Scientific Principles
Of Improvised Warfare
And Home Defense

The Advanced Biological Weapons Series

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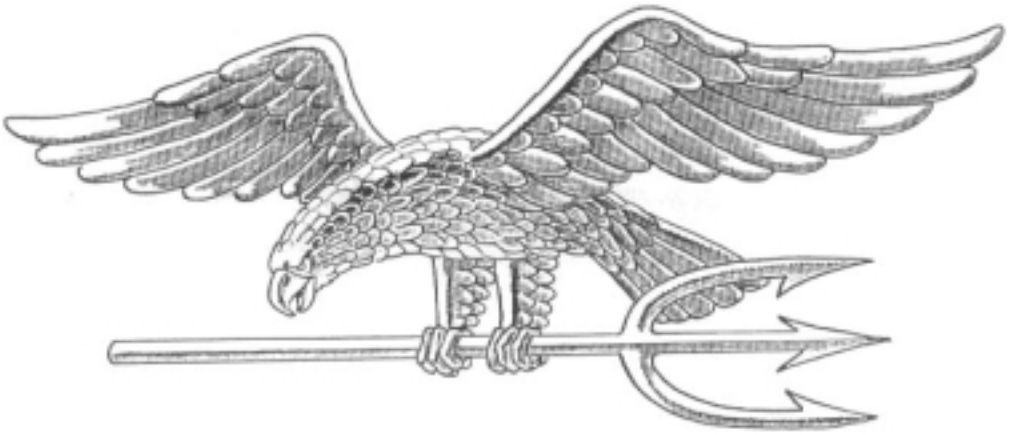
Mold Based Weapons



Some say “If life gives you lemons, make lemonade”
I say “If the warden gives you bread & water, make Aflatoxin”

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Chapter 1

A Short History of Molds & Fungi

Molds or Fungi, names used interchangeably to describe a group of living organisms, affect every aspect of human life. They have caused massive crop failure with resulting national calamity. They have saved millions of lives by producing antibiotics and their metabolic products have also been used to kill directly in biological warfare in Afghanistan (Yellow Rain). They are used to produce all kinds of organic acids, enzymes and food products.

Of several million species of organisms living on the earth, over 100,000 of these are called fungi. Most are scavengers. They eat away (or rot) almost every non-metal material on the planet, usually converting them into rich soil. Billions of years of plant debris would be accumulated and stand miles deep everywhere on the planet were it not for fungi breaking down the dead materials into simple molecules and using them for food.

When they attack living things, they affect mans ability to live and feed himself. The various rots and smuts live on various plants destroying crops and sometimes causing mass starvation. They decay wood in our homes, fabric, cloth, twine, electrical insulation, leather, all foods, and even the glass lenses of microscopes and binoculars when the humidity in the air is high enough.

All **Fungi** share three common characteristics –

1. They have no chlorophyll. This means they cannot take sunlight like other plants and produce their own food using carbon dioxide from the air and mineral and water from the ground. They live off the remains of other plants and animals (as saprophytes) or sometimes on living tissues (as parasites).
2. They reproduce by forming and spreading **spores**. These spores are produced in staggering numbers and can travel thousands of miles in the air before settling on the surface of a new home. The spores act like seeds and germinate to produce a new colony when they land in suitable environment.
3. The growing, food scavenging part of the fungus that we most often see consists of long filaments. These hollow, branched cells which form an entire mass are called **mycelium**. When humans look at this mass we usually call it mold or fuzz.

There are a few exceptions to fungi having all three of these characteristics. Yeast's are fungi but they do not form mycelium. They grow by a process called budding. Certain molds that live in liquids do not form mycelium either and a few species do not produce spores.

The three characteristics listed above describe almost all fungi. They also determine where, how, when, and why they live in certain environments. We encounter these molds and see them in these environments. The dusty little spots that spread over bread, cheese, oranges, and books can be a single cell of a mold with literally miles of mycelium covering the material and forming the visible mass. Some molds live on man in the forms that we call ringworm and athletes foot. A few can live inside of the lungs and other human tissues causing serious disease and death.

All material made by living things such as wood, leaves, rubber, textiles and dead bodies of animal and humans are broken down by the fungi into basic nutrients and used as food. The fungi leave behind the rich humus in the soil that is used over and over again and it is this nutrient cycling that led to the expression “earth to earth, ashes to ashes, dust to dust”.

Molds affect life in unusual ways at times. When water condenses in tanks containing jet fuel, certain fungi grow in the water using the kerosene for energy. The resulting mass or mats have been sucked into fuel lines and caused mysterious engine failure and plane crashes.

When the ancient tombs of the Egyptian pharaohs were opened, an ancient curse was sometimes found on the wall. In at least one case dating from the 1920’s, virtually every person involved in the opening of the tomb died soon after. At that time, medical science had not yet progressed to the point of being able to culture toxin producing molds that lived inside of lungs and slowly killed their hosts. Years later, the artifacts which still contained the dust and air from thousands of years before, were microscopically studied and the tiny spores that were seen were cultured in artificial media. The growing species were determined to be parasitic and accounted for virtually all the mortality. When the tomb was opened, the humidity and temperature were just right and the spores were breathed in and germinated. The curse of the mummy’s tomb lived on due to fungi.

Fungi reproduce themselves by forming billions of spores. Under the microscope, they are usually tadpole shaped with walls forming at the edge where new spores start to grow. The spores are so small (usually under 5 microns in diameter) that the small currents of air can pick them up and carry them in invisible clouds for thousands of miles. The stem rust of wheat has infected fields in Texas and produced spores that were carried into Canada producing epidemics there. They were found in the arctic circle and in a USDA test, the spores were counted exceeding one million per square foot near Fargo, North Dakota after an outbreak in Kansas. There were no closer sources for the spores for hundreds of miles.

Even in still air, some spores are so small and buoyant that a beam of light passed in a closed tube of still air created air currents that sends up clouds of spores like smoke from a oil fire. The largest spores that are up to 80 microns long and 20 microns wide have been measured to fall at a rate of one foot in 30 seconds in still air. The following table gives the calculated distance they can be carried in a 20 mile per hour wind from an altitude of one mile (from high wind driven storms).

	Rate of Fall	Time required to fall 100 feet	Miles carried
Alternaria	3 mm/sec	2 ½ hours	2,900
Helminthosporium	20 mm/sec	25 minutes	440
Puccinia graminis	12 mm/sec	42 minutes	740
Ustilago zeae	3 ½ mm/sec	2 2/5 hours	2,500

Spores have been recovered in air samples from planes flying over six miles high, sometimes in very large numbers. In a bio warfare test from the 1960's, spores with tracer or marker materials attached were released on the first floor of a four story building. In less than five minutes they were recovered in rooms and hallways on the second, third and fourth floors and five minutes later the number count reached hundreds per square foot on the third and fourth floors. This meant that spores were present at levels of thousands per cubic foot of air on the upper floors in 10 minutes.

If you go the local grocer you may see an orange on the shelf with a green spot forming. The spores are so thick that merely rubbing the spot sends a cloud of millions of them into the air. Hundreds of thousands of them will cling to your hand and you will carry them with you, transmitting them to everything you touch and almost everywhere you walk.

Around 1900, a fungi called Endothia parasitica was introduced into the United States from Europe. Within 40 years it nearly eliminated 100% of the commercially valuable chestnut stands in the entire country. It is known by its common name of Chestnut blight. Its mycelium invades the bark through small wounds made by the claws of squirrels or woodpeckers. Within 2 weeks they produce tiny pimple like fruit bodies just beneath the bark. Each of these clumps ruptures the bark and become exposed to the air. Stalks grow outward and spores form at the tip of each stalk and are soon released. Another spore forms beneath and so on until billions are released. More than 50 of these fruit bodies can be found in every square inch of bark of infested trees. It is little wonder that they could sweep an entire industry away in such a short time.

A spore called Tilletia tritici causes bunt, also known as the stinking smut of wheat. In a field with only 1% of the stalks infected, the fungus produces about 5 billion spores per acre. In the early 20th century it was not uncommon to find infection rates of 10-30%. During harvest, the combines would liberate huge, dark, musty clouds of spores that would ignite from static electricity on the combine causing explosions. The combines had to be grounded to prevent static electricity from igniting the spore clouds.

One type of fungi form fruiting bodies that we eat. These are usually called mushrooms or toadstools. They can grow on the surface where they are hunted and picked for food. Some of these produce the most deadly toxins known and have no antidote. The deadly ones will be covered in later chapters. One of the most interesting fungi grow on the roots of forest trees. They are called truffles and belong to a group of fungi called *Ascomycetes*. There are over 40 of these species in the US but none that are the choice edible kinds. The good ones grow mainly in Europe and the largest and

preferred ones and found in Southern France and Northern Italy. They were known and highly prized by the Romans more than 2,000 years ago.

Man first discovered them by watching wild pigs first smell them out and then begin rooting into the ground. The puffball-like fruit bodies grow from 1-6 inches beneath the soil and cannot be seen. In order to successfully find them, man soon used pet pigs on leashes to hunt and begin rooting them out. The hunter then feeds the pig a treat and ties it to a nearby tree while he digs up his treasure. This same routine has been used in Europe for over 20 centuries and the truffle continues to be one of the most highly priced vegetables on the market while forming a stable cash crop for farmers with the right stands of trees that have the ideal roots and soils. These are usually Oak and Beech trees that are planted and once they are established, pieces of the truffles are scattered about and then covered. The first harvest comes in 6-10 years and annual crops are recovered for about another two decades.

The interesting thing about the truffles is that the fruiting body is very tasty and appealing in odor but the spores in the truffles are not digested in the stomach. They pass through the intestines of the animals that eat them and in this way are spread throughout nature.

Today, humans mass produce mushrooms as a popular food crop, but we are not the only species that cultivates fungi for food. Ants are among the most highly developed social insects. Among the ants, the females do all the work and the males generally have only one function, to fertilize the queen (an arrangement that would be favored by this author and probably most other male members of our species). Ants have various social practices that involve slavery, spying, cannibalism, and growing fungi for food.

Most ants eat liquids only. They will take solids into their mouths and then store it in a small pouch or pocket in the lower part of the mouth. In this pouch, soluble materials are dissolved and this liquid fraction is swallowed. The screened out solids that do not dissolve eventually fill the pouch. Inside the solid mass are a few fungi spores that can grow on the mass. The ants spit out this mass as a certain location in their nests and the molds then begin to grow over the masses. The mold masses are both nutritious and tasty for the ants and provide a food supply from material that they could not digest (just like cows that graze on grass or mushrooms that use horse manure and that we eat in the next step of he food chain).

In a more advanced form of this practice, the leaf cutting ants of North America go out and bring back parts of leaves, chew them up and then place the mass into special compost piles. They enrich this compost with their own solid wastes thereby creating and depositing their own fertilizers (and you thought mankind was the only species with brains enough to figure out how to practice advanced agricultural methods). The ants also know enough to set up their compost piles in soil with just the right moisture content to grow the desired fungi correctly. Some of the ants have hanging gardens of fungus inside special rooms that they suspend from the ceiling. In the tropics, where leaves and moisture are abundant year round, some of these gardens become larger than many

peoples homes. The entire colony of more than a million ants feed solely on their cultivated fungus.

When a new queen moves from her nest to form a colony of her own, she fills her pouch with the fungus ball that the colony has cultivated for millions of years. Once she has found a new spot, she spits the wad of fungus on the floor, grubs out a small chamber, lays a couple of eggs and then crushes them to provide a starter food source for the mold. She adds her excrement to the compost to fertilize it and once the fungi garden is growing to her satisfaction, she concentrates on egg laying.

Some termites also cultivate fungi but unlike the ants, only the very young starting out are fed this food and then after reaching a certain age, they eat other foods. The rest of the fungus is fed only to the royalty and reproductive specialists and apparently are very rich in vitamins and have growth promoting properties. (Like humans, only the elite and powerful get the best foods and treatment).

Many fungi specialize in living only on one type of plant. The ones that effect man most are those that attack food crops. A wide range of mildews attack specific garden flowers, grapes and other plants. Powdery mildews grow only in the cells of living plants, while another fungus called *Cicinnobolus* lives only in the spore cases of the powdery mildews. These have been used to combat epidemics of the mildews.

Molds affect the crops in other ways. One of the most serious is parasitism of seeds. If you buy 100 seeds of pine trees, radish or tomatoes and place them on moist paper towels, you can count how many have germinated in a few days. This is normally 90% or higher. Planting the same number in a pot and keep them well moistened and the germination (emergence) rate will drop to as low as 25-50%. The others were killed and/or decayed by fungi before they broke the surface. In the 20th century, hundreds of fungicides have been developed as coatings for seeds to protect them from fungal attack during planting.

Before the 20th century, many food crops could be planted in the same field for year after year and in a few years the yields would begin to drop drastically. This would occur even with heavy fertilizers. At the time it was attributed to soil exhaustion. Around 1900 in North Dakota, an agriculture scientist named Bolley conducted an experiment. He grew flax in the same soil repeatedly in until the soil was saturated with the fungi *Fusarium lini*. By that time only a few plants survived the growing season. He then took these plants and used them as seedstock for the next planting. This resulted in a good crop. He discovered that a few of the plants had genetic resistance to the wilt and this resulted in the science of producing fungi resistant strains of crops. His primary method of commercially doing this was to grow crops in fungi disease gardens that were saturated with the disease pest and finding strains that were resistant. (This procedure can be reversed to find effective strains of fungi for crop based warfare).

Before 1500 AD, the potato was known only in a few regions of South America. It was cultivated by the Inca Indians in the Andes mountains for centuries before that

time. Today it sustains more people as a food crop than any other food on the plant, exceeding both wheat and rice. The Spaniards carried the Potato, Corn, Tobacco and other plants back to Europe where it was mainly a curiosity for two centuries. Finally it occurred to someone that the tubers of the roots were good to eat. It grew superbly in the cool, moist climate of Northern Europe and by 1800 had become a staple of the diet there. It was also used as animal feed and as feedstock for alcohol production.

The British Isles and especially Ireland converted to potatoes as a primary food source and both thrived and increased greatly in population as a result. Other parts of Europe grew potatoes but had diversified agricultural practices. In 1840, a fungus called *Phytophthora infestans* reached Europe, probably from South America where it has been found growing on wild potatoes. It caused some local epidemics in England and began to spread and grow in intensity. In July 1845, the weather was rainy and muggy and the blight hit with devastating results. Entire fields that were lush and green one week were brown and dead the next. In Ireland, nearly the entire crop was wiped out. The potato famine had begun. Millions starved and a half million died from the starvation and related disease. Nearly two million emigrated, most of these to America. It was a huge national calamity that took decades to recover from.

In 1855 a German called DeBary went to work on the problem. He quickly identified that it was a fungus but could not identify how it survived the winter. It did not live in the soil and rotted the tubers which were not used the following year as seed stock. They finally discovered that it would not always rot the tubers. The infection would be so slight that it would be present in the next years seed stock. When they were planted, the fungus would then infect the sprout and spread to neighboring plants. If the local weather was wet, an epidemic would be underway.

Seed tubers for the next years planting are stored in warehouses or cellars where potatoes are grown in large crops. In the spring they are sorted over and usually cut into pieces for planting. Decayed potatoes are thrown out on a pile. Many of these were infected with the fungi and in wet weather, these produced billions of spores to start the next epidemic. The solution became obvious. Eliminate the dump piles and pass laws to outlaw them. The potatoes have to be burned, buried, or otherwise destroyed. Modern use of fungicides will inhibit outbreaks when used at the right time.

The obvious lesson in biological warfare is to produce cull piles in forms that are not identifiable and that can be spread to cover entire regions without the target aware of the attack (blame it on nature) and make it self reproducing . This will be covered in later chapters.

Coffee has become a popular economic plant and spread worldwide by the 1800’s. By 1850 it was the primary economic plant in Ceylon. Between 1850 and 1870, coffee rust appeared and soon began to wipe out entire plantations. At that time only a handful of people on the entire planet knew what a fungus was and even fewer that it could cause plant disease. Within 20 years the entire coffee industry was eliminated and

the plantation owners and stockholders were ruined. The industry moved to the western hemisphere, without the rust and are principal economic crops today.

Cereal crops have always been the primary source of food for humans and animals since ancient times. Rice in the Orient, Corn in Central and South America, and Wheat and Barley in the Mediterranean. North America developed from a wheat culture. Failure of wheat crops has been described in the bible. Stem Rust became so bad in ancient times that the Romans established a god of rust and made sacrifices to him to protect their fields from this plague. In the first three centuries AD, the Mediterranean area received unusually high levels of rainfall which resulted in outbreaks of rust and led to areas of starvation, epidemics of cholera, breakdown of established rules and customs, and general outcry against government. Recurrent food shortages led to social unrest and turmoil that accompanied the decline and fall of the Roman Empire.

In the United States from 1925-1935 over 35 million bushels of wheat were lost annually to wheat rust. The financial loss was estimated at over $30 million in depression era money. The human cost was measured in black despair, financial ruin, and a struggle for survival.

Common species of rust show marked differences in their ability to infect crops. These are divided into races and varieties that have their own characteristics of ability to infect. Understanding how these different varieties came about fell to a Danish school teacher who around 1800, showed that the rust from barberry leaves would transplant to rye. He also discovered that many species of rust hybridize on the barberry leaves and they act as a plant breeding station for the various rusts. [This provides a common sense means for producing enormous numbers of hybridized rusts for use as agricultural type bio weapons]. Barberry has been eradicated as a plant in many areas to prevent local epidemics.

Many types of fungus can hybridize, mutate, adapt and attack wheat and other crops. These include stem rust, leaf rust, scab, root rot, and mildew. Once the grain is grown and harvested in good shape they face the next onslaught of fungi. In storage, as many as 50-100 species of fungi might be found in a single seed. At moisture of 14-15% or more, the fungi germinate and slowly grow inside the germs or embryos of the seed. The embryo is weakened and then killed and this changes the color of the seed to black. Elevator operators call this grain damaged or sick and the first appearance sets off alarms. Under the microscope, masses of *Aspergillus* spores are found, even on uninfected grain.

The storage fungi also cause the grain to heat since the fungi, like all living things generate heat when they respire. The action of fungi decomposing the seeds causes the release of moisture which then supports more fungal growth and so on. This cycle, if not arrested by drying and moving soon causes the grain to heat to 130 F and stay there for weeks. The rotting creates a stinking black mass. If the thermophilic bacteria take over, they turn the pile into compost raising it to 170 F and can cause spontaneous combustion or grain elevator fires.

All flour contains mold and if damp will begin show mold growth. The heat of baking kills all the fungus spores but can be contaminated after cooling from contamination in the mill. Even good housekeeping cannot reduce the mold level completely for reasons already described. Mold inhibitors such as calcium proprionate are added to bread to keep it free of growing molds for a few days.

A number of fungi affect animals and can be passes on to humans. These include *Aspergillus fumigatus* which infects the lungs of birds. It is one of those that has many strains and only a few infect humans. Mice, squirrels and other rodents in the western US also are affected by fungal lung disease which are sometimes passed on to man.

A variety of fungi also infect man directly. Some infect the skin, nails, and hair and are called dermatomycoses. These can cause disfigurement, irritation, and disability but seldom are fatal. Ringworm and athletes foot are the best known of this group. Others can cause lumpy jaw by infecting underlying tissues after dental surgery and can also infect the lungs. Madura foot was first observed in the region of India for which it is named. It infects the foot and leg and causes slow and painful disability and is sometimes fatal. It usually occurs in individual who walk barefoot and enters through tiny wounds.

In the San Joaquin valley in California, a fungus called *Coccidioidomycosis* occurs and causes several different diseases depending on what part of the body it enters. It can be found in various parts of the Southwestern US but is well established around Phoenix, Tucson and San Joaquin Valley. It produces aches and pains, fever, chills and cough. Similar in affect to the flu or pneumonia, most infections of the lung clear up. Some however spread throughout the body and is highly fatal. It was found that most of the people living in the area had been infected without knowing it and were resistant to it. Scientists have discovered that the fungus also infects many of the species of pocket mice and kangaroo rats of the region and heavily contaminate the soil around their burrow entrances. In the dry season, the dust is carried by the wind and is inhaled with spores attached by the local population. Those susceptible become infected. The infections remain local because the fungus does not survive air travel for long distances.

Candida albicans is a fungus that causes a range of infections in the mouth and lungs. These usually occur with other injuries and only certain strains appear to be pathogenic. These can be severe and resemble tuberculosis.

