Certain fleshy fungi have fruitbodies composed of two parts, one that grows above the ground, the mushroom, and the other a solid body of sterile tissue which grows below ground and is called the sclerotium. Such an example is the ‘Stone Fungus’ (*Polyporus tuberaster* Jacq.: Fr.). This is a fungus of Western Europe (although not Britain) and the northern parts of North America. In North America, the underground sclerotium was not immediately associated with the mushroom and it became known as the ‘Canadian Tuckahoe’.

The sclerotium is normally only found by accident, often dug up by farmers when ploughing. It appears as a hard, dark grey to almost black body, approximately round or slightly flattened, with a slightly wrinkled surface, and about the size of a fist. Exceptionally they may reach up to 40 cm across and weigh up to 4.5 kg. The fleshy interior contains sandy particles and it is difficult to cut, appearing very dark green in fresh specimens before becoming dark grey with a marbled aspect. It often comes as a great surprise to find that, when planted, these apparent stones grow! More importantly, they grow to produce excellent edible mushrooms. The round sclerotium can be planted about 8 cm deep in a buried pot or in garden soil and watered twice a day for about four days. A crop of polypore mushrooms is produced, which can be removed and, with luck, several further crops may result. The production of mushrooms can be controlled by the gardener reducing the rate of watering.

The normal function of a sclerotium is that of a storage body, able to withstand drought, frost or other unfavourable conditions. It therefore tends to be a perennial structure able to form fruitbodies over three or more years. The sclerotium can absorb up to fifty per cent of its own weight when soaked in water. The mushroom forms a pale brown cap, up to 15 cm across, covered with buff/brown scales and borne on a stout, central stem. The underside of the cap is poroid, placing the fungus in the family *Polyporaceae* a. str.

The underground sclerotium of *Polyporus tuberaster* is popularly known as the ‘Stone Fungus’. Fungus Stones were well known and much prized in Ancient Rome, where they were sold under the name of ‘Lapisdus’, and continued to be traded throughout the period of the Italian Renaissance, during the 16th and 17th centuries CE. In Italy it is still cultivated occasionally in cellars, under the name of ‘pietra fungale’. The hard, underground sclerotium may be found in the mountainous regions of southern Italy. The ball-shaped sclerotium was often collected, kept, and when watered could produce numerous, edible fruitbodies over a long period. This mystified early workers who gave several explanations, including the possibilities of either an animal or a mineral origin. The early mycologist Pier Andrea Mattioli, in his *Commentario* of 1566, referred to the sclerotium as ‘Lapis nymus’, or the ‘fossilised faeces of the lynx’. Ferrante Imperato in *Della historia naturalis*, first printed in 1590, gives mention to the ‘Fungo di pietra’ (fungus stone). Paolo Boccone in 1697 (Museo di fisica et esperieze, Venice) provided a stylised, wood-cut engraving of the ‘Pietra fungale’ (Fig 1), clearly showing the relationship between the sclerotium and the polyporoid fruitbody. This illustration depicts the fruitbody alone, without the sclerotium.

There are other fungi produced from an underground sclerotium. These include the ‘Umbrella Polypore’ (*Polyporus umbellatus* Fr.) the ‘Olatafa’ (*Lentinus tuber-regium* (Fr.) Fr.) of equatorial Africa and South-east Asia, and the ‘Black Fellow’s Bread’ (*Polyporus mycetates* Cooke & Massie) of Australia.

‘Blackfellows Bread’ or ‘Native Bread’ is fairly common throughout southern Australia, generally dug up as large, dense round lumps, which may be buried up to 60 cm below ground.
level. They vaguely resemble irregular loaves of bread, and there is evidence that they were used by the aboriginal tribes as a food source, particularly in Tasmania, when no other food was available because of adverse conditions. A fresh sclerotium is soft enough to be cut with a knife, and has been compared with ‘over-boiled white rice’ in texture and taste. The outer surface is dark and finely wrinkled, usually with soil particles adhering to it, but when cut in half the interior is seen to be creamy white and coarsely granular, rather like compacted lumps of sago. The structure grows up to 20 -30 cm in diameter and may weigh over four kg, but exceptionally they can grow to 60 cm across and weigh over 17 kg. The sclerotium first received a scientific description in the 1830s and was given the name of *Mytilita australis* by Berkeley. At the time, they were thought to be related to the truffles, which also grow underground. Another sixty years elapsed, however, before the fruitbodies were associated with the sclerotium, and the fungus was then given the name of *Polyporus myiliae*. The fruitbody is a centrally stemmed bracket fungus, with a white cap, about 10 cm across, a pale to egg-yellow centre and small, white pores on the underside. The cap is supported by a short, stout, central stem. As in the case of many Australian mushrooms, there appears to be the need of a heat stimulus to produce the fruitbodies, for they only appear either after a bush fire, or in very hot conditions. Blackfellows’ Bread has been also recorded from New Zealand, although it is thought to be introduced through the importation of eucalyptus railway sleepers.

