have been involved, we have found the data to give timings that proved to be accurate to the day.

Applications

Post-mortem intervals

The moulds found on human remains are generally not those causing diseases or other medical conditions in living people. They tend to be spoilage moulds able to grow on foodstuffs and damp surfaces; species of pin moulds (*Mucor*) and blue moulds (*Penicillium*), for example, are especially frequently encountered. In our experience in the UK, a corpse takes around a week to become amenable to colonization by moulds, although there are indications that the time may be much less in warmer countries. The reasons for this are obscure; temperature may be the key as many fungi will not grow optimally at body temperatures, but we have hypothesized that the break-down of the immune system, or conditioning of the skin after death, may be involved in this time-lag.

Fungi were first used to assist in the determination of post-mortem interval in the investigation of the murder of a Belgian baroness in 1982, but the first case of which we are aware of mould growths on a corpse being used in the UK was in West Yorkshire where a woman was estimated as having been murdered at least 3–4 weeks before her body was found in January 1996. In a case involving a headless and handless corpse that had been kept in a freezer and then dumped near a stream in Hertfordshire in 2007, entomological evidence for estimating post mortem interval was absent and this was probably because temperatures were too low to allow fly activity. A small *Mucor* colony had, however, developed on the torso, and the stage of development of the fungus indicated that the corpse had probably been in position for only 1-2 days; botanical evidence corroborated this observation.

We have found fungal growth data to be especially valuable in other cases where entomological evidence was lacking, either because flies had no access, or because conditions inhibited their activity. In a frenzied stabbing case in a closed flat in Dundee in 2009, we did not have access to the corpse, but fungal colonies had developed where body fluids had splashed and leaked onto furnishings and the carpet. Fungal growth had ceased after the corpse was removed and the furnishings and carpet dried out, and we established by further experimentation that this was because the humidity had been too low for continued fungal growth. We isolated and examined the growth rates of the fungi involved in Petri dish cultures kept at the same temperatures as in the flat, and came to an estimate of five days since the fungi had started growing. This time was confirmed by a subsequent admission of guilt. The following year we assisted in another case in Dundee where the corpse of a raped and murdered woman had been deposited in shrubbery by a roundabout; this was in February and again it had been too cold for flies. Fungal growth on exposed parts of the corpse were so extensive that they indicated a time of around two weeks. This proved to be consistent with the date of CCTV footage showing the accused close to the deposition site.

Food and materials spoilage

There is an enormous amount of information available on food spoilage fungi, mostly focussed on conditions that have led to deterioration of the foodstuffs, and the toxins (mycotoxins) some of the fungi involved can produce. We have found that food left exposed at a scene can give key evidence of timings. In a case for the Metropolitan Police in 2013, a mother had abandoned three children in an apartment, the youngest of which had been severely neglected and died while she was away. There was an issue over determining the length of time she claimed to have been absent. Extensive mould growths were present on plates with dried food remains, and in a deep cooking pan which also contained dried food. The food on the plates and in the pan supported extensive mould growths of five different species. From the sizes of the colonies and the known growth rates of the species identified at temperatures similar to those

recorded in the apartment, it was clear that the oldest had been growing for 10-14 days; this supported the view that the mother had been away for more than a long weekend.

Damp in buildings

Mould growth in buildings can pose health risks as well as causing damage, but the time of commencement of fungal growth can be crucial to some insurance claims for damages to inhabitants and (or) properties. In 2013 we assisted in a case where there had been sewage ingress into two adjacent houses in Hertfordshire, and the occupant of one was claiming for substantial damages to both health, and the structure and contents. In this instance, the moulds were identified microscopically and the colonies on the walls in different rooms measured. The largest sizes of fungal colonies revealed that the extensive growth of two species in particular had happened much more recently than when the ingress had occurred in 2010. The problems could actually be attributed to a combination of rising damp, a flooding episode from an upstairs bathroom, and the home being neglected and unoccupied for several years. The case went to court, and the judge found in favour of the water company rather than the claimant.

Conversely, fungal growth data may also provide evidence to support claimants as well as defending insurance companies. A home-owner in West Lothian was away for almost three months, but on return found that there had been a water leak which had not been noted when his brother checked the property three weeks before his return in February 2013. In this case, colonies formed by eight different moulds were identified microscopically. From what was known of their growth rates and the sizes of the colonies developed on walls and carpets, and the temperature in the property, it was apparent that these had developed in not more than 2-3 weeks, and perhaps less. This result was consistent with the leak having developed after the brother's visit on 2 February 2013, as had been reported by the home-owner, and the claim was met by the insurance company.

Further information

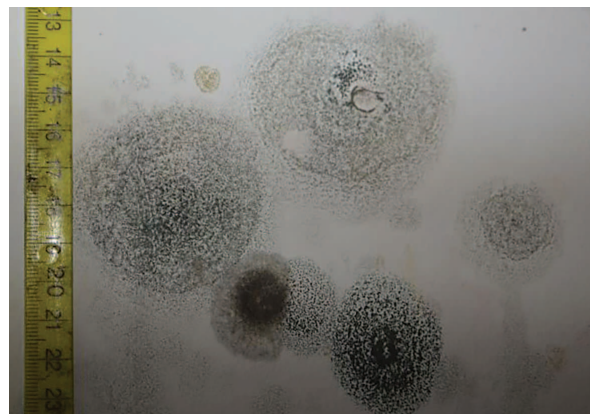
Further information on the use of fungi in forensic cases can be found in the following publications:

Hawksworth DL, Wiltshire PEJ (2011) Forensic mycology: the use of fungi in criminal investigations. *Forensic Science International* 206: 1-11.

Hawksworth DL, Wiltshire PEJ (2015) Forensic mycology: current perspectives. *Research and Reports in Forensic Medical Science* 5: 75-83.

Wiltshire PEJ, Hawksworth DL (2014) Palynology and mycology: forensic evidence from soil, clothing, corpses, carpets, walls, and food. *Expert Witness* 1 (7): 28-31.

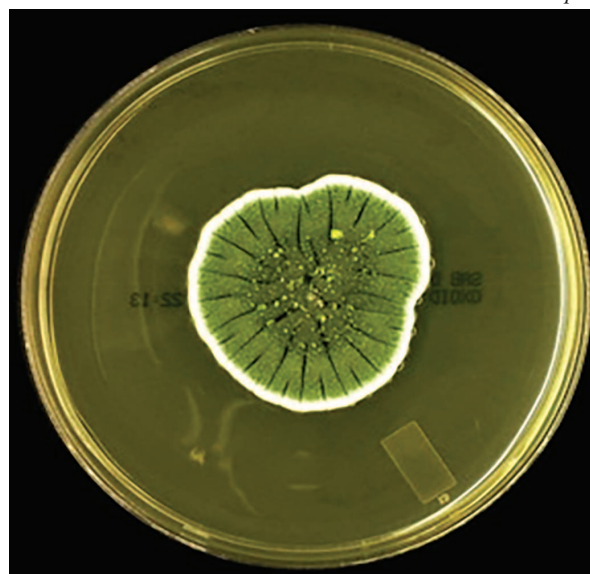
Opposite, Penicillium stoloniferum (green) and other fungi growing on remains of a meal.



Above, Aspergillus niger colonies developed on a wall following a water leak.



Above, Mucor hiemalis (arrow) growing on the abdomen of a human corpse.



Above, Penicillium brevicompactum colony growing in a Petri dish.