Edible fungi have already been mentioned in the form of mushrooms. Fungi are also used industrially for different food, chemical and commercial products. The same techniques used for producing these fungi can also be applied to weapons production. These applications will be covered later in the book but a few of the beneficial industrial mycology practices deserve mention here.

Soybeans and Rice have been modified in the Orient by the use of molds to manufacture sauce, saki and cheeselike foods for over 2,000 years. They evolved economies in which most of their protein is plant based rather than animal and the use of fungi have helped convert plants into palatable, storable and nutritious human foods.

Western science has learned to use fungi to produce antibiotics, citric gluconic and other organic acids, ripen cheese, and make enzymes. The waste of fungus material is often rich in growth promoting factors and vitamins which is used in animal feed.

When food becomes moldy, it is usually thrown away as garbage. In some cases, hungry humans have eaten the garbage and found something new and tasty due to the mold. One of these advances from antiquity is the discovery of cheese. Fungi that spoil and rot other foods were found to ripen (rot) cheese and give them their characteristic flavor and texture.

In the area of Roquefort France, several hundred years ago, sheep milk curd was not deliberately inoculated with mold. Farmers milk would often contain debris and offal that was not intended to be present and when it arrived at the dairy processor it was strained out. The milk was not pasteurized at this time so any microorganisms present would remain in the milk. What organisms survived and flourished would depend on the storage conditions and this would determine the ultimate character and quality of the newly made sheep-milk cheese. In the Roquefort region of France, it was soon learned to store the cheese in the limestone caves in the area. Water would percolate through the rocks and created cool, humid air which permitted a fungus called *Penicillium roquefortii* (a cousin of Penicillium notatum used in making Penicillin) to become the dominant organism in the cheese.

After several months of storage, this growing mold would give the cheese a soft texture and tangy flavor. It grew in and partly digests the milk curd and fat. We call it ripening the cheese because saying it rots the cheese would make consumers uncomfortable. The molds would also produce masses of bluish-green spores distributed in irregular veins and pockets throughout the curd which gives the cheese its mottled appearance.

The first time this happened, the cheese makers were trying to produce the old cheese that their forefathers had made. They just happened to store the cheese in the location that let the mold grow preferentially. They did not even know that molds existed and would have considered the study of this new mold to be a waste of time. They did know of the unique storage and location requirements so the making of the cheese soon became a trade secret and mysterious art. If you had the right conditions with the right contamination, you could make good Roquefort cheeses. Some cheese would have the wrong bacteria or mold and this would spoil the cheese and have to be thrown away. They would be uncertain every time they made the cheese as to how many would turn out good and how many would be lost. Because they did not know the science of mold production they did not know how to consistently make Roquefort cheese. Sometimes they made it and sometimes they didn’t. That is why they called it an art.

Modern science has turned the manufacture of cheese into a science. Around 1900, Charles Thom, working for the US Department of Agriculture, began a study of cheese fungi. He isolated the molds responsible for good cheese, learned how to collect

the spores so they could be inoculated into the next batch of cheese, and discovered how to prevent contamination of undesirable organisms so that the production of cheese could be reliable and failure free. His research led to the success of the blue cheese industry in the United States. Details like the strain of the fungus, the amount of salt used to inhibit bacteria, the number and sizes of the holes punched into the cheese to give the mold air to breathe, the temperature, humidity and time of storage all affected the outcome. Blue cheese can now be bought with different physical properties such as hard and crumbly, soft and smeary, with different sharpness or bitiness.

This type of science can be applied to mold based weapons as well. Molds that help the final material self dry into a crumbly, powdery material allows weapons to be made and handled safely as a semi-solid, and self dry to a powder that distributes into the wind in the target area. It can be applied to vehicles and doorways like paint. It can be dropped off along highways in gravel like spheres and then dry into the desired consistency just like the cheeses produced by farmers.

Mushrooms are also molds. The only difference is that the fruiting body or toadstool is large enough to be seen without a microscope and often good enough tasting to be eaten as a food. We have been growing mushrooms in the western hemisphere for about 500 years. Out of several thousand wild mushrooms, only a few hundred are big and tasty enough to be used for food and only a couple are cultivated as a crop.

The one that is commonly grown in Europe and America is called *Agaricus campestris*. It can be found growing on lawns and compost heaps and produces 4 spores per basidium. A variety of it found only on the compost heaps or manure piles has only 2 spores per basidium and in Europe it is called *Agaricus bisporiger* and is the only 2-spored variety that can be cultivated.

It was first grown in France in limestone caves in and near Paris. The temperature in the caves is uniform, cool, humidity is just right, and there is a gentle and constant circulation of air. Until 1900, the French had a virtual monopoly on the world mushroom market. Once again the scientists at the USDA produced the scientific foundation for mushroom cultivation and converted it from art to science.

The primary process uses fresh horse manure, and straw or wood shavings are added. The mix is piled on the ground where it begins to heat because of the growing molds and bacteria. It usually reaches 140 degrees F, where it remains for about 10 days. The manure is then repiled and allowed to heat again which conditions the manure and makes it favorable for the growth of *Agaricus campestris*. It also kills off insects, competing bacteria and fungi, and nematodes that interfere with mushroom production. Proper heating is essential to the process.

Once cured, the manure has been transformed into a rather pleasant smelling compost of crumbly texture. It is piled into beds 6” to 2’deep, and up to several feet wide. Manufacture is also done in shallow trays which are stacked on top of each other. The compost is then inoculated with the mycelium or spawn of the mushroom. It is scattered

on the compost and in about 2 weeks, the fungus mycelium permeates the bed. The bed is then covered in black soil. If the manure and composting were “right”, and the spawn and casing soil “right”, mushrooms begin to appear at 5-6 weeks to three months at which time the compost is exhausted. It is then removed and replaced and the process repeated.

Mushrooms, like other plants are affected by disease, insect pests, and other fungi which can ruin crops. If the temperature or humidity fluctuate it can cause partial crop failure. This is why few people are able to commercially grow mushrooms in their basements or shed.

For centuries, mankind has used barley malt to convert starch into sugars that yeast can then ferment into alcohol. Around 1800 BC, the Japanese began using a fungus to do the same thing. Instead of using corn or wheat to make beer, the Japanese learned to use rice to make rice wine. They would wash the rice, then pile up the moistened batch and inoculate it with *Aspergillus oryzae* or *Aspergillus flavus*. In a few days, the fungus has converted most of the starch into fermentable sugars. The rice is then put into vats, yeast is added and the fermentation produces the wine we call “Saki”. This is the same fungus we have already mentioned that causes the spoilage in stored corn and wheat crops. When just the right water content is present in the seeds, the Aspergillus takes over fermentation from all other organisms. The Japanese learned long before 1800 BC what these correct conditions were and by then had perfected it into a household art without knowing what fungi were. Almost 200 years before Louis Pasteur had invented pasteurization, they heated the finished wine to kill the microorganisms to prevent later spoilage.

Citric acid was isolated in pure form in 1784, long before any commercial use could be found for it. Until 1922, Italy was the principal supplier of it, extracting it from lemons and reacting it with lime to make calcium citrate salt. Most was shipped to the United States. It is used in medicines, foods, soft drinks, silver plating, engraving, dyeing, and printing of cloth. In 1893, scientists found it being produced in molds. Because of high prices, methods were learned to commercially manufacture it from molds. By 1944, over 90% of the worlds citric acid was being produced by mold fermentation.

Aspergillus niger is a common black fungus that is found on decaying vegetation and is a common contaminant in laboratory cultures. A few select strains of it produce large amounts of citric acid under the right conditions. In the processes of the mid 1900’s, a liquid medium is prepared and placed in shallow pans. The spores are sown on the surface like grain seed in the fields. The liquid is kept acid so that competing organisms will not grow. In a few days, the mold forms a thick mat of mycelium on the surface of the liquid and has excreted most of the citric acid that it can produce into the medium. The liquid is then drained off and the powdered calcium is added to form calcium citrate. This is then precipitated out as a solid and purified, packaged and sold. Acres of pans are used to produce the citric acid on large scale. By the same method, biological intermediates and weapons can also be mass produced by anyone, anywhere.

In 1922, a worker in a citric acid plant noticed that not all the acid was citric. Some of it turned out to be a contaminant called gluconic acid. The calcium salt of gluconic acid is calcium gluconate, an excellent form of calcium for pregnant women and young children. It would cure dairy cows of milk fever. Before its accidental discovery in the citric acid pans, it was produced in a very expensive chemical process from dextrose. High producing strains were found to yield it commercially and a new industry was born.

Many fungi have been used since antiquity as drugs in ancient medicines. In 1852, a German book mentions ergot, the hallucinogen that grows on rye and other grains, as a useful aid to accelerate childbirth and by 1800 it was in common use among midwives throughout Europe. They simply picked the ergot grains from harvested kernels of rye. Then they were ground into powder and materials added to give it a medicinal odor and flavor. Alcohol was often added to provide a mild pain killer as part of the "secret" formula. By the 1800's the medical profession found ergot an acceptable part of their practices.

Commercial ergot comes from the infected flower of rye and related grasses. The fungus replaces the seed with a long black spur of fungus tissue. It is commonly found in wild rye even today. The rye plant seems to support the production of ergot at levels greater than that produced in other grasses. In fog bound northern Europe, rye became the principal food crop during the middle ages. The climate would aid in producing epidemic levels of ergot in the harvested grains. The wealthy and powerful would take the clean grain and leave the infected rye for the hungry workers to live on. Taken continuously in small doses, the ergot causes convulsions, gangrene and painful death. Ergoty bread was consumed with fingers, toes and even arms and legs sloughing off as a result. In 944, more than 40,000 died of ergotism in France. Repeat epidemics occurred in 1039 and 1085 making it one of the most dreaded and fearsome scourges of the dark ages. This alone commends its consideration as a tool of biological warfare.

The most famous of all mold products is the story of a Dr. Fleming, who, in 1929 noticed a mold that had contaminated one of his bacteria culture dishes. It had floated in from the air and began growing with the bacteria. He noticed that the bacteria around it would not grow leaving a halo or "zone of inhibition" around the mold. Ten years later, workers in Oxford, England isolated the substance that inhibited the bacteria. They grew the mold in a liquid vat and caused it to excrete the antibiotic that we know today as *Penicillin*. The nutrient liquid medium (beer) is pressure cooked to kill all the other microorganisms. It is then inoculated with a special strain of *Penicillium notatum* (from more than 50,000 tested strains).

During the first years of production, milk bottles were used. Every day, the workers had to empty, wash, fill, sterilize, and inoculate thousands of bottles. The original strain would only produce penicillin when growing on the surface of the liquid. By 1944, strains were found that would grow in submerged vats which allowed for mass production of the material. Sterilized (filtered) air would be pumped into the vats to provide oxygen. A gallon of air per gallon of liquid per minute was used. The vats were agitated to provide maximum surface area for mold fermentation and penicillin

production. One of the best strains of submerged penicillin production was actually found on a rotten cantaloupe in a Peoria, Illinois grocery store by workers at the regional laboratory for the antibiotics production who were stationed there.

It took several years of research to improve production levels to tons and to find ways to purify and improve the shelf life of the antibiotic. It took scientists trained in fields of mycology, chemistry, engineering, physiology and other disciplines working together to solve all the problems that came up.

When you take a deep breath of soil, it has the familiar earthy odor. This odor is not the soil, but the odor of the *Actinomyces*. Molds of this genera are common in soil, manure and decaying vegetation. You can produce this odor at home by simply growing a culture of actinomyces with the lid off. One of the species, *Actinomyces scabies* causes common scab of potato tubers. This is the rough patch that looks like a skin disease you see at the store and is caused by this fungus. Actinomyces bovis causes the lumpy jaw described earlier.

A scientist, Dr. Waksman of the New Jersey Agricultural experimental Station, collected and studied soil bacteria and fungi. He specialized in Actinomyces and the related Streptomyces. In the mid 1900's he found a *Streptomyces griseus* isolated from a manure pile, that produces substances that could kill bacteria not affected by penicillin. This became the antibiotic we know as streptomycin.

Some fungi, especially certain mushrooms, produce toxins that are as deadly as any nerve agent, plant poison, or bacteria infection. Some produce poisons that do not show up in effect for many months allowing large scale warfare to take place without anyone (except those involved in attacking) even being aware that a war is even going on.

More than 5,000 mushroom (or gilled fungi) species have been catalogued and described. Around 40-50 of these produce potent toxins and all were discovered due to the eating of the poisonous mushroom caps. Almost all deaths from eating poisonous mushrooms comes from *Amanita phalloides* and *A. verna*. As little as one third of a cap or even bread soaked in the juice of the mushroom has caused fatalities. Symptoms do not appear for 8-16 hours. By then the victim cannot be helped. Intense pain, vomiting, and diarrhea with greenish liquid, blood and mucus may be voided. Cramps, convulsions, jaundice, delirium, and coma are followed by death which comes only after 8-20 days of horrible suffering. The poison degenerates internal organs and mortality ranges from 60-100% depending on the dose ingested. Those who survive often lose control of their limbs for weeks or months.

Three poisons in Amanita phalloides are phallin, phaloidin and amanitin. The last one mentioned has a chemical formula of C43H45O12N7S. Only 5 micrograms or 1/6,000,000 of an ounce is 100% fatal when injected into mice. This is considerably more deadly than Sarin on a same weight basis.

Amanita muscaria is less toxic but produces an intoxication greater than that of alcohol. It also produces hallucinations. It Siberia, natives would pay up to $20 for a single cap and up to 10 would be needed for a real rousing "high". The toxin would be excreted in a very short time in the urine and this would be consumed by others at the same party to continue the high. It has been reported that the experience could pass through as many as five people before being diluted to an ineffective dose. [The reality of life in Siberia (especially under communism) can force extreme methods of escape.]

Some mushrooms produce substances that are poisonous only when consumed with alcohol. *Coprinus atramentarius* and *C. micaceus* are two of these edible species. Other species contain water soluble poisons that can be boiled and the poisonous water filtered out, making them edible. (The water is thrown out or dried for the poison).

The strongest hallucinogens from fungi include LSD (d-lysergic acid diethylamide) from the ergot fungus *(Claviceps purpurea)* mentioned earlier, and Psilocybin and Psilocin found in several species, most notably *Psilocybe mexicana & Stropharia cubensis* found primarily in central and southern Mexico. They have been consumed by the natives for thousands of years, often as a community or religious ritual. Tests by physiologists and psychologists suggest that these hallucinogens do not provide any universal truth but causes most subjects to get out of touch with everyday reality (which for some provide an alcoholic like escape from the harsh reality of life).

In 1934, a veterinarian in Illinois reported more than 5,000 horses dying from an unknown cause. They all consumed corn or cornstalks that were possibly invaded by fungi. It was called "cornstalk disease" or "moldy corn disease". It produced "staggers or blind staggers" and autopsies showed internal effects similar to that of a virus that causes encephalomyelitis. It was not until many repeats of these losses occurred and studies in the 1950's finally showed that the cause was feed invaded by fungi.

In 1957, it was reported that one species of *Aspergillus flavus*, isolated from damaged corn, could be inoculated into moist autoclaved corn and then be 100% fatal to pigs by ingestion after one months incubation. Only one of nine isolates from the corn was toxic and they very nearly missed this one.

In 1960 in England, more than 100,000 turkey poults died of an unknown cause. The disease, now called turkey-X (no relation to the X-files) was eventually traced back to a lot of peanuts imported from Brazil by the feed manufacturer. As a last resort, scientists began looking at molds as a possible cause. The peanuts were found to be infected with A. flavus and they soon learned that it favored potent toxin production when grown on (or in) peanuts.

In 1527, an Italian botanist Micheli, grew some common fungi, including Penicillium on freshly cut pieces of melon rind. He proved almost 500 years ago that these fungi were living and growing plants. It was a new idea and it took over 300 more years for anyone to discover that these fungi can cause plant disease or rots.

A simple test exists for finding out the cause of disease. Go to the store and pick out some clean oranges and then find one or two with a moldy spot on it. Rub a toothpick or needle into the spores on the decaying spot of the moldy orange. Stick the point of the needle into a sound orange. Attach a small tag to the needle with the date written on it. Then put the inoculated orange into a small, clear, plastic bag with a sound orange and then seal or close the end. Within a few days, a discolored spot appears on the skin of the inoculated orange forming a circle around the point of inoculation. It enlarges every day and in a week or less produces a heavy crop of spores on the decayed portion. Eventually the entire fruit decays and the decay spreads to the other orange (only after the spores appear). You can then take the fungal spores from the orange and grow them on a petri dish. In this way you can see if there is only one fungi or if many strains are growing on it. Once you have a pure culture from an agar plate, you can take its spores and inoculate more oranges to see if they decay as well. If they do, you have now proven that a pure culture of this Penicillium causes decay of oranges.

If you had done this experiment in the 1800's, you would have become the leading biological thinker of your time. It was not until 1880 that a German bacteriologist called Kock formed his "rules of Proof". The rule states that to prove that any specific fungus or bacteria causes a specific disease, it is necessary to –

1) Find the fungus or bacteria in constant and regular association with the disease
2) Isolate the organism and grow it in pure culture (to make sure you do not have a mix of organisms with one causing the disease and the others following it)
3) Inoculate sound specimens with pure cultures of the organism and cause the typical symptoms of the disease in or on the inoculated specimen.

This "science" can be learned and reproduced by anyone anywhere. It is not an art or hocus pocus. Anyone can learn to grow, produce, and handle bacteria and fungi. You can learn how to manufacture toxins and use these organisms and toxins reliably as weapons. This knowledge enables any person, who lives under any government, anywhere, to arm themselves to fight back against tyranny. You do not need guns which throw metal out of long hollow tubes. You do not need explosives or other chemicals used in bombs. All you need to arm yourselves and fight back is a basic understanding of scientific principles. This knowledge is the only thing that will allow people to arm (or rearm) themselves under any form of government and enable them to fight back. That is the purpose of these books. Knowledge is the key to power!

Chapter 2

Basic Biology of Molds & Fungi

Most fungi are *Saporophytes* which means *rotten plant*. It means that they live on dead material which they consume as food. The results of digesting the dead material are something that we call rotting or decaying. We rot or decay the food that we eat inside of our stomachs. The acids and enzymes we produce in our intestinal tract break down the foods we eat inside of the tubelike container of our organs. This broken down material is absorbed through the wall and into our bloodstream for use by the body.

In fungi, the digestion takes place outside the fungal cell wall. Digestive enzymes break down the plant material and then it is absorbed through and into the walls of the fungi. Once inside, they are used by the fungi to build more mass.

Some fungi live on living plant or animal (or human) tissues and are called parasitic. These cause disease in the affected plants or animals and these fungi usually have life cycles specific for the host organism.

Fungal life cycles are extremely varied and can be complex. We will try to keep the basics as simple as possible in this text. There are very detailed books that cover the biology and identification of molds in far greater depth should the reader be interested.

The growing, food getting part of the fungus is a group of long, hollow, branching cells which are called **mycelium**. The individual long tubes (cells) which make up the mycelium grow at astonishing rates. Under the microscope you can actually see it grow. The mycelium can cover a petri dish in 2-3 days and with all the branching and growth, a colony under ideal conditions can produce up to ½ mile of length in only 24 hours. By 48 hours, with no limit of food, a colony can reach several hundred miles of cells.

Fungi can rapidly grow through a loaf of bread in a few days, and in butter from cream that has become infected, it may contain several miles of mycelium per pound. The mycelium of mushrooms and wood rotting fungi can extend for many yards under ground through the soil and decayed wood. Different molds produce different mycelium which is one of the ways that molds can be identified and distinguished from each other. It is through these tiny pipelines that the digestion of nutrients in the environment takes place and further growth occurs.

The individual cells of the mycelium can be as small as 1/100,000 of an inch thick. Like the cells of all living things, they are miniature chemical factories. They produce many enzymes that they excrete outside of the cell walls into their surroundings and on occasion they also produce and excrete acids. These enzymes and acids break down the materials around them into basic chemicals that can then pass through the cell wall membrane and then be used as food. This food goes into little factories that convert the absorbed chemicals into more cellular material which is how the mycelium reproduces itself so rapidly.

An example of mold mycelium seen on grass and on a log.

 A colony of Aspergillus fumigatus grown on culture media. Active mycelial (hyphael) growth is seen in the white region at the edge. Pigmented spores are seen developing behind the sterile hyphae at the colony margin.