In Australia there are one or two other bracket fungi which similarly develop from an underground resting stage. One of these is the ‘Stonemaker Fungus’ (*Polyporus tumulus* Cooke & Massee) in which the fruitbodies are produced by a large, elongated false sclerotium consisting of a mixture of the fungus mycelium and sand. This species is to be found growing in the sandy areas of southern Australia.

In equatorial Africa, fruitbodies belonging to the genus *Lentinus* Fr. are a familiar sight in the forests. Mostly they grow on the dead stumps and fallen tree branches, although a very few arise from an underground sclerotium. One of these is *Lentinus tuber-regium*, which is also known under the Madagascar name of ‘Olatafa’. The word ‘Olatafa’ is a corruption taken from an early 19th century account of the discovery of the fungus along the east coast of Madagascar, where the indigenous name ‘hola-tafan’ was used. The local word ‘holatch’ refers to a mushroom, and ‘atafan’ to a species of tree (*Terminalia catappa*), thus the ‘Terminalia Tree Mushroom’. The large sclerotia, up to 50 cm across although mostly smaller, may be found throughout central Africa. The distribution extends to much of south-east Asia, tropical Australasia, including Papua New Guinea, and some of the Pacific islands, such as Samoa and the Solomon Islands. In Nigeria, it is called ‘ohu’ (Yoruba tribe), ‘o’usako’ (Urhobo) or ‘osu’ (Ibo), whilst in the Solomon Islands, it is called ‘poekala’.

The mushroom emerges from the buried sclerotium that is large and spherical. These sclerotia develop within fallen tree-trunks and when these have rotted away, the sclerotia fall to the ground where they lie spaced at intervals in long straight lines as an indication of their origin. In the course of time, they become covered with soil and rubble so that when the mushrooms emerge from them they may appear to be growing on the soil. Mushroom formation is initiated by the appropriate temperature and the availability of water, usually coming from the first rains, and takes about seven days. The mushroom has a pileus up to 15 cm across, which soon forms a shallow funnel-shape, and is rather scaly. When young it is whitish, soft and tender, but becomes yellowish brown, hard and leathery. There is a central, solid stipe that is also scaly, and the lamellae are very narrow and densely crowded. The flesh is white and slightly peppery to taste. The sclerotium has been variously described as resembling a small bread loaf or ‘a small child’s head’.

Probably the main use of the sclerotium in Madagascar has been as an antidote for a number of plant poisonings. The flesh is crushed on a stone and added to a little water before ingestion. In most African countries, only the sclerotium is used, usually ground down to a fine meal and then either mixed with peppers and onions or added to a soup. In some countries, such as Nigeria, both the mushroom and the sclerotium are consumed. As in the case of the European ‘Stone Fungus’ and the Australian ‘Blackfellows’ Bread’, the underground sclerotium is often gathered and taken home. It is then watered to produce mushrooms as the need
arises, maybe over several years. The mushrooms may be eaten whilst they are young and fresh or, alternatively, the sclerotium is crushed and milled to a powder, when it can be consumed as a cereal and added to soups. The tougher mushrooms are sometimes boiled in water, to which has been added the ashes of plants, in order to soften them. This is an African practice for a number of tough fungi, especially for the small 'split-gill' (Schizophyllum commune Fr.), which is frequently chewed and used in stews and soups. In Zaire, only the sclerotium is eaten, when it is sometimes cut into pieces and fried in oil-palm oil. The witch-doctors of the Yoruba tribe in Nigeria have been reported to cover their face with a paste made from the powdered sclerotium and various herbs, in order to help them to see into the future. There are also many accounts of medicinal uses. In a number of African countries the crushed sclerotium, mixed with peppers and onions, is administered to treat pains in the head and the stomach, and against infection. This has also been applied for the treatment of asthma and for hypertension. Additional uses of the sclerotium in West Africa include use for brushing teeth, and for the manufacture of corks and plugs. In the Solomon Islands only the mushroom is eaten and the sclerotium is ignored, whilst the Gogogola tribe of Papua New Guinea carve the hard, old sclerotium for the manufacture of club-heads.

In North America, the name 'Canadian Tuckshoe', given to the sclerotium of Polyporus tuberaster, was used to avoid confusion with another fungus. 'Tuckahoe' was a name given to a sclerioid fungus by the indigenous North American tribes of Virginia, although the term was also used to describe a range of buried roots and tubers. It was also known under the name of 'Indian Bread' because it vaguely resembles a bread loaf and has a flour-like, starchy quality when ground down. It represented an important element of the native diet. The distribution extends from New Jersey down to the Gulf of Mexico, and westwards as far as Kansas. When first discovered and described in 1762, it was thought to be a type of puffball. When fresh, the sclerotium is moist and pliable and can be cut with a knife but it quickly dries to become very hard and tasteless. It is eaten either by roasting and adding salt or it is dried and pounded to be used for making a kind of bread. The flesh is known to contain pectin and this has made it suitable for use as an arrowroot substitute, after boiling. Although originally used by the indigenous tribes, it was later adopted by the expatriate African slaves. It is usually found attached to tree roots in low-lying marshy places, often a metre or more below the surface. The form and size are that of the potato or sweet potato, although the largest recorded specimen was 104 cm long and about half as broad, and they may weigh up to 175 kg. The inner flesh is compact and white, but this is covered with a coarse, brown skin, somewhat resembling a coconut. For this reason it was given the scientific name of Plocyma cocos (Schwein.) Fr. meaning a thick-skinned coconut. For many years, scientists only knew this fungus from the sterile sclerotium form, but eventually it was linked to a bracket fungus, a polypore named Wolfiporia cocos (Schwein.) Ryv. & Gilb, which normally grows on trunks and tree branches appearing as effused, pinky buff patches. The fungus originates from a mycelium in the tree roots, forming a brown cubical root and butt rot on a wide range of trees.

It was only after the fruitbodies were discovered.
An improved technique for obtaining single ascospore cultures of ascomycetes

PEDRO W. CROUS

Dept. of Plant Pathology, University of Stellenbosch, P. Bag X1, Matieland 7602, South Africa
e-mail: pwc@naties.sun.ac.za

A revised method for obtaining axenic, single spore cultures of ascomycetes is described. The proposed method greatly simplifies the technique for picking up single ascospores, helps to clearly distinguish different taxa occurring on the same substrate, and also enables mycologists to easily compare spore germination patterns.

Keywords: ascospore germination patterns, Botryosphaeria, Mycosphaerella.

One of the primary concerns of mycologists in establishing a new anamorph-teleomorph connection is that it has to be confirmed in culture. Cultural studies are, however, also fallible, and therefore we have come to insist on single spore cultures for confirming such links, and also for subsequent molecular studies. For several years I have used a relatively simple technique to culture ascomycetes associated with leaf spots and stem cankers. Host tissue with ascomata is soaked in water for 1-2 hrs, and then attached to the lids of Petri dishes containing various media (Crous et al., 1991). In doing such studies one invariably comes across species with spores that do not germinate on nutrient-poor media such as water agar; and therefore it is best to use a richer medium such as 2% malt extract agar (MEA) as standard. Once ascospores have shot out onto the surface (after 24 hrs in the dark), single, germinating ascospores can be transferred to fresh MEA plates. Previously, in producing slides for microscopy, a large block of agar containing ascospores was flooded with lactophenol and covered by a coverslip (Crous et al., 1991). This method proved unsatisfactory, however, as these slides did not preserve well. A better option is to take a small block of agar with germinated ascospores, add one drop of lactophenol, cover it with a coverslip, and gently heat over a flame to melt the agar. The slide can now be sealed normally, retained along with other slides of the fungus in question and compared with slides of spores produced from cultures or used in descriptions.
Another problem that may be encountered is that certain species will have ascospores that germinate at 25°C, only to lyse soon after. To compensate for such taxa, I now use a rule of thumb: at least one plate of five singly transferred ascospores at 15°C (Crous, 1998), as I have already encountered several species of *Mycosphaerella* both on Myrtaceae and Proteaceae that need to initially grow (for 7–10 d) at 15°C before they will readily resume growth at 25°C, which is usually the ambient temperature in the laboratory. This technique has now become a standard for obtaining single ascospore cultures and confirming anamorph-telemorph connections in *Mycosphaerella* (Crous et al., 1991; Carnegie & Keane, 1994; Crous, 1998), and has also been used with great success in genera such as *Pyrenophora* (Campbell et al., 1999), *Botryosphaeria* (Denman et al., 1999), and *Tapesia* (Moreau & Mariete, 1996) to name but a few.