Fungal growth is affected by temperature, water, oxygen, pH, food, minerals, vitamins and growth promoting substances found around them.

Most fungi grow best at 70-90 F but will grow more slowly at 50 F. They stop growing at 30-40 F but do not die. They become dormant and wait for better conditions and most will survive freezing for several years. They resume growing as soon as the temperature becomes warm again. Some molds can grow at below freezing which is why meat is kept frozen at 20 F.

Heat will kill off fungi quickly. Growth stops at 1001-110 F and is destroyed at 130-150 F for a minute or two.

Some fungi grow in water while others can grow in seeds, flour, wood or leather at moisture content of only 12-15%. They also often require humidity of 70% or more to grow efficiently.

Nearly all fungi require oxygen to grow and live. Only a few can live on low oxygen content and most are killed by high CO2 content in the air just like human beings, although it usually takes longer.

Ultraviolet light from the sun can kill some molds, enhance the growth of others, but in most it has little effect.

The parts of fungi life cycles will be explained in the following sections-

1. Hyphal growth
2. Colony growth
3. Chlamydospores & Sclerotia
4. Mycelial strands & Rhizomorphs
5. Spores
6. Classes of Fungi and their life cycles

1. Hyphal Growth

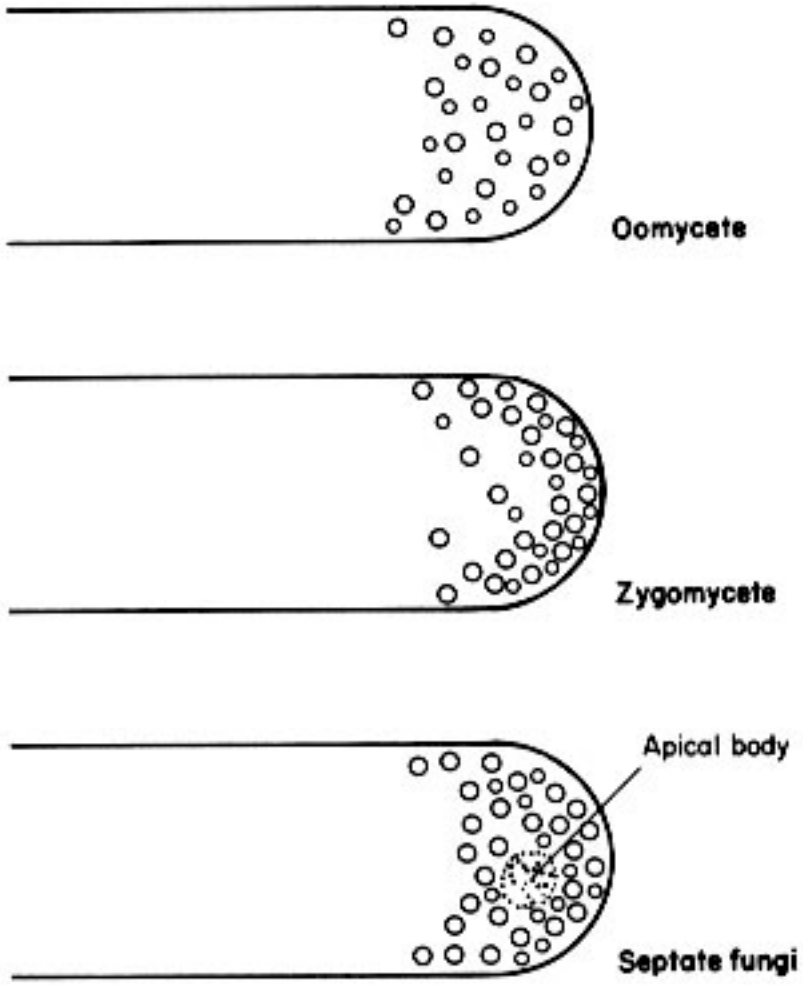
The thin walled tubes of the mycelium are called **hyphae**. The fungi vary in hyphae chemistry and structure which aids in identification. The hyphal walls have layers

in the walls which serve different purposes and these will not be covered here. The act of growth is important in understanding molds and we will explain it.

When the hyphae grow, they add new mass only at the tip. Once branches or septa are formed, they do not change in length or size in any way. Only the tip segment will increase in length and then only at the tip. It does not increase in width or diameter, but only in forward extension of the tube. The cell wall at the tip is usually thinner than the wall behind it. During growth, the cell wall behind the growing front becomes more rigid as food is converted to additional cell wall components.

The hyphae at the tip are very efficient at absorbing nutrients from the surrounding environment. If the hyphae are punctured at the tip, its contents pour out indicating pressure inside. This pressure helps to force the extension of the tip during growth. It also forces new nutrients into the direction of the tip for use in forming new cell mass.

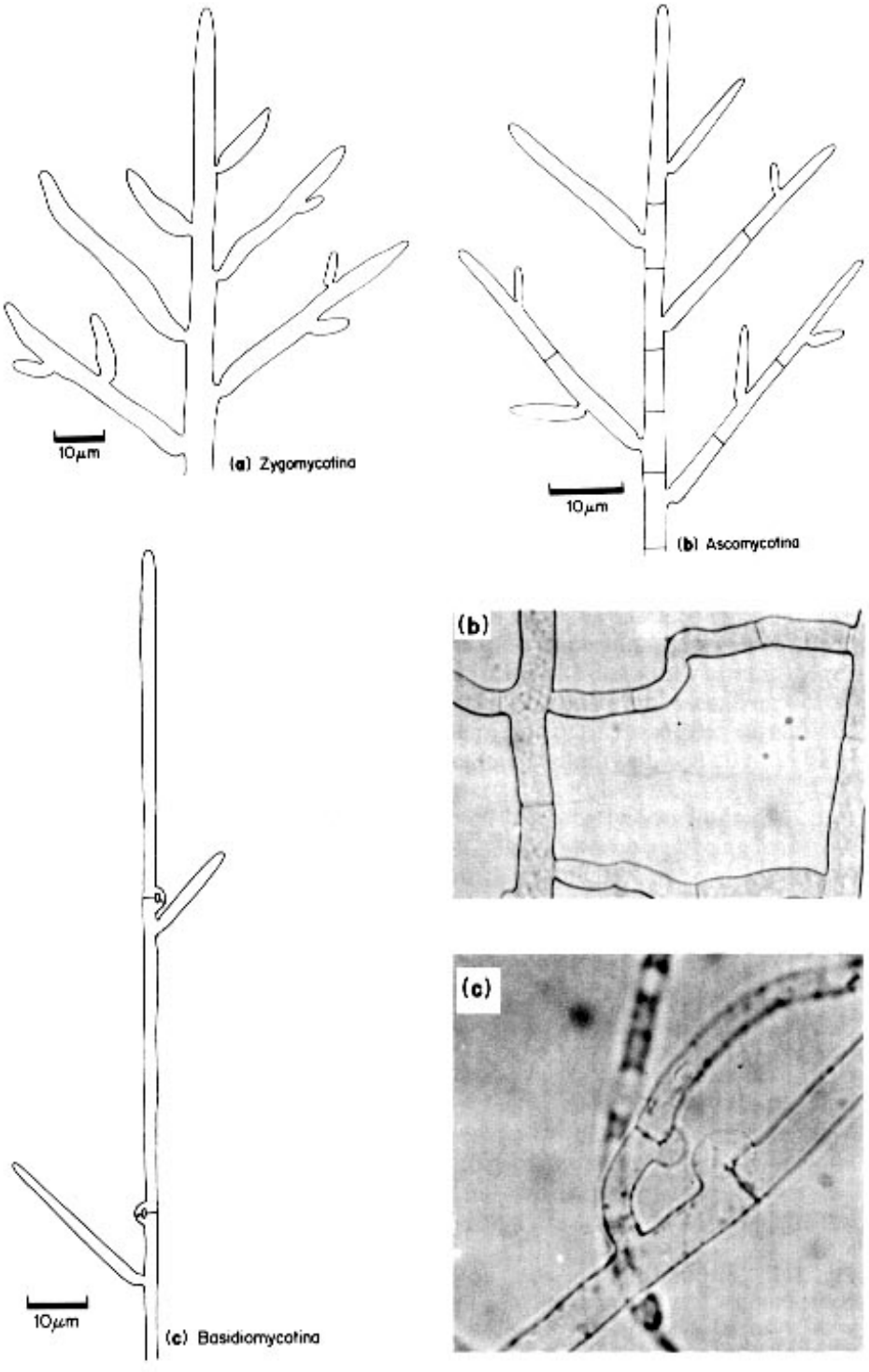
At the apex, or the curve on the leading tip of all actively growing hyphae, a dense cluster or complex of cytoplasmic vescicles can be seen. These move forward with the hyphael growth. The center of the vescicles contain an area that has only small or is completely free of vescicles. These disappear when growth is checked and reform just before growth is resumed.



Examples of vescicles in the tips of different classes of fungi seen under the microscope.

The hyphae form branches where and when the wall is bulged out behind the tip and a new hyphal apex is created. This new apex also contains vescicles. On a flat surface, a leading hypha develops with a series of alternate branches. The lead hypha extends at a more rapid rate than the branches into areas of uncolonized food (substrate) while the branches will extend into areas that have already been colonized. Some primary branches will form and fill in the gaps as the colony develops with a circular outline growing outwards in all directions.

When hyphae are examined under the microscope, separations that appear like walls or divisions can be seen in the long tubes. These are called septa. One of the major differences between the families of fungi is whether they have septa, or cross walls. In members of the Basidiomycotina, a form of septa occurs called a clamp connection.



Examples of Septa and “clamp connections” of Basidiomycetes (C) and other classes. Many fungi can be classified or identified during their life cycles by the branching, septa, and other forms of hyphae they produce.

2. Colony Growth

In nature, colonies can be rarely seen as a single entity. They grow through wood, soil and other substances that are opaque. We grow them in petri dishes to see what they look like as they grow. In the petri dish, after you inoculate the center with spores, you can see a circular outline and growth spreading across the plate. This growth is relatively constant until the edge of the dish is reached. By then, the food has been consumed and sugars have been converted to acids which slow the fungi growth. In petri dish cultures, the oldest hyphae at the center become sealed off with septa, and the individual cells or compartments die from exhaustion of the food supply and accumulation of toxic metabolites.

Mycelium can spread thinly and rapidly or thick and slowly across a dish depending on the nutrients in the dish and the type of fungi. In soil, the mycelium typically grow dense and slowly in nutrient rich substrates and sparsely with a rapid and longer reach in poor environments. As the mycelium grow outwards and food supply is exhausted, the older hyphae separate forming septa, and typically die off and become food themselves for other species. This leaves a ring of living fungus outside of inner rings or circles of dying or dead mycelium.

The best example of this seen in nature is the “fairy rings” produced by various mushrooms. The fairy ring is a circle of dead or dying grass bordered on both the inside and outside by darker green, more vigorous grass. This is caused by the mycelium continuously growing outward and dying off in a circular pattern behind. The ring of best growth competes for all the soil nutrients causing the grass to starve and die. Once the mycelium has grown pass this point, the grass can feed on the nutrients from the dead hyphae. This process is invisible under the soil but the effect is seen in the rings above ground. They will often expand at a rate of about 200mm per year and rings 10 meters in diameter can often be seen on golf courses, in woodlands and lawns. The mushrooms will appear on the inner side of the dead zone in June to November if the moisture is sufficient.

3. Chlamydospores & Sclerotia

The inner protoplasm of the hyphae in some fungi are not completely evacuated as the tips grow outward. In these fungi **chlamydospores** may be produced. When a short length of a hyphae has an accumulation of protoplasm at a particular point, it can round off and become surrounded by a thick wall that is often pigmented. This wall is rich in resrves of glycogen or oil and when the surrounding hyphae die off and decay, these chlamydospores persist as survivial spores. When fresh food or substrate appears in the environment again, they “awaken” and grow anew. Many soils are saturated with spores of a wide variety of fungi, each one waiting for the right food, humidity, temperature and pH to live again.

Instead of Chlamydospores, some fungi form **sclerotia** which are hyphael aggregates. These can be small, irregular, loose clusters of cells or they might be

rounded, compact structures. They can vary from tiny (<100 microns) in size to large (>200mm) or massive bodies.

Sclerotia contain large amounts of food reserves and are designed to survive in drought, intense heat and cold and extreme soil variations. Sclerotia are common in the fungi that act as plant parasites. They allow them to survive for long periods in the absence of a suitable living host. Many will often only germinate in the presence of living tissue of the host plant. Most can survive for years and even decades with very high germination rates.

Most mature sclerotia have an outer rind made up of swollen, globose, cells with very thick, melanized (pigmented) walls. An inner wall contains many storage hyphae. Three different types of sclerotia are seen in fungi.

1. Loose type-where increased and localized irregular branching and septation of adjacent hyphae occurs. Loosely arranged barrel shaped cells are formed.
2. Terminal types-formed by the prolific and often dichotomous branching of the tip of one or several hypha. Numerous septa are laid down forming short celled branches which fuse together forming a compact knot.
3. Strand types-with numerous small, lateral branches forming in a localized region of a hyphael strand. Frequent septation, fusion and interweaving of branches takes place to form a hyphael aggregate from which the sclerotia develops.

In vegetating mycelium, the hyphael strands appear to repel eact other or form away from each other. In sclerotia formation, they are attracted to each other. Numerous fusions occur between the branches and the final structure is able to take in and store water from its surroundings. Its exterior acts as a chemical wall preventing attack by other microorganisms and enzymes so it does not easily become someone else's food.

4. Mycelial Strands & Rhizomorphs

Hyphae may produce cord or strand like structures which are aggregates of many hyphae and function as organs that are different in purpose. They can be composed of only a few to as many as thousands of hyphae. When they form together, they can bridge over areas that contain no nutrients and would not support the expanding mycelium. These structures are often used interchangeably and are called mycelial strands and/or rhizomorphs.

Most are capable of unlimited extension over many kinds of surfaces. They usually form from scerotia or the at the edge of vegetating mycelium and grow away from the colony. Ultimately, they fan out forming vegetative mycelium in new substrate or produce reproductive structures if new food cannot be found. Most of the fungi that produce rhizomorphs are colonizers of trees, leaf litter and woody plants.

The photographs earlier show silky, cotton-like cords that are typical of mycelial strands. They are formed by multiple branches of hyphae forming and intertwining or coiling around themselves. An adhesive forms to stick the branches together and provide structural strength.

The branches of strands can be “vessel”, “tendril”, and “fibrous” in nature and provide both conductive and supportive elements.

True *Rhizomorphs* have a different development. They grow in a coordinated fashion with thousands of closely associated, parallel, unbranched septate hyphae expanding at 5-6 times the rate of ordinary hyphae. Mature rhizomorphs have a hollow center filled with air, surrounded by a long system of wide, thin walled, elongated cells. The outside walls become much thicker and black with deposits of melanin and they serve to protect and support the rhizomorph.

Many rhizomorphs are hard to distinguish from tree roots. If they have branches or trees fall on them, they become flattened and look like bootlaces and the common name for *Armillaria* is the “bootlace fungus”.

The rhizomorphs can grow up to 10-20 feet in length an can move nutrients through it like tree roots to the main colony or mushroom. The internal airway provides oxygen in water saturated soil that would suffocate and starve other fungi.

5) Spores

Most fungi are dispersed throughout the world by spores. They reproduce both sexually and asexually. There are literally thousands of types of spores that have different functions and physical characteristics. The different sub divisions of the fungi are based on these different types of spores that are formed, especially the sexually produced ones. In many fungi, sex organs are not produced and the “sexual spores” are spores produced after a nuclear fusion and meiosis.

The best way to learn about how fungi produce spores is to simply grow and watch them under the microscope and on the culture plates.

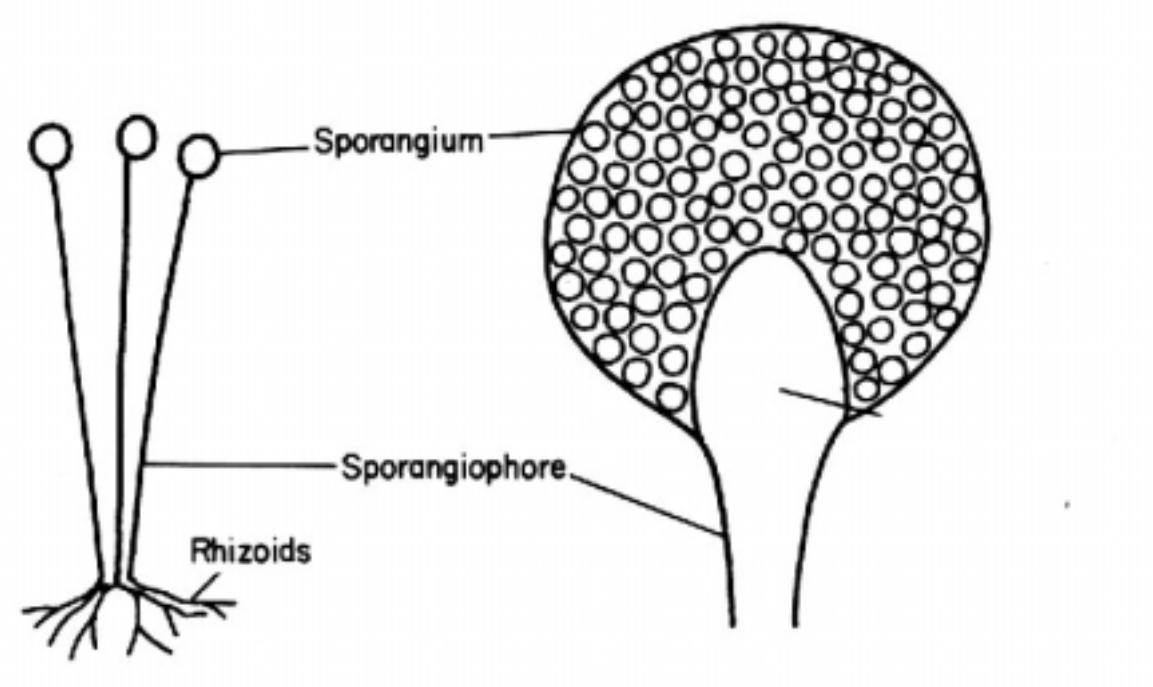
Asexual Spores

There are two types of asexual spores, *Sporangiospores* and *Conidia*

Sporangiospores are enclosed during development inside of a “sporangial wall”. It is released only at maturity when the wall fragments or when pores develop in the wall. There are two types of sporangiospores-

Zoospores- which have one or two flagella (tails) and swim about using these. All zoospores make up the group of fungi called Mastigomycotina. No other fungi have motile spores that let them move in their environment under their own power.

Aplanaspores-or planospores as they are sometimes called are non-motile, and form singly or in groups on the branches of upright hyphae. All planospores belong to the Zygomycotina.



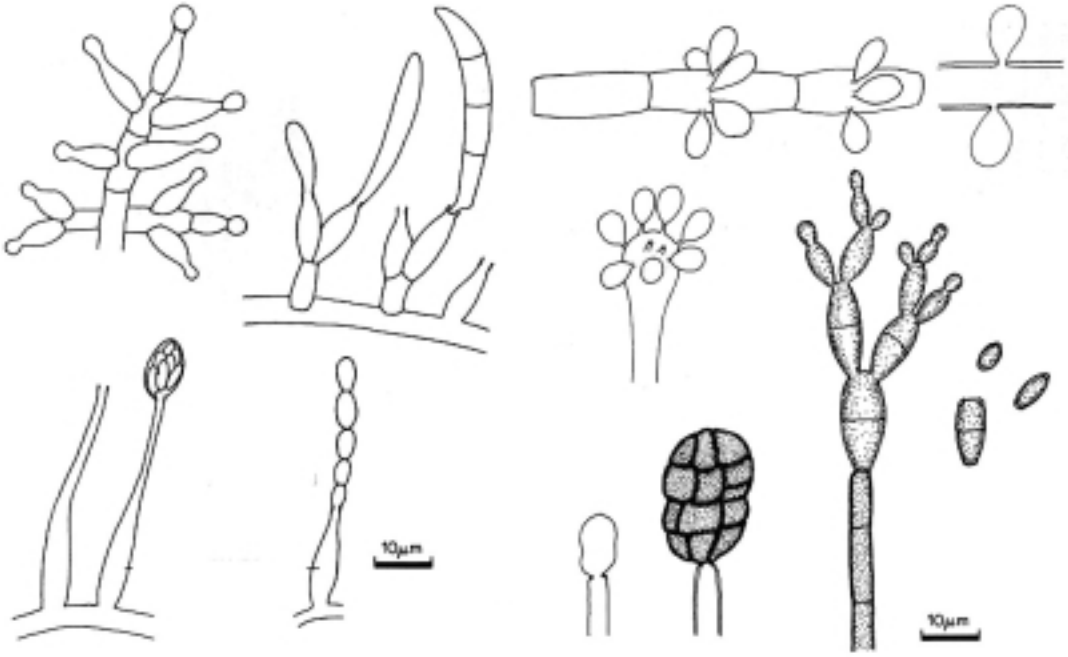
Conidia are distinguished from non motile sporangiospores by the fact that they are not enclosed by a separate sporangial wall. The are usually produced externally at the tip of the hyphae.