An important issue has recently been encountered during these studies, which can have severe implications for establishing anamorph-telemorph connections. It has been noted (Crous et al., 2000) that up to four species of *Mycosphaerella* can be encountered on leaf spots of hosts in the Myrtaceae and Proteaceae. The possibility exists that this may be the norm for most hosts that have more than one species of *Mycosphaerella* infecting them. The worrying fact, however, is that these lesions may appear uniform in colour, size, border and surrounding tissue, creating the impression that possibly only one species may be present. Now that we are aware that different ascospore germination
patterns in fact do represent different species of *Mycosphaerella* (Crous, 1998), the trained mycologist can easily recognise the germinating ascospores as representing different species. When re-examining the slides prepared of the ascii and ascospores (on which the new description or identification will be based), you may be faced with the shock of seeing several species germinating from this apparently uniform lesion. Although ascospores vary in size within a species, they usually tend to retain their shape and taper. I find that ascospore shape is a more reliable feature than size. When sporulating in culture, the shape has thus far always remained stable for the species I have dealt with. Incidentally, the conidium shape of *Mycosphaerella* anamorphs also tends to remain stable, though length, septation, colour and surface texture do not. Thus, anamorph-teleomorph connections, even those based on single ascospore isolates, may quite easily prove incorrect, as is evident from the huge number of weird and wonderful anamorph genera linked to *Mycosphaerella* (see compilation in Corlett, 1991), versus the 23 form genera we now accept (Crous et al., 2000).

Another problem that we encounter with the technique as outlined by Crous et al. (1991) is that ascospores of different species frequently lie mixed in one spot, as well as germinating ballistospores of several yeast species that also discharge minute spores, which can contaminate apparent single ascospore transfers (Fig 1A). Recently, I decided to amend this technique by sticking the host tissue to the upper half of small glass coverslips, and then inserting these vertically into the agar, on the edge of the dish, with the host tissue facing towards the centre (Fig 1B). I found that ballistospores do not discharge very far, whereas ascospores of *Mycosphaerella* could discharge for up to 2 cm from the coverslip. A further interesting observation was that not all species had the ability to shoot ascospores the same distance, making it somewhat easier to recognise different species on the agar. This modified technique is also convenient when eight ascospores need to be obtained from one ascus. In the case of letting them shoot down onto the agar, they form a tight cluster that is difficult to separate. In contrast, when they are discharged over the length of the dish, they spread over some distance, but are still recognisable as spores coming from one ascus. Finally, having the coverslip inserted vertically into the agar also greatly simplifies the initial focusing of the dissecting microscope onto the agar surface.

In conclusion, different species of ascomycetes (especially in *Mycosphaerella*) frequently colonise the same lesion either as primary and secondary pathogens, or perhaps even as saprophytes. By discharging ascospores over the length of the Petri dish, instead of downwards onto its surface, the ascospore germination pattern as well as the distance discharged may now be used in the identification of different species. Be warned, however, that each new taxon will have its own humbling lesson, and that different species can frequently have the same germination pattern. The ultimate proof remains, therefore, to induce sporulation in culture, and link this to the original slides if possible. Most taxa tend to form ascomata more frequently when inoculated onto sterile host tissue (autoclaved tissue works fine) on water agar, or carnation leaf agar (Fisher et al., 1982), whereas the anamorph usually readily sporulates on standard media such as MEA.

References