Many of the Ascomycotina and some of the Basidiomycotina produce conidia.

There are three types of conidia-

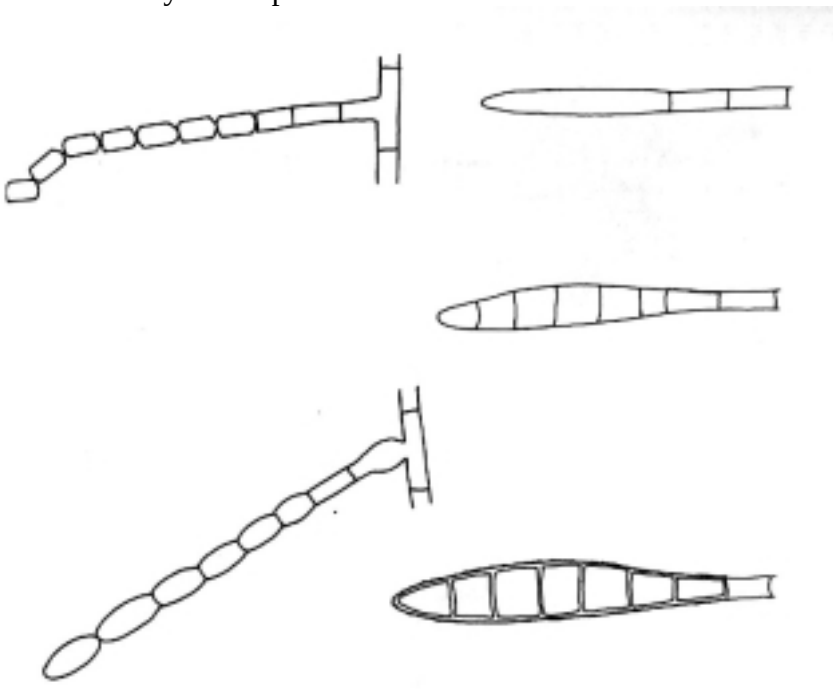
Blastoconidia form buds where a limited area of the wall of a hypha becomes plastic and balloons out to form a bud. They can be produced singly but are most often produced in straight or branched chains

Phialoconidia are produced by a special bottle shaped cell called a phallide. Each conidium forms fully and then is cut off by a septum with another one starting to form underneath. This results in long chains with the youngest at the base. They can also become aggregated into sticky heads at the apex of the conidiaphores, forming stalked spore drops.



Examples of Blastoconidia and Phialoconidia

Thalloconidia form where the hypha separate at the septa and the cells themselves become the conidia. They can be short or long, dry or slimy. They separate at the cross wall when fully developed.



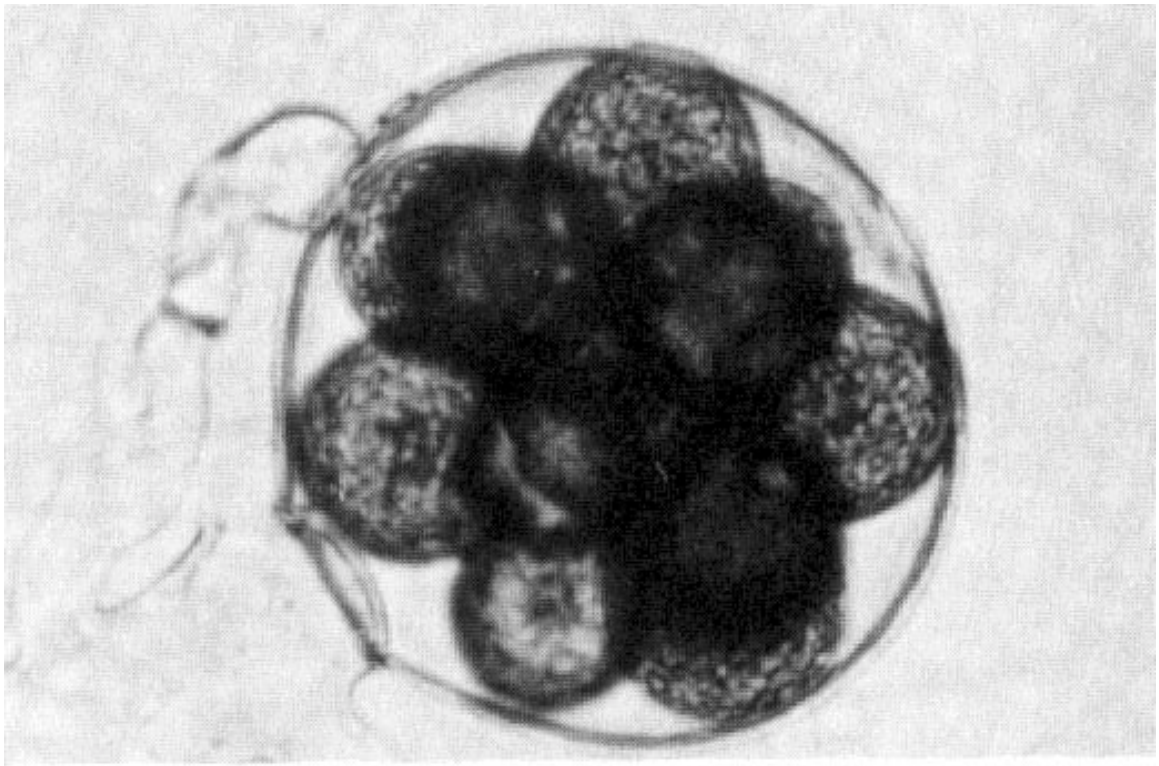
Examples of Thalloconidia

Sexually Produced Spores

The four main groups of fungi are characterized by the spores they sexually produce.

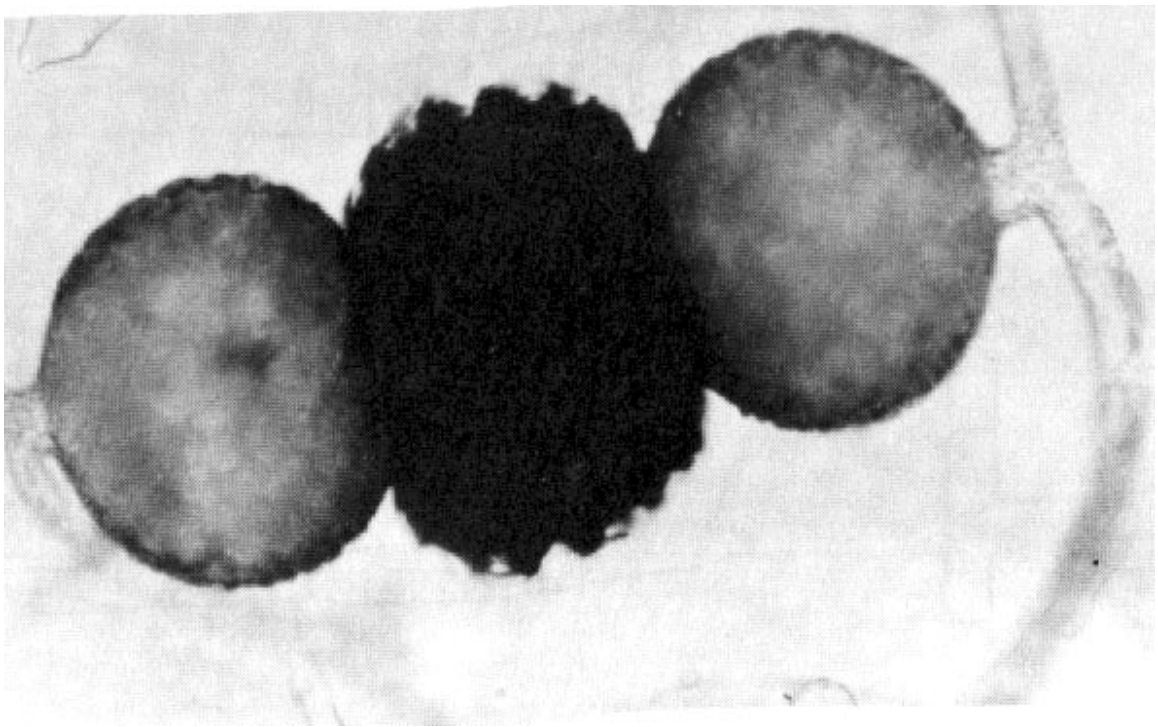
Oospores- Oomycotinia/Oomycetes
Zygospores- Zygomycotinia/Zygomycetes
Ascospores- Ascomycotinia/Ascomycetes
Basidiospores- Basidiomycotinia/Basidiomycetes

The Oomycetes get their name from the Oospores that they produce. Oospores form in a spherical body that grows from a hyphal tip. Cytoplasm inside the sphere cleaves into 5-10 eggs called oospores. The hypha also produce an *antheridium* that attaches to the sphere at a thin area in its wall. It then produces fertilization tubes which penetrate the wall and into the eggs. A male nucleus enters each egg and fuses with the nucleus. These then each develop into thick walled oospores which are released when the sphere wall breaks down.



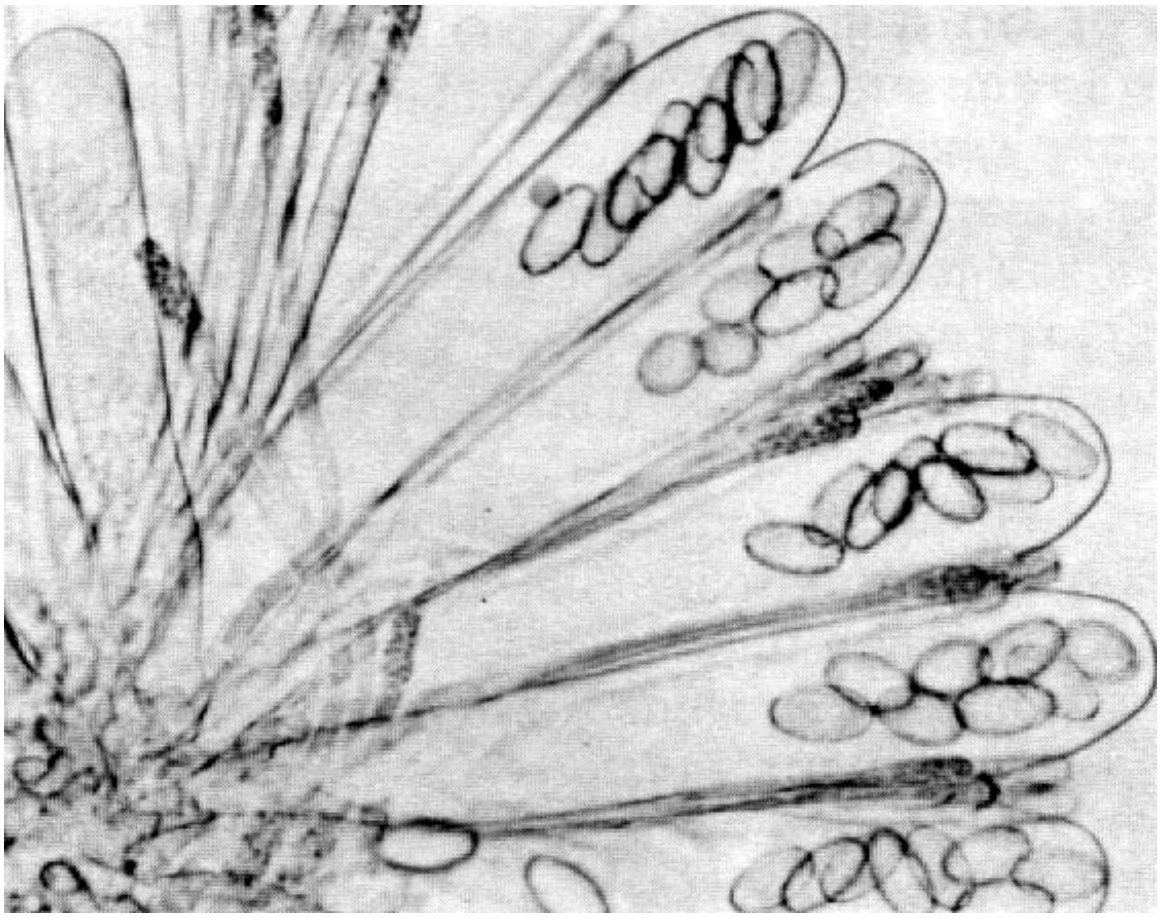
An example of mature Oospores

Zygospores form as protrusions from the hyphae. They contain gametangia, a pair of cells with a wall between them. They are cut off from the supporting suspending hyphae by a septum. The walls between the gametangia breaks down and the two cells fuse to form a new single cell which develops into a thick walled, warty, black zygospore. It is a survival spore. All the zygomycetes produce these types of spores.



An example of a mature zygospore.

Ascospores are produced in a sac-like pear shaped ascus, although a few are born unenclosed. The asci are released when the sac wall breaks down or they may burst the wall liberating the ascospores. All the ascomycotinia produce ascospores.


Ascus containing 8 ascospores each.

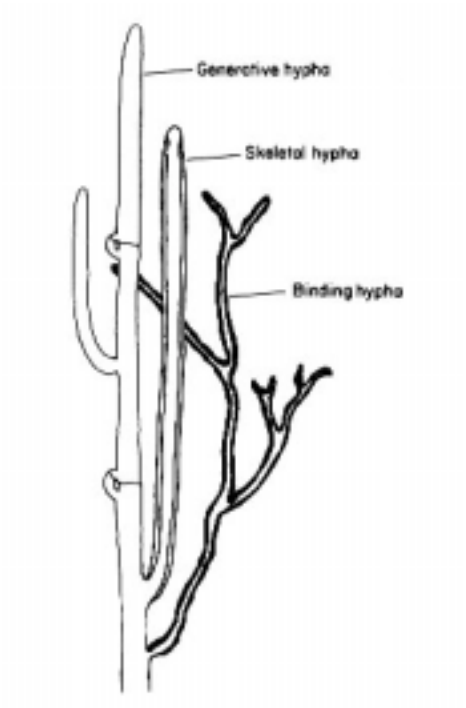
All Basidiomycotina produce basidia or basidiocarps. Like the ascomycotina, the hyphae become aggregated to form small to very large structures. As a group, they have a huge range of size, complexity, and degree of hyphael differentiation. The hyphea form aggregates, branch, fuse swell and may thicken the walls to form these structures.

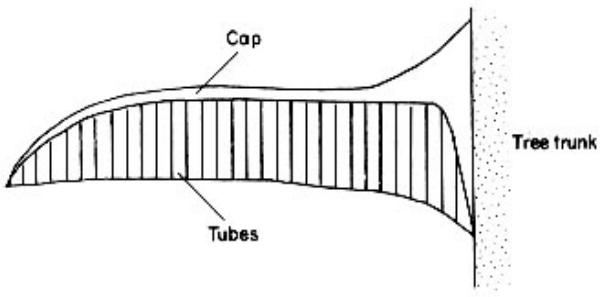
All mushrooms and toadstools form *Agaric* type basidiocarps. It is the disc or domed shape cap that we see growing under trees, and that we eat at the restaurant. They are also called fruiting bodies. The cap is supported by a stalk. On the underside of the cap are the basidia, the wedge shaped gills, tubes, or spines that radiate outward. The spores fall from the cap and are caught and transported in turbulent air and are carried away. The spores are only released in conditions of high humidity.

The Aphyllophorales, another member of the Basidomycotina, also known as the bracket fungi or polypores, produce basidiocarps that are often membranous, corky, leathery, or woody in texture. Some may even be perennial. Three different types of Hyphae are seen this group. *Generative* hyphae are thin walled, branched and septate, most often with clamped connections and are always present. In the basidiocarp, they form *skeletal* hyphae which are thick walled with a narrow lumen, non-septate and usually unbranched and have unlimited growth and act as strengthening structures making the basidiocarp rigid. *Binding* hyphae may be produced instead of skeletak

hyphae and are limited in growth, are very thick walled, narrow, and rarely septate. They are highly and irregularly branched and devlop some distance behind the growing margin. In Basidiocarps with all three, the generative hyphae are the ground plan, sketetal hyphae form the constructional framework and the binding hyphae firmly cobweb them together.

These types of fungi are solely restricted to woody food sources with most seen on a tree trunk or branch above the ground. The more skeletal and binding hyphae present, the more rigid, longer and narrower the tubes become.





6) Classes of Fungi and their life cycles

It helps to be able to identify and tell apart different fungi if you plan to learn how to grow them and produce toxins for weapons. This book will not be a book on mold classification. Only the basics will be described here. For a number of simple, mold based weapons we will describe later in the book, very little identification knowledge will actually be necessary.

Most texts on fungi describe them as free living, parasitic or mutualistic symbionts. They produce no chlorophyll. Some are yeast like but most produce thread like filaments called hyphae which branch and produce mycelium. They produce spores in various reproductive structures. Differences in these structures and their life cycles is how the different species are identified. In many cases (except for mushrooms) a microscope is necessary for learning these physical differences. A cheap microscope from Wal-Mart is usually sufficient.

The fungi are divided into two broad categories

Myxomcota which are wall-less, unusual organisms. They form a mass of protoplasm which feed by ingesting matter and move like amoeba. The slime molds belong to this group.

The plasmodium of a typical slime mold.

Eumycota which are all the true fungi which have cell walls. Modern texts usually recognize five separate branches.

1. Mastigomycotina- which are zoosporic fungi that are mainly aquatic. The types are broken down according to the distinctive type of zoospore produced.
2. Zygomycotina- which have aseptate hyphae, asexual spores nonmotile aplanaspores and a vegetative haplophase.
3. Ascomycotina- also vegetative haplophase, hyphae septate, with simple pore. They produce ascospores within an ascus. Asexual conidia are often present.
4. Basidiomycotina- are vegetative dikaryophase, th mycelium septate with dolipore septa and often with clamp connections. Produces basidiospores on basidia. Discharge is usually violent.
5. Deuteromycotina- also known as “fungi imperfecti” due to asexual (imperfect or anamorphic) mycelial state. They are solely conidial or mycelial.

Chapter 3
Isolation, Cultivation & Identification of Fungi

As the title of the chapter suggests, we will cover the contents in four parts-

1. Isolation of Fungi and dealing with contaminants
2. Cultivation of Fungi on Culture Medium
3. Using the microscope for fungal ID
4. Using Identifications Keys

1. The Isolation of Fungi and dealing with contaminants

Almost every single substance on earth has not only mold spores, but often entire communities of molds and spores growing on and around it. Sometimes, one species will dominate for a time and can be seen as a mushroom, a mycelium growing on rotting corn, or a black mass on rye (ergot). In all three cases, with some very simple skills, these molds can be seen with the naked eye, easily isolated and converted into weapons without a college degree. A microscope is usually necessary for serious work so you can see the spore bearing structures and can use a sterile needle to remove the correct ones for inoculation and then growing pure colonies.

Isolation can be accomplished usually by transferring the candidate mold from its natural habitat to your lab. The candidate sample can be a poisonous mushroom cap, a soil or feces sample from rodent hole entrances in an area known to harbor a toxic fungus, a sample of ergot on a rye seed, and using a blacklight, a sample of aflatoxin producing corn. Once in the lab you can use a number of methods to isolate the desired mold.

The easiest method is too look at the sample of the material under the microscope and see the individual strands and spores. If the structures are the right ones for the desired species, you can take an inoculating needle or loop, heat the tip to red hot to sterilize it and then transfer the spores or mycelium into a sterile plate of culture medium. Usually you can put a tiny speck of the agar, jello or other semi solid medium you plan to use on the tip and then the spores or mycelium will stick to it. Jam or jelly will even work. You can also moisten the tip with a small amount of glycerine. Once this transfer has been made, you only need to wait a few days for the mold to grow and form its pure colony.

You can use this technique to obtain pure mushroom cultures by breaking open the fruiting body and transferring some of the tissue into the culture medium directly. Some of the most poisonous mushrooms produce toxins roughly equivalent in toxicity to Sarin or Ricin and make excellent weapons.

Another excellent method for growing fungi for isolation is to use a “moist chamber”. It can be any container in which you can put water soaked cloth, cotton, paper,

peat moss, or soil into. A sponge will even work. This provides the necessary moisture that molds need to get started. You then add the sample of material with the suspected mold culture in it. You also want a lid.

If you do not have a black light to see aflatoxin producing species growing on corn, you can place kernels of corn into the moist chamber, put a lid on it and wait a few days. Water is added to the container to saturate the material in the chamber and then a paper towel or filter paper is placed on top of the wet layer. This keeps the specimen from coming into direct contact with the moisture bearing layer.

It is best to use a clear glass or plastic container so you can watch the progress without opening the lid and introducing more contamination. It is best to add the specimen on top of the filter paper and moisten it slightly to get it started. Keep the temperature warm and constant.

In a few days molds will begin to appear in the chamber on the specimen. They are usually very small and should be studied with a microscope before the colony's begin to grow together. A magnification of only 15-20 times is all that is needed (and a bright light). Once you have located your candidate colony, you can simply transfer the spores or mycelium from the chamber to the culture plate by the method described above.

You can use any candidate material for the moist chamber. Peeled skin from athletes foot. The kernels of corn, rye or other grains. Wood, leaves, soil, foods, and other sources can also be used for the learning student to practice with. In high school, the author grew his first mold by moistening bread and putting it under a glass dish for a few days.

For those with laboratory agar, simple water agar can be used and the specimen placed directly into the petri dish. If you add any nutrients to the agar, only the fastest growing molds will usually be seen and will overgrow everything else.

If the mold produces a large structure that is spore bearng, the structure will usually be contaminated with other spores. It can be sterilized by soaking in 10% chlorine bleach for 60 minutes. Then its surfaces are sterile and the structure can be placed into the culture plate and cracked open to liberate the pure candidate colony material inside. You can also often clean the surfaces by smearing the structure against agar to clean off the foreign spores and bacteria.

If you have a mold that prefers a particular growth medium (such as ergot on rye) then this can be incorporated into a culture plate and you can directly place the specimen into a medium containing the food or nutrient (such as sterilized rye seed).

Antibiotics can be purchased at any local pharmacy or livestock store. These can be added to a medium to stop bacterial growth and will stop or slow down undesirable competing molds.

If you have a mix of colonies grown together and believe that your candidate is present you can use a method called dilution plating. You simply take one gram of the solid mix of mold growth, mix it into 9 ml of sterile water. Then one ml of this solution is mixed into 9 more ml of water. This dilutes the original mass by 100:1. You can continue this dilution as far as necessary. A desired dilution is then taken and inoculated onto a plate or other medium with an eyedropper. A single drop of diluted material spread over an entire plate allows distinct and separate colonies to grow.

You can also create moist chambers in nature. You can take a water sprayer and soak rye grain heads in the field to foster ergot growth on the plant. You can do the same with grains of corn. This is the easiest method of recovering aflatoxin bearing material without a lab. You can visibly see the infecting mold on the grain in a few days or weeks. A farmer will not notice a few ears of infected corn and these can be recovered for plating once the molds are visible.

You can grow the molds at different temperatures in the moist chambers. At each different temperature, some molds will grow much faster than others. This provides another method for separating colonies.

Another method is streaking where a mixed cultured is streaked across a culture plate in a line. Another sterile tip is used to cross over the first line forming a second. This massively dilutes the culture. The technique is illustrated in V6-1 of this series on bacteria based weapons.

If you are trying to grow a colony from a mushroom cap, the cap can be suspended overhead from the roof with tape or if it is a small cap, vaseline can be used as an adhesive.

A wide variety of selective agars are used to isolate and grow certain species. These will be covered later in the chapter.

When working with microbials contamination is always a problem. The air is filled with millions of spores in every cubic foot. Dust carries thousands of spores on every speck. Bacteria and yeast can overrun mold colonies quickly using the mold itself as part of the food.

The best way of preventing contamination or minimizing it is cleanliness. Keeping all surfaces wiped down with chlorine bleach solution. When working with materials in the lab, the windows should be closed and no fans of any type should be operating. This minimizes air turbulence. All contact parts of tubes, plates and media should be boiled or flame sterilized to kill contaminants. Rose Bengal Dye is added to many media because it kills almost all bacteria and inhibits the growth of many molds thereby allowing some selectivity in isolating the target material.

2. Cultivation of Fungi on Culture Media

Identifying most molds depends on being able to observe their methods of spore production and life cycles. You cannot always see these in their natural habitat. Growing molds and their toxins for use as weapons also requires being able to observe and identify them. To this and to grow them for commercial and military use, you need to be able to provide them with a "substrate", a material that they can grow on that meets all their nutritional sources. Like the bacteria that are produced on culture plates, man has developed various culture media that feed the fungi and permit them to grow through all parts of their life cycles. Some fungi need specific forms of nutrients like people do. Amino acids are an example. Others can take raw minerals and energy and build the nutrients from scratch.

To supply the necessary nutrients in a laboratory setting we produce a *medium* which supplies the nutrients, usually in a semi solid surface like agar, jello, or fried egg white. The nutrients and water are mixed into the base and then solidified. The mold that grows on this medium is called a *culture.* Culture media can also be liquid, but solid media is used most often as they allow sporulation to take place more easily. Gelatin has been used to grow fungi but some fungi produce enzymes that dissolve gelatin and turn it into food for them and the medium turns from semi solid to liquid. Other materials that emulsify, thicken or gel water can also be used in the field.

Media are made with nutrients that can be pure chemicals such as nitrogen from ammonia, synthesized B vitamins like you buy at Wal Mart, and carbon in the form of sugar. The fungi can take these and make all the carbohydrates, proteins and other parts they need to grow and reproduce. This usually slows growth because it takes time for the biological processes to do this. Many media use natural substances like yeast extract or potato, bread, carrots or other plant or animal parts and most often they make very good sporulation media.

We will describe the basic mold media that can be used for colony growth.

1. Czapeks Solution Agar

   This is a semi solid that has been widely used in laboratories. Many molds produce characteristic colonies on it and may also exude pigmented substances. Aerial growth is usually suppressed and sporulation enhanced with some molds. Some fungi will grow poorly on this media if they cannot synthesize their own vitamins from the raw materials used here.

Sucrose	30g
Sodium Nitrate	3g
Potassium Phosphate	1g
Magnesium Sulfate	.5g
Potassium Chloride	.5g

Iron Sulfate	.01g
Agar	15g
Distilled Water	1,000 ml

The material is boiled so the agar dissolves and then it is cooled and solidifies like jello.

2. Potato Dextrose Agar (PDA)

A good all purpose formula that has been used in laboratories for over a century, PDA has been widely used and can be used by anyone at home.

Thinly sliced, peeled white potatoes	500g
Glucose (sugar)	20g
Agar	15g
Distilled Water	1000 ml

The potatoes are heated for 1 hour at 60C and then filtered through cheesecloth and the water added to reach 1000ml. Add other ingredients and cook one hour.

3. Sabourads Agar

This has been a standard medium used in medical mycology for molds that grow on human tissues. It is a standard used for colony identification in hospitals and the colonies pictured in a later chapter on human infectious molds will be mostly on this media.

Glucose	40g
Peptone	10g
Agar	15g
Distilled Water	1000ml

4. V-8 Medium

This medium is used for molds that do not sporulate on PDA. They will often sporulate well on this one or vice versa.

V-8 Juice	200ml
Calcium Carbonate	3g
Agar	20g
Distilled Water	1,000 ml

One of the most useful field improvised mediums that the author has used is simply buying yeast culture off of the store shelf, bake it in the oven at 300F until you

detect a burnt odor and then add water to soak the mix. I almost always get excellent mycelium growth with this formula but sporulation can be a problem. To improve this, several additions can be made and usually one of this will yield good sporulation results.

a. Add baby oatmeal (2%), and tomato paste (2%) to the yeast before baking
b. Add V-8 juice (20%) before baking
c. Add the filtered potato extract from PDA at 20%.
d. Store bought vitamin packs can also be added at 1%

Usually one and sometimes all of these will work. In some mushroom formulas you may need to actually add horse manure (from any horse activity like racing stables, fairs, etc.) to the mix to produce the fruiting bodies with the highly concentrated toxins.

For large scale production, you can buy yeast culture at feed mills by the ton or truckload. This is usually yeast grown on a fine ground corn substrate and has added vitamins and minerals.

The best way to prepare agar or jello media is to boil water on the stove and then place the agar or jello and water mix into a flask and then immerse the flask into the boiling water for one hour. This prevents the agar from burning on the bottom. The other materials are then added and stirred in and the mix is cooled and solidified.

The agar, while still hot and liquid is usually poured into a clear glass or plastic test tube (at a slant of 30 degrees), petri dish or other clear container. If glass is used, it should be heated in an oven at 450 F for one hour to sterilize it before use.

To sterilize plastics and glass, you use a pressure cooker and to kill all possible contaminants before culturing. The contents are heated to 250F (121C) for 20 minutes with the pressure raised to 15# per sq. inch.

When egg white is used, the ingredients are added and then the mix is fried and added to a clear container and then inoculated. A small amount of additional water can be added for some formulas.

Alcohol can also be used to help sterilize a medium since it will evaporate away when heated. If cultures are contaminated during growth, the desired colony can be transferred with an inoculating needle from the media to a new culture plate. The tip of the needle should be put in a flame (a lighter or match will do) to sterilize it.

When working with molds (or bacteria) that can be deadly to the handler, a double or triple plastic bag method is recommended. The media, culture or sample, and tools are placed inside a plastic bag which is sealed like shrink wrap or folding the end over many times and taped shut. A second plastic bag is placed around this and sealed. A third one can also be used. The bags are kept loose with minimal air so that the media and colonies can be worked with by hand. The pure culture can be removed using a

needle from inside another plastic bag which can be removed and remain self contained for transfer.

3. Using the Microscope For Fungal ID

Anyone can go to Wal Mart and buy a cheap microscope. For bacteria cultures these are inadequate because the optics at 1,000 magnification are poor and the bacteria cannot be seen easily. Molds often require only low magnification to identify basic structures and for these, the cheap microscope is usually adequate and very educational. The purchased microscope usually comes with instructions and there are books that can be obtained from the library on microscopy so I will cover only a few of the basics here.

The part of the mold that is mounted on a slide should be from the actively growing, young portion at the margins where spores are being produced. The older hyphae and colony parts may be partially decomposed and become unrecognizable. The sample should be taken off with a needle near the margin of the colony. A small amount of the agar at the surface is usually taken up with the sample. If the colony is thick and wooly, this may not be necessary. A second needle is used to spread out the filaments into a thin flat section. A cover slip is then placed over the slide lowering one edge and then the other so that the air bubbles are pushed out. Remaining air bubbles can be removed by gently heating the slide over an alcohol flame until it steams slightly (do not let it boil).

Some molds have spores connected in very fragile chains that disintegrate at the slightest movement of air. In these cases, the entire petri dish can be placed under the microscope or a slide can be placed in the dish and removed with some growth on it. In this way the colony can be observed undisturbed. A slide can also have a colony sample and agar place on it and a cover slip lightly placed on top. At the first signs of growth, the slide can be taken out of incubation and observed.

Water is usually used as a mounting medium but they can dry up quickly. Several improvements have been made which slows water evaporation and improves the view. These include-

a) adding a few drops of photographic wetting agent to the distilled water
b) using prepared stains from medical supply companies
c) Potassium hydroxide (10%) and Phloxine (.025%) which is a pink dye that stains hyphae a bright pink and makes them easier to see.

Melzers solution can also be purchased from a supply company. It will turn some tissues blue to blackish and are called amyloid, while others stain red and are called dextrinoid. This solution contains chloral hydrate which is a very toxic poison itself and should be handled with care.

Many fungi are difficult to “wet” and it can be helpful to use a drop of ethyl alcohol for a few seconds onto the slide and then before the alcohol completely evaporates or dries, add the mounting medium.

4. Using Identification Keys

Identifying molds are based almost entirely on the spores and the structures which bear them. Huge textbooks are written, many with illustrations or accompanying photographs which aid in identifying each species by their anatomy. It would be nearly impossible to simply go through endless photographs though to try and find a matching picture. Over time, a device was developed which allowed for a common and simple means of identifying molds and this is called "*dichotomous keys*".

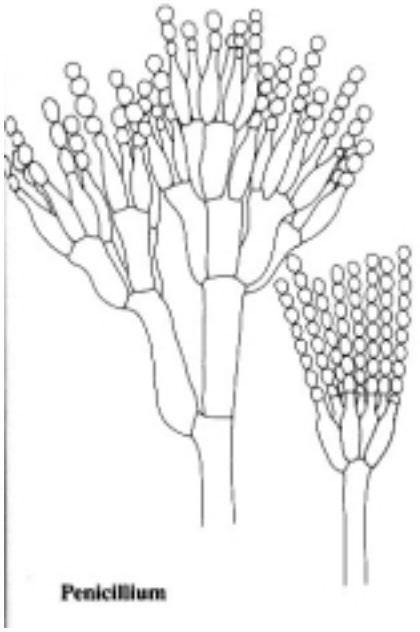
Dichotomous keys are a device which presents a series of alternatives or choices which you make, one at a time based on what you see under the microscope. The following is an example from the start-

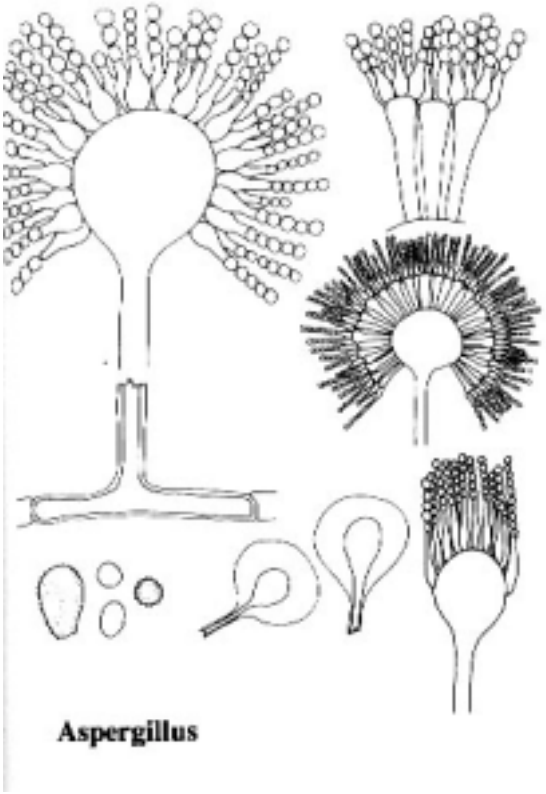
Group 1

1.	Spores 1-celled	go to 2
	Spores with more than one cell	go to 12
2.	Colonies, spores and other tissues colorless or brightly colored	3
	Colonies, spores and other tissues dark colored	8
3.	Spores produced in chains	4
	Not produced in chains	6
4.	Conidiophores with a swollen head or vescicle Bearing bottle shaped phialides	*Aspergillus
	Conidiphores not swollen at apex	5
5.	Spores in unbranched chains, borne from clusters Of cylindrical to bottle shaped phialides Colonies are usually green	*Penicillium
	Spores borne in branching chains from undifferentiated conidiophores: often growing very fast and pink	Monilia

As you can see, in only a few steps through the key, you can reach a group or genera. In the next chapter, a glossary describing what the words mean and complete basic keys of most common fungi and the poisonous fungi will be presented.

In textbooks on mold identification, they will often use diagrams or photos of their actual appearance –





Chapter 4

Classification of Fungi -Keys, Features and Glossary

The fungi can be identified as we have already seen by the use of identifying keys. We will begin this chapter with a glossary of terms to aid in understanding some of the language that has not been illustrated yet.

Then we will illustrate the main identifying features of the fungi that are commonly known as mushrooms.

After that we will provide three sets of keys.

1. Key to the basic fungi groups and genera
2. Key to the mushroom fungi
3. Key to the poisonous fungi

Acanthophysis: bottle-brush cell
Accumbent: lying against
Aculeate (spore): with radially arranged protuberances or spines;

Acyanophilous (opposite of cyanophilous): cell wall of spores, etc., are not stained blue with Cotton Blue-lactic acid

Agaricoid: fruit-body divided into pileus and stipe, with lamellae on the underside of the pileus
Allantoid: sausage-shaped and rather narrow
Alveolate: pitted like a honeycomb
Ampullaceous: swollen, especially below, so as to be flask-shaped
Amygdaliform (spores in side view): almond-shaped;
Amyloid: yielding a blue-black colour with Melzer's reagent;
Anastomosing: lamellae joined together by cross connections
Angiocarpous: with the hymenium remaining in a closed cavity during the development of the fruit-body
Annulate: having an annulus or ring
Annulus: ring-shaped, membranous or lanose-fibrillose, sometimes also slimy, structure on the stipe, arising either from further growth of the cortical layer as far as the stipe or from the veil [M40]
Apex: end of the spore opposite the apiculus
Aphyllophorous: belonging to a group of basidiomycete families based on features of the macroscopic basidiocarp; the taxonomy of the group is in a state of flux;
Apical (spores): at the opposite end of the spore to the apiculus
Apiculus: small, often somewhat laterally positioned, conical appendage to the spore by which it is attached to the sterigma of the basidium

Appressed: pressed flat against
Arboriform: applied to cystidia that have an elongated part branching in a tree-like fashion

Areola: a space marked out on a surface, separated from other spaces by cracks or chinks

Ascendent, ascending: when the edges of the lamellae are lower at the margin of the pileus than at the apex of the stipe; when an annulus extends upwards towards the apex of the stipe or when it can be pulled off the stipe in a downwards direction

Ascomycetes: fungi that form asci (see: ascus)

Ascus (plural, asci): characteristic, mostly sac-like, spore-forming organ that after maturation division produces spores inside by 'free cell formation' (= meiosporangium of Ascomycetes; cf. basidium);

Attenuate: narrowing gradually, becoming smaller and thinner

Azonate: opposite of zonate (q.v.)

Barbate: having groups of hairs; bearded

Basal mycelium: mycelium at the base of the stipe

Basal root: base of the stipe when root-like and narrowing towards the bottom, mostly in the soil

Basidiole: sterile or young basidia, visible as club-shaped elements without sterigmata

Basidiomycetes: fungi that form basidia (see basidium)

Basidium: characteristic spore-forming organ mostly in the hymenium of lamellate and tubulate fungi (= meiosporangium of Basidiomycetes), i.e. in which the maturation division is followed by exotopic spore formation

Bifurcate: forked in twos, like a tuning-fork

Bilateral (lamellate and tubulate trama): hyphae of the trama diverging towards the hymenium

Binding hyphae: thick-walled, abundantly branched hyphae without septa

Boletinoid (tubulate fungi): tubes that are wide and with mouths that are extended radially

Boletoid: with a tubulate fruit-body
***Botrydina* type**: globose algae at the base of the fungal stipe; in lichen-forming fungi (*Omphalina*)
Brittle (lamellae): when the lamellae are readily broken up on being rubbed
Breadth: lamellae measured from the base of the pileus to the edge
Broad (lamellae): broader than the thickness of the context (measured half-way along the radius)
Broom cell: a cell with apical appendages giving a characteristic broom-like appearance, occurring in the pileus and sometimes at the edges of lamellae
Brown rot: rotting of wood in which only cellulose is decomposed (see White soft rot)
Bulb: thickened bottom part of the stipe

Caespitose: growing in compact groups
Calyptrate: spore with the exosporium raised more or less as a hood over the rest of the spore membrane

Cantharelloid: a stipitate fruit-body with ridges on the underside of the pileus
Capillitium: a mass of sterile thread-like tubes and fibres mixed with the spores
Capitate: having a head; see also, muricate
Capitulate: having a small head
Carminophilous: see, siderophilous
Carpophore: entire fruit-body of higher fungi
Cartilaginous: flesh, especially that of the stipe, hard, but at the same time easily broken (*Collybia*)
Caulocystidium: cystidia at the surface of the stipe, sometimes only to be seen at the apex of the stipe
Centre: middle of the pileus, about a quarter of the radius from its middle point
Ceraceous: waxy
Cerebrose: convoluted like the brain
Cheilocystidium: a hymenial element at the edges of the lamellae that differs in size, appearance, and/or chemistry from basidia
Chlamydospore: thick-walled, asexual spores (reproduction cells) formed from hyphae by constriction
Chrysocystidium: cystidia with internal amorphous bodies that become (often faintly) yellow on treatment with potassium hydroxide or ammonia
Ciliate: when the edges of the lamellae are covered with fine hairs
Clamp-connexion: outgrowth at hyphal septa
Clavate (stipe, basidia, etc.): broadening in the shape of a club;

Claviform: club-shaped
Clothed: surface covered with scales, flocks, hairs, etc.
Clustered: growing in compact groups
Collar: ring-like structure towards which the lamellae face, round the apex of the stipe but leaving it free
Compound tube: tube divided internally by longitudinal walls
Compressed: when the stipe is flattened laterally
Concave: lamellae with edges that are concave in outline
Conchate (pileus): with the shape of a shell
Concolorous: having the same colour, e.g. pileus and stipe

Conidium (plural, conidia): a specialized non-motile asexual spore
Context: body tissue supporting the hymenophore, especially in pileate species; sometimes applied only to the body tissue of the pileus, sometimes taken to include the inner tissues of the stipe; often synonymous with 'trama'

Coralloid: fruit-body coral-like, without a special hymenophore, i.e. smooth hymenium
Corky: flesh tough like cork
Corneous: of a horn-like texture; horny
Corrugate: coarsely ridged or wrinkled
Cortical layer: layer at the surface of the pileus (except the hymenium) and stipe, when differentiated from deeper tissues
Corticate: fruit-body on the outside with a distinct, more or less sclerotized outer layer
Corticoid: fruit-body lying flat against the substrate, hymenium smooth
Cortina: cobweb-like connection between the marginal zone of the pileus and the stipe, which, in sufficiently young fungi, can be recognized as forming the partial veil (velum partiale); in older specimens visible as a fibrous zone on the stipe and sometimes also on the marginal zone of the pileus
Costate: with raised ribs, ridges or veins
Crateriform: shaped like a cup or goblet
Crenate: edges of the lamellae scalloped or round-toothed
Crenulate: finely crenate
Cristate: crested; spores with various kinds of amyloid ridges on their surfaces, found in the genera *Russula* and *Lactarius*
Crowded (lamellae): almost touching each other, so that the base of the pileus can be seen hardly or not at all
Cupulate: cup-shaped
Cutis (cuticle): layer covering the pileus, when consisting of elongated, horizontal, radially arranged hyphae
Cyanophilous: walls (of spores, hyphae, etc.) are stained blue with Cotton Blue-lactic acid Care should be taken not to confuse the blue-coloured cell content (plasma) with the cyanophilous cell wall

Cylindric (pileus) (stipe) having the form of a cylinder, i.e. round in outline and of the same diameter throughout its length; if the term refers to the surface of the stipe, it is not essential for the stipe to have a central hollow
Cystidium (plural, cystidia): sterile, mostly enlarged, cells and filaments of various forms which are located among the basidia and often project beyond them; see also: dermato-, caulo-, pileocystidium

Decurved (margin of the pileus): bent down
Deliquescent: lamellae decomposing to a liquid mass; liquefying
Dendriform: branched like a tree
Dendrophysis: tree- or antler-like branched cystidium or element of the cortical layer
Dentate: edge of lamellae with fine, even teeth [finer than serrate, according to M40]; of a hymenophore arranged in the form of teeth
Depressed: with a depression in the middle of the pileus

Dermatocystidium: cystidium formed in the cortical layer
Detersile: easily removed, leaving the pileus or stipe naked
Dextrinoid: see, pseudoamyloid
Digitate (cystidia): with finger-like outgrowths

Dimitic: trama with generative and skeletal hyphae or with generative and binding hyphae

Distant: lamellae arranged with spaces in between so that the base of the pileus can be seen (opposite of crowded)
Diverticulate: branched elements (cystidia, hyphae)

Echinate: with sharply pointed spines
Ectomycorrhizal: when a fungus is associated with the roots of a plant but remains on their surface and forms a Hartig net (inter-cellular hyphal network)

Elastic: flexible without breaking
Ellipsoidal (pileus), (spore), elliptical in outline when viewed from the side
Encrusted, incrustation: cell walls with special crust-like formations
Encrusted primordial hyphae (pellicle): intensely coloured hyphae in the cutis of *Russula* species whose walls on observation in water are seen to bear wart- or crust-like deposits
Endosporium: innermost membranous layer of the spore
Entire (edge of lamellae): with a continuous, not indented, outline
Epicutis: outermost layer of a multi-layered cutis
Epimembrane pigment: pigment located on the surface of the cell wall (hyphae)
Equal: (stipe) diameter the same throughout its length
Evanescent: of an organ or structure disappearing in the course of the development (e.g. maturing)
Even (opposite, uneven): e.g. of the surface of the pileus when without depressions or unevennesses of any kind
Everted: turned inside out
Excentric: stipe not attached at the centre (but also not at the edge) of the pileus
Excoriate: becoming peeled or stripped off; with a roughened surface

Exsiccatum (plural, exsiccata): dried specimen of a plant, in the present context a fungus. Prepared by drying in a rising current of warm air (40°C)
Exosporium: outermost membranous part of the spore after disappearance of the perisporium (= membranous part of the spore which in the young stages forms a coherent cover over the entire spore)
Eyepiece micrometer calibration factor (microscopy): the factor by which measurements made with a microscope eyepiece micrometer are converted to microns

Farinaceous: as if dusted with flour; finely dusted; (odour, taste) like that of meal or flour
Farinose (odour): like that of flour
Farinaceous-furfuraceous: as if dusted with flour and in between small particles or flocks
Fasciculate: several fungal stipes fused at the bottom to form a tuft
Faveolate: honeycombed
Favoid: like a honeycomb
Fibril: thin and thread-like fibre
Fibrillose: with more or less thin and thread-like filaments on the pileus and stipe

Fibrillose-glabrous: fibrils integrated into or embedded in the surface layer
Fibrillose-squamose: of scales composed of fibrils
Fibrous: surface covered with fibres or composed of them
Fimbriate: edge, e.g. of pileus, annulus, etc., irregularly lacerated
Fissured concentrically: when fissures occur concentrically round the centre of the pileus, etc.
Fistulose: hollow, like a pipe
Flabelliform: fan-shaped
Flattened: an originally convex outline, e.g. of the pileus, becomes flat; see, applanate; in the case of the stipe,
Flattened convex: shape of pileus
Fleshy: trama is both soft and relatively thick
Flexible (stipe): moving without breaking
Flexuose: zigzag, bent in alternate directions
Floccose: with soft, small bundles of hair
Flocculose: finely floccose
Foveate: surface (pileus, stipe) with small pits

Friable: breaking up into smaller pieces, crumbling
Front view: view of the spore positioned so that the apiculus appears to be in the middle of the end of the spore and not at the side
Fruit-body: macroscopic body for producing, protecting and discharging spores
Fugacious: disappearing early on and rapidly; evanescent
Fuliginous: a dark, sooty colour
Fulvous: reddish cinnamon-brown; tawny; reddish yellow
Furcate (lamellae): forked
Furfuraceous: covered with fine particles (pileus, stipe); branny, scurfy
Fuscous: dusky, more grey than brown
Fusiform: cigar-shaped; with a narrow, elongated appearance and at the same time tapering ends (spores, cystidia)
Fusoid: when the stipe tapers towards the bottom or at the top and the bottom

Gasteroid: largely globose fruit-body; basidia formed in the inside of the fruit-body perishing; spores enclosed in the fruit-body until mature
Gelatinous: of a pliable consistency and when dry able to swell again; macroscopic: of flesh with a hyaline appearance and capable of swelling; microscopic: of a hyaline mass between hyphae capable of swelling
Generative hypha(e): thin-walled hypha(e), often bearing clamp-connexions. When thick-walled and bearing clamp-connexions = sclerified generative hyphae
Geniculate (pileus): of the edge when it is abruptly bent downwards (at a right angle); (stipe) when it has a sudden bend
Germ pore: hyaline coloured or pore-like spot in the spore membrane at the upper end of the spore opposite the apiculus. Even using an oil-immersion lens, often unclear and difficult to recognize
Gill(s): knifeblade-like structure(s) on the underside of the pileus of Agarics
Glandiform: acorn-shaped
Gleba: spore-containing mass in Gasteromycetes, often decomposing to a powdery mass

Gloeocystidium: a cystidium with oily, resinous, or granular, mostly yellow, content; thin-walled
Glutinous: coated with a jelly-like substance

Metachromatic: usually in connection with cresyl-blue staining, when the wall shows a blue- and a red-coloured layer (spore wall of *Macrolepiota*); in practice, seldom used
Metuloid: more or less thick-walled cystidia arising deep in the trama;

Micron: 1/1000 mm = 1 µm; unit for microscope measurements
Miniate: colour of red lead, orange-red
Mitrate: shaped like a mitre
Monomitic: consisting only of generative hyphae (opposite of di- and trimitic)
Movable: when the annulus can be shifted up or down the stipe
Muricate: (cystidia) having an apical encrustation
Mycelium: vegetative mass of hyphae or fungal filaments; hyphae in the fruit-body are not called mycelium
Mycology: science or study of fungi
Mycorrhiza: so-called fungal root, i.e. symbiosis between fungi and higher plants in the region of the underground organs (mostly roots)

Naked: surface without any covering (hairs, felt, scales, etc.)
Napiform: top-shaped fruit-body; stipe likewise
Narrow (lamellae): when lamellae are less broad than the thickness of the pileus (measured halfway along the radius of the pileus); sometimes also the insertion of the lamellae (see below)
Narrowly adnate: insertion of lamellae
Necro-pigment: pigment that first appears after the fungus has died
Nodulose: having broad-based, blunt, wart-like protuberances

Obsolete: poorly developed, rudimentary, hardly perceptible
Obtuse: margin of the pileus forming a broad angle with the lamellae;

Oidium (plural, oidia): sterile spores in the form of chains (reproductive units)
Ornamentation (spores): small structures on the wall that do not form a continuous layer on the surface of spores
Ovoid: shape of pileus

Pannose: like felt or wool in texture

Paraphysis (plural, paraphyses): a sterile, basally attached hyphal element in a hymenium, particularly in Ascomycetes, usually clavate or thread-like and branched or unbranched
Parasite: species that grows on other living organisms and that is dependent on the host for its nutritional requirements
Pectinate: like the teeth of a comb
Pedicellate (spores): borne on a slender stalk or pedicel
Pellicle: gelatinous membrane on the surface of the pileus
Pellucid-striate: of the pileus when it is so thin that the lamellae are visible through it as striae

Peridiole: a division of the gleba having a separate wall, often acting as a unit of distribution
Peridium: covering of the fruit-body in Gasteromycetes
Perisporium: the spore membrane that envelops all the other membranes in the young state
Peronate: stipe at some distance from the base covered with a fibrous, floccose, or granular velum which forms a more or less enclosing ring at the top
Phaseoliform: bean-shaped

Phragmobasidium: a basidium divided by walls
Pileate: fruit-body with a pileus; mostly divided into pileus and stipe
Pileocystidium: see, dermocystidium
Pilose: covered with long, soft hairs
Plage: (supra-hilar spot): a more or less clearly delimited zone on spore walls above the apiculus, which in verrucose spores is smooth or distinctly less verrucose

Pleurocystidium: cystidium on the face, but not the edge, of a lamella
Pleurotoid: having one or more characters of the genus *Pleurotus*, e.g. lateral stipe
Plicate: with radial folds, folded
Pore: mouth or bottom end of a tube,
p.p.: see, pro parte
Primordial hypha: see, encrusted primordial hypha
Pro parte: in part, partly

Pruna: surface powderiness
Pruinose: as if covered with a fine powder that can often be wiped away, as in the case of plums
Pseudo-amyloid (dextrinoid): when walls (spores, hyphae) on treatment with Melzer's reagent become a deeper brown than the surrounding medium
Pseudo-rhiza: a root-like extension of the stipe; a connection between the fruit-body and the mycelium in the soil
Pulveraceous: pulverulent: powdery
Punctate: with fine points on the spore membrane; in the case of the surface of the pileus, when this has small raised points
Puncticulate: minutely punctate
Pyriform: pear-shaped

Radial: radiating from the centre towards the margin of the pileus
Radially fibrillate: with fibrils in the radial direction of the pileus
Radially parallel (hyphae of the cortical layer): hyphae lying closely parallel and extending in the radial direction of the pileus
Radially rugose: with radially directed corrugations
Radially striate: with striations arranged in the radial direction

Ramealis structure: when a hyphal cell is repeatedly furcate at right angles
Ramificate: branched
Reflexed (pileus): with a turned-up or turned-back margin

Reniform: kidney-shaped
Repand (pileus): having a wavy margin and turned back or elevated
Repent: creeping, prostrate
Resupinate: fruit-body lying flat on the substrate without raised edges or with the apex (dorsal surface) of the pileus sessile
Reticulum: a network or net-like arrangement
Reviving: after being dried, assuming its original appearance in water and then often again able to discharge its spores; not decomposing, only wilting, and on moistening appearing fresh
Revolute: rolled up or back
Rhizoid: mycelial strands like root-hairs at the base of the stipe
Rhizomorph: mycelial strands with a thickened outer layer; root-like
Rimose: cracked in all directions or by radial fissures
Rimose-areolate: surface divided into more or less regular areas
Rimose-fibrillose: cortical layer becoming fibrillose through the occurrence of numerous cracks

Rostrate: having a beak
Rostrum: a beak-like extension
Ruderal: growing in waste places or among rubbish
Rugose: surface roughened by the occurrence of wrinkles
Rugulose: finely wrinkled

Saccate: with a cup- or sheath-like volva at the base of the stipe
Saprobe (saprophyte): living on dead organic matter and dependent on it for its nutrition
Sclerotium (plural, sclerotia): a bulbous, hard body which represents a survival state or storage organ in many fungi
Scutellate: shaped like a small shield
Sellaeform: saddle-shaped
Sensu latissimo: in the broadest sense
Sensu lato: in the broad sense
Sensu stricto: in the narrow sense
Septum (plural, septa): cross-walls in hyphae
Sericeous: silky; surface densely covered with fine shiny threads, like silk threads
Serrate (edge of lamellae): with coarse or regular teeth [M40]
Setose: covered with bristles, bristly
Setum (plural, seta): coloured, more or less thick-walled hair that is often brush-, awl-, or hook-shaped (hymenium, etc.)
Siderophilous: black granulation in basidia when treated with carmine-acetic acid

Sinuous (stipe): bent back and forth, flexuose
Skeletal hypha: thick-walled hyphae without septa and clamp-connexions
s.l.: see sensu lato
Solid: refers to the consistency, when this is neither brittle nor spongy; more or less compact flesh is solid, but compact flesh must not be tough; when the flesh fills the stipe so that there is no hollow present
Spathulate: fruit-body with spatula-like shape
Sphaerocyst: globose cell of the trama or the epicutis (except spores, etc.)
Spinose: having spines
Spinulose: having small spines
Squamose: covered with scales
Squamulose: a smooth surface that has broken up into very fine scales
Squarrose: surface covered with erect, recurved scales
s.s.: see sensu stricto
Stellate: star-shaped
Sterigma (plural, sterigmata): outgrowth of the basidium on which the spore develops
Stipitate: having a stem or stipe
Stipitiform: stalk-like
Striate: having striations, e.g. at the apex of the stipe from the insertion of the lamellae
Strigillate: stiff-haired
Sub-bulbous: broadening of the lower part of the stipe in the form of a club; clavate
Subcutis: layer of thread-like cells between the epicutis and trama of the pileus; only applied when such a layer can be differentiated from the trama of the pileus which (also) has thread-like cells

Subgleba: sterile, non-spore forming, part below the gleba of a Gasteromycete
Subhymenium: layer immediately below the hymenium which can sometimes be distinguished from the trama by its different structure

Subiculum: hyphal mass lying on the substrate which gives rise to the fruit-body
Sub-limoniform: of spores which in side view are almost lemon-shaped
Substrate: nutrient medium of the mycelium: earth, plant remains, wood, etc.
Substratum: particles of earth, needles, leaves, etc., adhering to the base of the stipe

Sulcate: grooved
Supra-hilar spot: see, plage

Terete (stipe): circular in cross-section
Tessellate: like a mosaic, checkered
Tomentose: densely matted and woolly, like a wollen blanket
Tomentum: a tangled or matted covering of the pileus or stipe comprising long soft hairy filaments with thick walls, like wool
Tough: flesh form, neither breaking nor tearing, and not woody
Trama: in the wider sense the substance (flesh) of the fruit-body; in the narrower sense the trama of the lamella, stipe, and pileus excluding the surface layer
Trama (lamella): layer in the lamella between the hymenia;

Transversely undulate: with waves across the longitudinal direction of the lamella
Trichoderm: cortical layer of the pileus with hyphae mostly at right angles to the surface, but not strictly parallel and not all arising at the same level; elements involved not like a hymenium
Trichodermal palisade: cortical layer of the pileus with vertical hyphae that have a fairly strict parallel arrangement and do not all arise at the same level (not as in a hymeniderm); terminal members sometimes develop as dermatocystidia
Trimitic: trama with the following kinds of hyphae: generative, skeletal, and connective
Truncated bulbous: when the bulb appears as if abruptly cut off flat at the bottom
Tube: element of a tubulate hymenophore
Tuberculate: surface of spore (or other flat microscopic structure) with very fine protuberances almost like warts
Tubulate: when the hymenophore is composed of numerous tubes
Turbinate (pileus): top-shaped

Umbilicate (pileus): having a central navel-like depression somewhat funnel-shaped
Umbonate (pileus): with a boss in the centre
Umbraculiform: umbrella-shaped
Uncinate: attachment of lamella
Unctuous: greasy or oily to the touch
Undulate: edge of pileus with a wave-like form
Uneven: opposite of even (q.v.)

Urceolate: urn-shaped

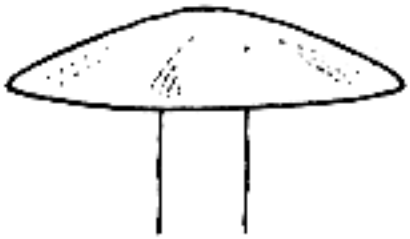
Veil: see, velum
Velar: appertaining to a velum or veil
Velum: a covering that completely or partly surrounds the fruit-body; velum universale (universal veil) completely surrounding the fruit-body, velum partiale (partial veil) partly surrounding the fruit-body

Velutinate: thickly covered with short soft hairs, and comparable with velvet; velvety
Ventricose (stipe): swollen in the middle
Ventricose-rostrate (cystidium): like the stinging hair of the nettle, i.e. having a broad to swollen or tubular base with a long narrow neck
Verrucose: used especially for the surface of spores when covered with small warts; see **15e** (oil immersion); see also punctate, verruculose
Verruculose: minutely warty
Versiform: variable in form; changing in shape with age
Vesicular, **vesiculose**: composed of vesicles
Villous: covered with long soft hairs
Vinaceous: wine-coloured, the colour of cloth stained with (red) wine
Viscid: sticky or tacky when moist; slippery

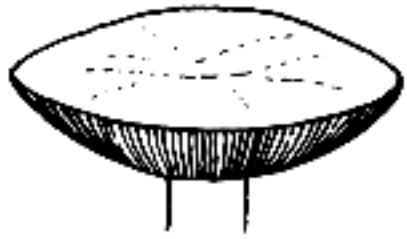
Volva: sac-like structure at the base of the stipe, originating from the velum universale

Warty: see, verrucose
White soft rot: rotting of wood in which cellulose and lignin are decomposed; the rotted wood becomes soft, fibrous, and whitish

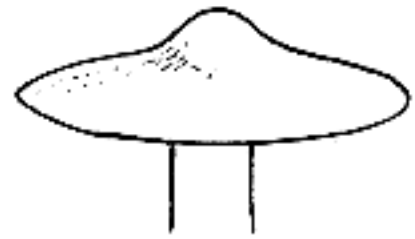
Zonate: surface of the pileus with one or more annular concentric zones that are more or less delimited by colour from the rest of the surface



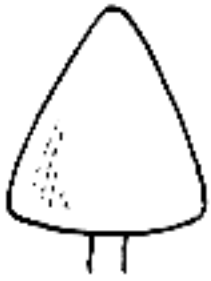
Regular and Convex



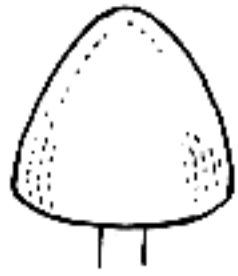
Plane or Expanded



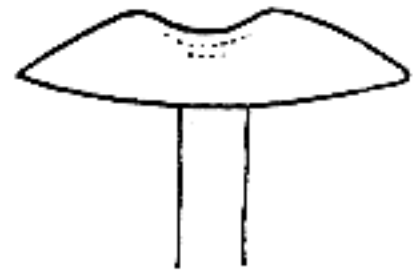
Umbonate



Conical



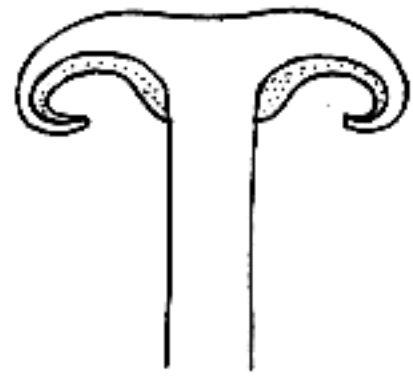
Bell-Shaped or
Campanulate-



Centrally Depressed

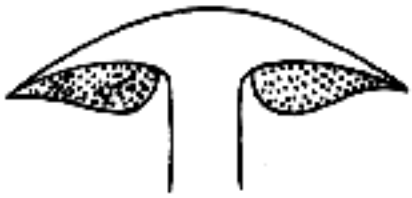
Infundibuliform or
Funnel-Shaped
(*in section*)

Cylindrical



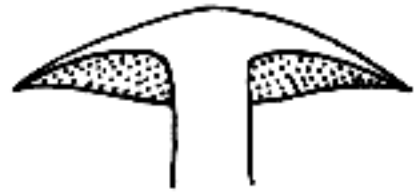
Involute
(*in section*)

Cap Shapes in Agarics

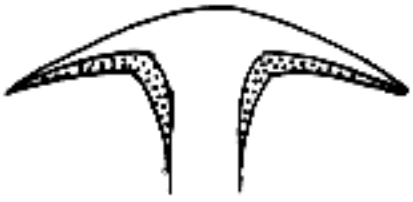


Free

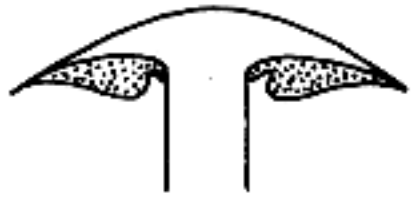
Adnexed



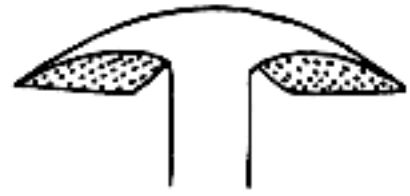
Adnate



Decurrent



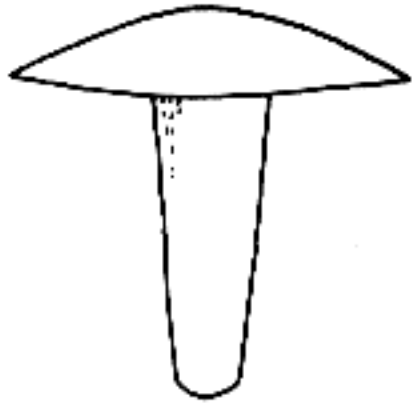
Sinuate



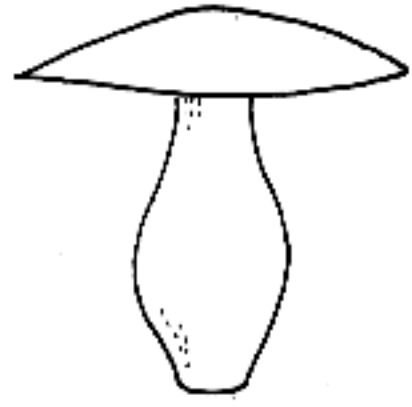
Emarginate

Gill Attachments

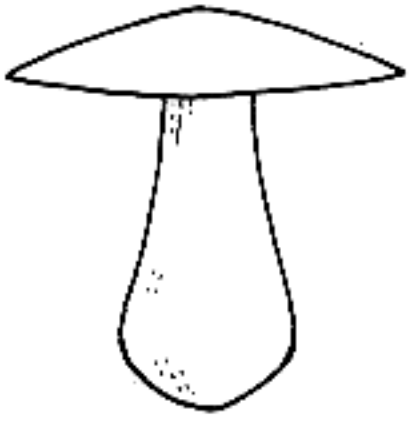
Equal



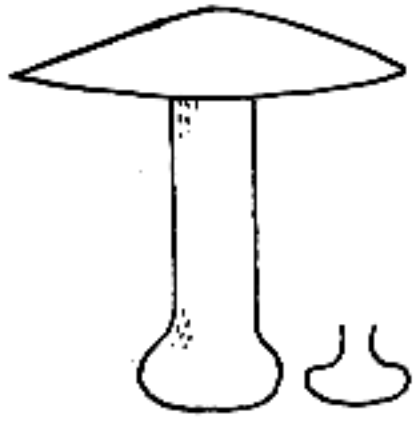
Attenuating
Downwards



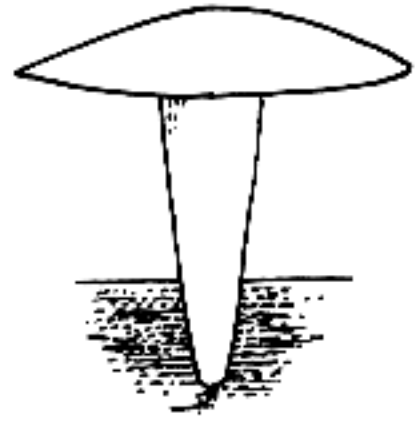
Fusiform or
Spindle-Shaped



Club-Shaped or
Clavate

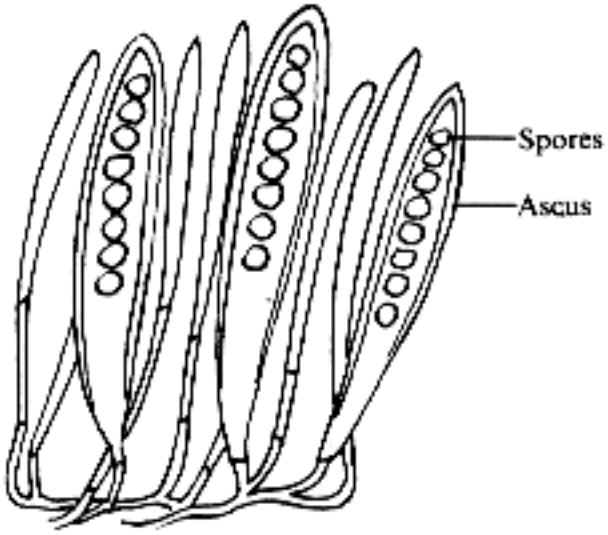


Bulbous &
Marginate Bulbous



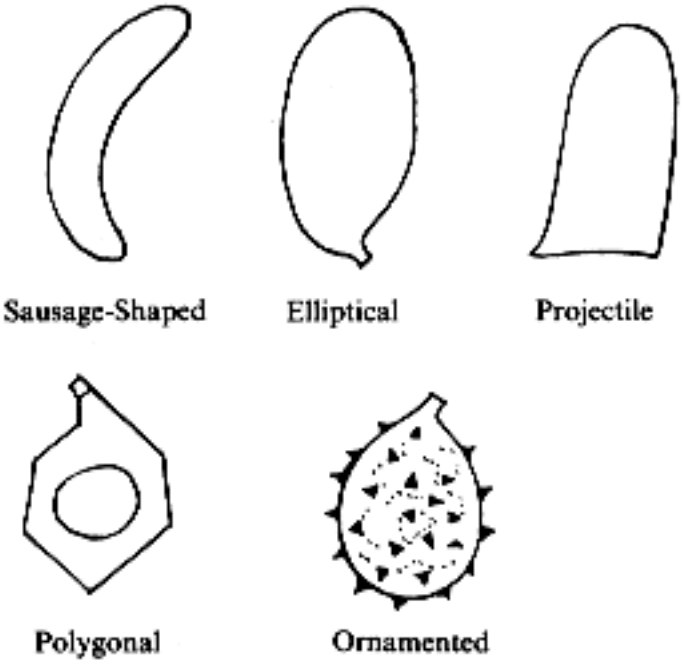
Rooting

Stipe Characters

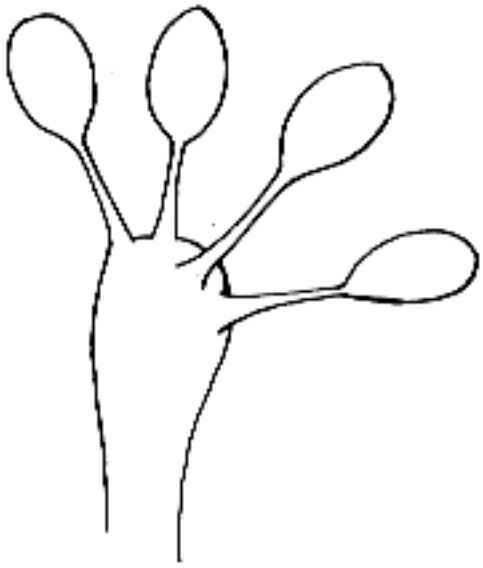


Asci and Sterile Filaments
Highly Magnified

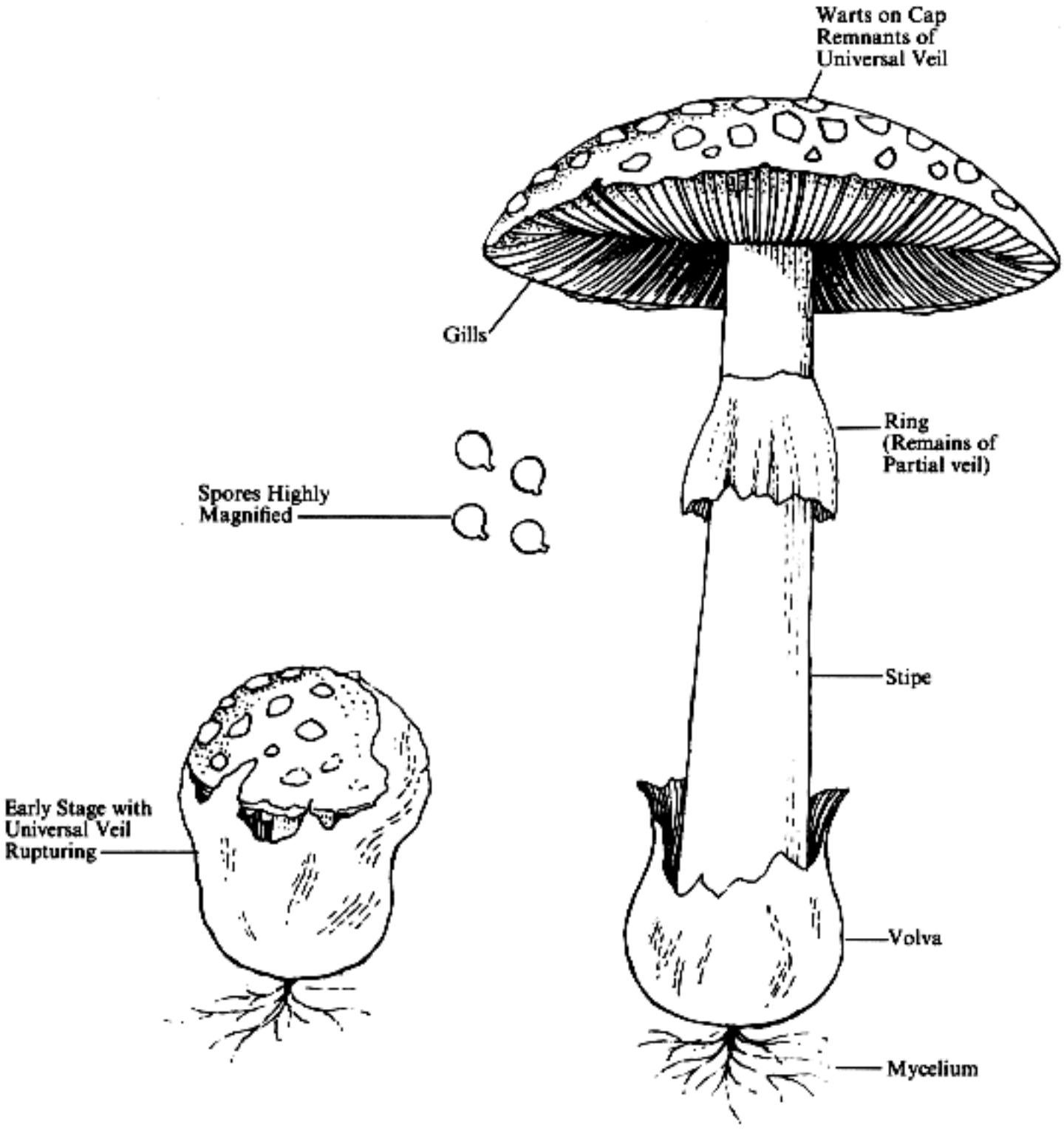
4-Spored Basidia of a Basidiomycete
and
Sterile Cells
(Agaricale Aphyllophorales)



Various Spore Shapes

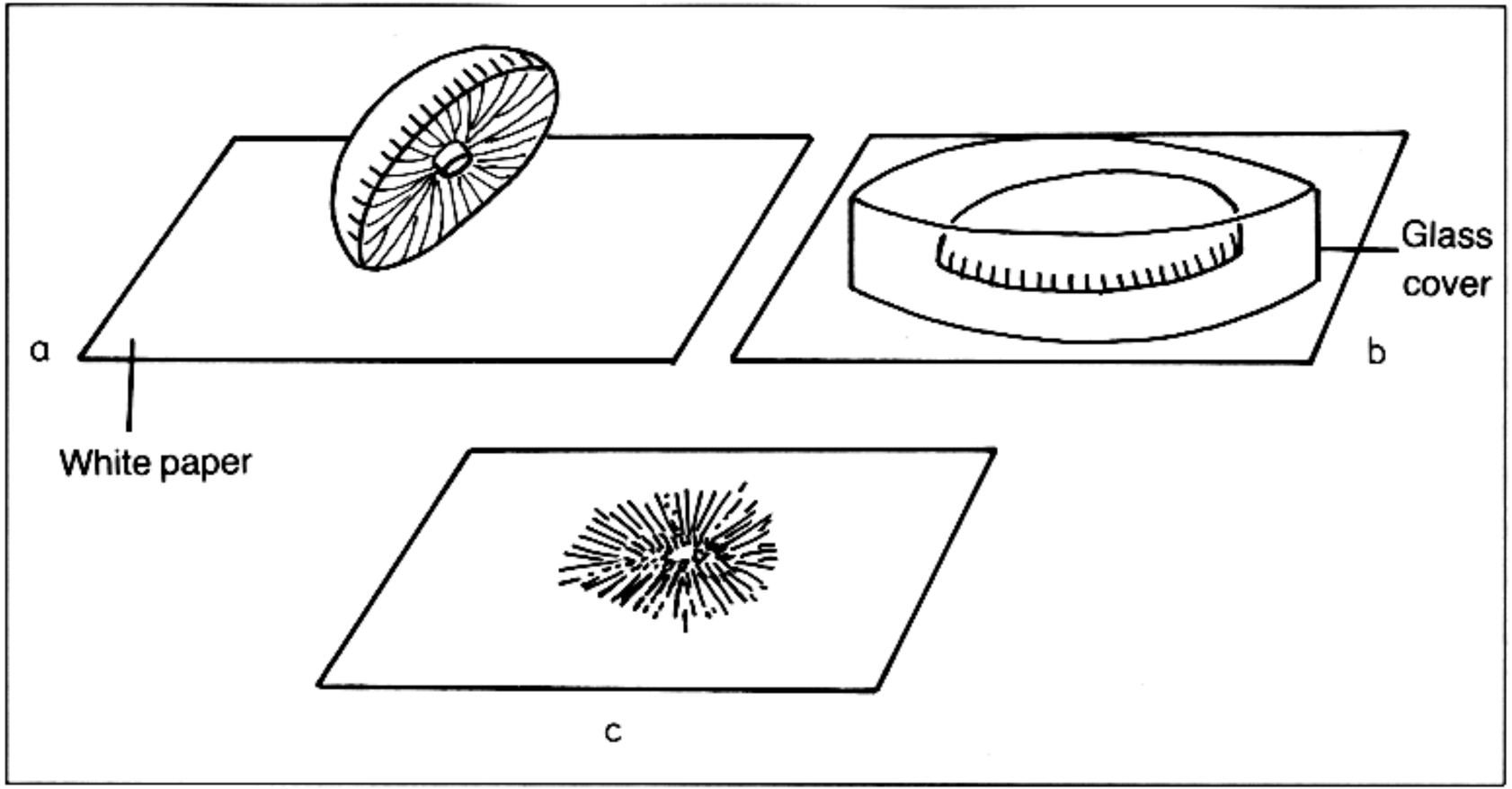


4-Spored Basidia of a
Gastromycete



Fruit-Body Structure of a Gill-Fungus (Amanita)

Obtaining a spore print from a large lamellate (or tubulate) fungus: a) the pileus of the fungus with its stipe removed and its lamellae (or tubes) pointing downwards is laid on a sheet of white paper, b) a glass cover is placed over the pileus, c) the deposited spores.



GROUP I

1 Spores 1-celled **2**

Spores with more than one cell **12**

2 Colonies, spores, and other tissues colourless or brightly coloured **3**

Colonies, spores, and/or other tissues dark coloured **8**

3 Spores produced in chains **4**

Spores not produced in chains **6**

4 Conidiophores with a swollen head or vesicle bearing bottle-shaped phialides **Aspergillus**

Conidiophores not swollen at apex **5**

5 Spores in unbranched chains, borne from clusters of cylindrical to bottle-shaped phialides; colonies usually green **Penicillium**
Compare with *Paecilomyces* (group II), *Gliocladium* (group III), and *Scopulariopsis* (group III)

Spores borne in branching chains from undifferentiated conidiophores; colonies often very fast growing and pink **Monilia**

6 Spores borne in a sporangium with a columella; often with only the columella evident as a swollen hyphal tip; hyphae not septate **Mucor**
Compare *Rhizopus* (group I), *Mortierella* (group II), *Absidia* (figure 2B), *Circinella* (group V), and *Zygorhynchus* (figure 2C)

Spores produced externally; hyphae septate **7**

7 Conidiophores well-developed and usually with a central axis; very fast growing and with conidiophores usually produced in small cushions of hyphae; often green **Trichoderma**
Compare with *Verticillium* (group II) and *Gliocladium* (group III)

Conidiophores poorly developed or lacking; phialides produced singly along the vegetative

hyphae; hyphae often aggregated into 'ropes'; seldom or never green **Acremonium**
Compare with *Verticillium* (group II), *Sporothrix* (group IV), and *Phialophora* (group IV)

8 Spores in chains, produced externally **9**

Spores not in chains, produced inside sporangia or fruiting bodies (pycnidia) **10**

9 Conidiophores with a swollen head or vesicle bearing bottle-shaped phialides; conidial chains unbranched **Aspergillus**

Conidiophores lacking a swollen apex; spore chains often branched; spores often both 1- and 2-celled **Cladosporium**

10 Spores produced inside a fruiting body (pycnidium) with a cellular wall; hyphae septate **Phoma**
Compare *Pyrenochaeta* (group IV) and *Coniothyrium* (group IV) and also be sure that asci are not present at a very early stage

Spores produced within a sporangium with a columella, often with only the columella evident as a swollen hyphal tip; hyphae not septate **11**

11 Sporangiophores with rhizoids (branched 'roots') at base **Rhizopus**
Compare with *Absidia* (figure 2B)

Sporangiophores lacking rhizoids **Mucor**
Compare with *Mortierella* (group II), *Absidia* (figure 2B), *Circinella* (group V), and *Zygorhynchus* (figure 2C)

12 Spores with transverse septa only **13**

Spores with both transverse and vertical septa **14**

13 Spores dark, produced in branched chains **Cladosporium**

Spores colourless or brightly coloured, mostly with more than two cells, often canoe-shaped, usually produced in slimy masses; colonies often pink **Fusarium**
Compare *Cylindrocarpon* (not treated here), *Candelabrella, Dactylella, Monacrosporium* (all group III), and *Trichophyton* (group V)

14 Spores usually in chains, usually club-shaped; colonies grey to brown **Alternaria**
Compare *Ulocladium* and *Stemphylium* (group II)

Spores in clusters but not in chains, usually spherical; colonies often (but not always) bright orange or yellow and purplish in reverse **Epicoccum**
Compare with *Stemphylium* (group II)

GROUP II

1 Colonies composed of hyphae, or at least with some hyphae present **2**

Colonies lacking hyphae; short chains of 'budding' cells may be produced **16**

2 Spores 1-celled **3**

Spores with more than one cell **14**

3 Spores and hyphae colourless or brightly coloured **4**

Spores and/or hyphae dark coloured **10**

4 Spores produced in chains **5**

Spores not produced in chains **7**

5 Spores produced from small clusters of tapering phialides, often rather pointed at the ends **Paecilomyces**
Compare with *Penicillium* (group I) and *Verticillium* (group II)

Spores produced by the simple fragmentation of hyphal segments into individual cells **6**

6 Colonies very slow growing (slower than 5 mm/week), often grey, often with a strong earthy odour; hyphae usually less than 1 μm in diameter **Streptomyces**

Colonies growing faster, with a fruit-like odour or odourless, hyphae larger **Geotrichum**
Compare with *Geomyces* (group IV)

7 Spores produced in sporangia, with sporangia often broken and represented only by simple blunt sporangiophores (no swollen columella); colonies often velvety in texture and pink to brown **Mortierella**
Compare with *Mucor* (group I) and *Absidia* (figure 2B)

Spores produced externally **8**

8 Spores produced in large numbers and completely covering the surface of large terminal cells; cells of conidiophores often flattening in alternating planes as they dry; colonies often producing black stony sclerotia **Botrytis**
Compare with *Chromelosporium* (not treated here)

Spores produced at the tips of terminal cells and never covering them; cells of the conidiophore not flattening characteristically upon drying **9**

9 Conidia produced in small round masses at the tips of phialides; phialides in whorls, tapering gradually to a very narrow tip **Verticillium**
Compare with *Acremonium* (group I)

Conidia produced singly at the ends of short branches; or in short chains, not on phialides; spore-producing cells not in whorls **Chrysosporium**
Sepedonium and *Trichophyton* (both group V) and *Geomyces* (group IV) are similar

10 Spores produced in sporangia or in fruiting bodies **11**

Spores produced externally **12**

11 Spores produced within densely hairy fruiting bodies (perithecia), very dark; asci present when young **Chaetomium**

Spores produced in sporangia **Go back to 7**

12 Conidiophores united to form large synnemata that have a sterile base and a spore-bearing upper part, often accompanied by spores of *Echinobotryum* (group V)
Compare with *Trichurus* and *Graphium* (both group III) **Doratomyces**

Conidiophores never united to form such structures **13**

13 Spores arising in dense masses directly from swellings on the vegetative mycelium; colonies usually rather flat and moist **Aureobasidium**
Compare with *Exophiala* (not treated here)

Spores completely covering the terminal cells of erect conidiophores; colonies cottony and rather dry; black sclerotia often present **Botrytis**

14 Spores with transverse walls only, colourless; colonies often pink; often associated with eelworms **Arthrobotrys**
Compare with *Trichothecium* (group V), *Candelabrella* and *Geniculifera* (both group III)

Spores with transverse and vertical walls, dark brown **15**

15 Conidiophores more or less straight because of their elongation directly through the scar of the previous spore, bearing only one spore at a time **Stemphylium**
Compare with *Pithomyces* (group IV)

Conidiophores often with a slight zigzag appearance due to new growth from just below the tip, often bearing a spore at each bend **Ulocladium**
Compare with *Pithomyces* (group IV) and *Curvularia* (not treated here)

16 Cells very small, seldom more than 1–2 μm in diameter, dividing by simple fission into two equal-sized daughter cells, sometimes containing a single internal spore **Bacteria**

Cells usually larger than 1–2 μm in diameter, dividing by budding, with the daughter cell seen as a small 'bubble' arising from the wall of the parent cell, sometimes containing one or more internal spores (ascospores) **Yeasts**
Compare *Aureobasidium* (group II), *Candida* (group III), and *Exophiala* (not treated here)

GROUP III

1 Spores 1-celled **1**

Spores with more than one cell **9**

2 Conidiophores united into complex synnemata with a sterile base and fertile upper part **3**

Conidiophores solitary, never forming complex structures, sometimes not present **4**

3 Spores produced in a large colourless drop of fluid at the tip of the synnema **Graphium**
Compare with *Pesotum* (not treated here)

Spores produced along the sides of the upper part of the synnema, dry, interspersed with loosely coiled hairs **Trichurus**

4 Spores produced in chains **5**

Spores not produced in chains **6**

5 Spores brown, produced from a cluster of strongly swollen cells (phialides) **Memnoniella**
(see *Stachybotrys*)

Spores usually grey, tan, or colourless, produced from clusters of bottle-shaped cells (annellides) **Scopulariopsis**
Compare with *Penicillium* (group I)

6 Spores produced in small clusters at several 'nodes' along the length of the erect conidiophores **Gonatobotrys**

Spores apical or conidiophores not obviously well-developed **7**

7 Spores borne along the length of the hyphae from apparently undifferentiated cells; colonies white, moist, and flat **Candida**

Spores produced at the apex of the distinct conidiophores; colony appearance various **8**

8 Conidiophores unbranched or rarely very simply so; spores arising from an apical cluster of swollen cells (phialides) **Stachybotrys**

Conidiophores highly branched; spores borne from clusters of narrow cells (phialides), produced in a slimy mass **Gliocladium**
Compare with *Penicillium* (group I) and *Leptographium* (group V)

9 Spores dark brown, rather large, several-celled **Bipolaris**
Compare with *Pithomyces* (group IV) and *Trichocladium* (group V)

Spores colourless; usually associated with eelworms **10**

10 Spores solitary at the tip of a long unbranched conidiophore (sometimes weakly branched) **11**

Several spores on each conidiophore **12**

11 Spores spindle-shaped (tapered toward the ends), with one cell markedly larger than the others **Monacrosporium**

Spores cylindrical to club-shaped, without any of the cells markedly enlarged **Dactylella**

12 Spores produced along the length of an elongating and more or less zigzag conidiophore **Geniculifera**

Conidiophores producing a series of short branches from a single locus (candelabrum-like), with each branch bearing a spore **Candelabrella**

GROUP IV

1 Spores 1-celled **2**

Spores with more than one cell **11**

2 Spores produced within a distinct fruiting body having a hyphal or cellular wall **3**

Spores borne externally **6**

3 Fruiting bodies or spore mass brown or black **4**

Fruiting bodies and spore mass colourless or brightly coloured **5**

4 Spores brown; fruiting bodies (pycnidia) lacking spines **Coniothyrium**
Compare with *Myrothecium* (group V)

Spores colourless or brightly coloured; fruiting bodies (pycnidia) with spines around the apical opening **Pyrenochaeta**

5	Fruiting bodies (cleistothecia) composed of hyphae; associated with *Penicillium* (group I) Compare with *Gymnoascus* (group V) and *Arachniotus* (not treated here)	**Talaromyces**
	Fruiting bodies (cleistothecia) with a distinctly cellular wall; associated with *Aspergillus* (group I) Compare with *Neosartorya* and *Emericella* (neither treated here). Similar forms associated with *Penicillium* are probably *Eupenicillium* (not treated here)	**Eurotium**
6	Spores distinctly dark brown or black	**7**
	Spores colourless or quite pale	**8**
7	Spores usually spherical and roughened, with two hyphal connections; hyphae not septate Associated with *Mucor, Zygorhynchus, Absidia, Rhizopus*, etc.	**Zygospores of Mucorales**
	Spores discoid or egg-shaped, often with a colourless band, usually smooth, with only one connection to the conidiophore; hyphae septate Compare with *Wardomyces* and *Nigrospora* (both group V)	**Arthrinium**
8	Spores in chains (sometimes interrupted by sterile cells)	**9**
	Spores not in chains	**10**
9	Spore chains often characterized by an alternating series of spores and narrow sterile cells (bead-like in appearance); filaments never dark Compare with *Chrysoporium* (group II)	**Geomyces**
	Spore chains composed of uniformly cylindrical spores, never with alternating sterile cells; conidiophores often dark	**Oidiodendron**
10	Spores borne from the apex of flask-shaped phialides with a flaring collar	**Phialophora**
	Spores borne at the tips of somewhat jagged conidiophores	**Sporothrix**
11	Spores borne in fruiting bodies (pycnidia), 2-celled	**Diplodia**
	Spores borne externally, with more than two cells Compare with *Trichocladium* (group V)	**Pithomyces**

GROUP V

1	Spores 1-celled	**2**
	Spores with more than one cell	**11**
2	Spores borne in dense masses within some kind of structure	**3**
	Spores produced externally, never from any kind of compound structure	**5**
3	Spores produced inside thin-walled sporangia that are recurved on short hooks Compare with *Mucor* (group I)	**Circinella**
	Spores never produced in recurved sporangia	**4**

4 Fruiting structure a sporodochium containing a layer of conidiophores; spores in slimy masses, very dark green **Myrothecium**

Fruiting structure a cleistothecium containing asci (when young) and ascospores; spores dry at maturity; never associated with phialides **Gymnoascus**

5 Spores brown to black **6**

Spores brightly coloured or colourless **8**

6 Spores roughened, with a prolonged apical snout; often associated with *Doratomyces* (group II) **Echinobotryum**

Spores smooth **7**

7 Spores usually borne in small clusters, usually egg- or bullet-shaped, with a narrow colourless band (germ slit); may be associated with *Scopulariopsis* (group III) **Wardomyces**

Spores solitary, usually spherical to somewhat flattened spherical, often with a germ slit **Nigrospora**

8 Conidiophores dark brown, densely branched at the apex and bearing the colourless spores in a drop of fluid **Leptographium**
Compare with *Verticicladiella* and *Phialocephala* (not treated here)

Conidiophores colourless **9**

9 Spores completely covering a large swelling at the apex of an erect conidiophore **Oedocephalum**
Compare with *Cunninghamella* (figure 2D)

Conidiophores not well-developed and lacking a terminal swelling **10**

10 Spores relatively large, usually nearly spherical, roughened **Sepedonium**

Spores quite small, usually egg-shaped, smooth; often pathogenic to man **Trichophyton**

11 Spores dark (at least some of the cells) **12**

Spores colourless **13**

12 Spores with long appendages at the apex, borne in sporodochia **Pestalotiopsis**

Spores lacking appendages, not in sporodochia **Trichocladium**

13 Spores 2-celled, produced in chains from the apex of erect conidiophores **Trichothecium**

Spores usually more than 2-celled or irregularly 1- to several-celled, not arising from distinct conidiophores; often pathogenic to man **Trichophyton**
Compare with *Microsporum* (figure 7A)

A—*Key to major groups based on character of basidium and fruit-body shape*

1 Basidia produced in a layer of cells (hymenium) and exposed to the air before the maturity of the spores. (Hymenomyctes) ... 2
Basidia either produced in a hymenium or in a mass, and until maturity contained within a closed fruit-body. (Gasteromyctes) ... 6

2 Basidia simple; a single cell. (Homobasidiae) ... 3
Basidia usually septate, or if simple then fruit-body gelatinous and often collapsing to form a skin when dried. (Heterobasidiae) ... 4

3 Fruit-body usually fleshy, soft and easily decaying (putrescent); spores produced on the surface of gills or ridges, or within tubes. (Agaricales) ... (10)
Fruit-body with spores produced on smooth surfaces, teeth, ridges or plates or if within tubes, then fruit-body tough and leathery. (Aphyllophorales) ... (68)

4 Basidia divided. ... 5
Basidia simple and apex drawn out into two long necks. (Dacrymycetales) ...(111)

5 Basidia divided into two or four cells by vertical cross-walls. (Tremellales) ...(115)
Basidia divided transversely by one to three horizontal cross-walls. (Auriculariales) ...(113)

6 Fruit-body growing beneath soil-surface (hypogeous).
Hymenogaster & *Rhizopogon*. (False Truffles)
Fruit-body not growing beneath the soil-surface. ... 7

7 Spores in a slimy mass on a specialised fruit-body arising from an egg-like structure *Phallus* & *Mutinus*. (Stinkhorns)
Spores powdery at maturity or in small capsules. ... 8

8 Spores powdery at maturity and contained within the fruit-body. ... 9
Spores enclosed in a small capsule, or group of capsules in a cup-like structure resembling the eggs within the nest of a bird.
Crucibulum, Cyathus & *Nidula*. (Bird's nest fungi)

9 Spores intermixed with threads within the fruit-body from which they are dispersed through a specialised pore at its apex. (Outer surface intact or flaking away) ... *Lycoperdon*.
(Outer surface folding back to form a star-like pattern).
... *Geastrum*. (Puff-balls and Earth-stars)
Spores not mixed with threads within the fruit-body and not dispersed through a special structure, but through cracks as the fruit-body weathers.
... *Scleroderma*. (Earth-balls)
(If resembling an unexpanded mushroom, compare with *Endoptychum agaricoides*.)

10 Spores produced on gills, ridges or veins but never in distinct tubes, although gills may become poroid at stem-apex. ... 11
Spores produced in tubes. ... (62)

11 Spores distinctly coloured in mass and coloured individually under the microscope. ... 12
Spores not coloured, or only faintly in mass and hyaline under the microscope. ... (35)

12 Spores pinkish. ... 13
Spores blackish or some shade of brown. ... (17)

13 Stipe laterally attached to the cap or absent. . . . *Claudopus*. (and some species of *Clitopilus*)
Stipe centrally attached to the cap. . . . 14
14 Stipe with a cup-like structure enveloping the base. . . . *Volvariella*.
Stipe lacking any special structure at its base. . . . 15
15 Gills not attached to the stipe (free), or with part attached to and descending down the stipe (decurrent). . . . 16
Gills attached to the stipe but not descending down the stipe. . . . 17
16 Gills remote to free from the stipe. . . . *Pluteus*.
Gills distinctly attached and descending down the stipe. . . . *Clitopilus*. (see also *Eccilia*)
17 Gills broadly attached to the stipe (adnate). . . . *Entoloma*.
Gills narrowly attached to the stipe (adnexed). . . . *Leptonia* & *Nolanea*.
18 Stipe laterally attached to the cap. . . . *Crepidotus*.
Stipe centrally attached to the cap. . . . 19
19 Spore-print some shade of brown. . . . 20
Spore-print blackish to purplish black. . . . 28
20 Spore-print bright rust-brown. . . . 21
Spore-print dull clay-brown or ochraceous. . . . 26
21 Stipe with the veil girdling the stem to form a ring or cobweb-like (cortina). . . . 22
Stipe without a ring, or if present then easily lost. . . . 23
22 Stipe with distinct ring or ring-zones. . . . *Pholiota* & related genera.
Stipe with cobweb-like veil or faint filamentous ring-zone. . . . *Cortinarius* & *Gymnopilus*.
23 Gills attached to the stipe but not descending down the stipe (adnexed to adnate). . . . 24
Gills free of stipe, or distinctly attached to and running down the stipe (decurrent), and then often joined together at the apex of the stipe, or at their base. . . . 25
24 Cap-surface composed of rounded cells. . . . *Conocybe*.
Cap-surface composed of filamentous cells. . . . *Galerina*.
25 Gills free of the stipe and the whole fruit-body very fragile. . . . *Bolbitius*.
Gills attached to and running down the stipe (decurrent), easily separable from the cap-tissue and frequently veined at apex of stipe. . . . *Paxillus*.
26 Cap scaly, fibrillose and roughened. . . . *Inocybe*.
Cap smooth, greasy or viscid. . . . 27
27 Cap-surface composed of rounded cells. . . . *Agrocybe*.
Cap-surface composed of filamentous cells. . . . *Naucoria* & *Hebeloma*.
28 Gills or complete fruit-body becoming liquified. . . . *Coprinus*.
Neither the gills nor the fruit-body collapsing into a slurry of tissue. . . . 29
29 Gills free to remote from the stipe or attached and descending down the stipe (decurrent). . . . 30
Gills attached in some way to the stipe but not descending down the stipe (adnate to adnexed). . . . 31
30 Gills decurrent; stipe possessing a cobweb-like veil. . . . *Gomphidius* & *Chroogomphus*.

Gills remote or free; stipe usually possessing a persistent ring. (If unexpanding, compare with *Endoptychum agaricoides*) . . . *Agaricus*.

31 Gills distinctly spotted or distinctly mottled; stipe stiff but breaking with a snap when bent; growing on dung or in richly manured areas. . . . *Panaeolus*.

Gills not spotted nor distinctly mottled; stipe cartilaginous or not; and fruit-body rarely growing on dung. . . . 32

32 Gills broadly attached to the stipe (adnate) and with a veil girdling the stipe. . . . *Stropharia*.

Gills narrowly attached to the stipe (adnexed) or with concave dentation near the stipe (sinuate), or if adnate lacking a ring. . . . 33

33 Gills with concave indentation near the stipe (sinuate) and cap and stipe with a cobweb-like veil. . . . *Hypholoma*.

Gills attached to the stipe but lacking a distinct concave indentation near the stipe. . . . 34

34 Stipe stiff but breaking with a snap when bent; edge of cap incurved at first and cap-surface composed of filamentous cells. . . . *Psilocybe*.

Stipe fragile, edge of cap straight even when young and cap-surface composed of rounded cells. . . . *Psathyrella*.

35 Fruit-body fleshy and readily decaying, often firm but never tough. . . . 36

Fruit-body tough and not easily decaying. . . . 37

36 Growing on other agarics. . . . *Asterophora* (Nyctalis). (and some *Collybia*)

Not growing on other agarics. . . . 37

37 Spore-bearing layer (hymenium) on fold-like often forked gills or simply on irregularities. . . . 38

Spore-bearing layer (hymenium) on distinct well-formed gills. . . . 39

38 Spore-bearing layer on fold-like gills. . . . *Cantharellus*.

Spore-bearing layer on smooth or irregular surface. . . . *Craterellus*.

39 Cap easily separable from the stipe. . . . 40

Cap not easily separable from the stipe. . . . 41

40 Stipe with girdling veil (ring) and/or with a persistent cup-like structure at the base (volva); cap usually with warts or scales distributed on its surface. . . . *Amanita*.

Stipe with ring but lacking the volva; cap surface powdery, hairy or scaly. . . . *Lepiota* & related genera.

41 Cap, stipe and gills brittle; stipe never stiff and either exuding a milk-like juice or not; spores with spines or warts which stain blue-black in solutions containing iodine. . . . 42

Cap, stipe and gills soft or if stipe is stiff then snapping when bent and gills never brittle. . . . 43

42 Fruit-body exuding a milk-like or coloured fluid. . . . *Lactarius*.

Fruit-body not exuding fluid. . . . *Russula*.

43 Gills thick, watery and lustrous (waxy) or with a bloom as if powdery with talc; often brightly coloured. . . . 44

Gills not waxy and rarely over 1·5 mm thick. . . . 46

44 Gills rather watery and lustrous (waxy); spores smooth. . . . 45

Gills rigid not watery, with powdery bloom; spores with distinct spines.
. . . *Laccaria.*

45 Fruit-body with a distinct veil and growing in woods; cap often viscid or pale coloured. . . . *Hygrophorus.*
Fruit-body lacking a veil and usually growing in fields; cap usually brightly coloured and sometimes viscid. . . . *Hygrocybe.*

46 Stipe with girdling veil (ring) and/or stipe not attached to the centre of the cap (eccentric). . . . 47
Stipe central and lacking a ring. . . . 48

47 Stipe central and possessing a ring. . . . *Armillaria.*
Stipe not centrally attached to the cap (members of the Pleurotaceae) including *Pleurotus* (Oyster mushroom).

48 Stipe fibrous. . . . 49
Stipe stiff only in the outer layers. . . . (52)

49 Gills with a concave indentation near the stipe (sinuate). . . . 50
Gills attached to and descending down the stipe (decurrent). . . . 51

50 Spores with warts which darken in solutions containing iodine.
. . . *Melanoleuca.*
Spores not so colouring in solutions containing iodine.
. . . *Tricholoma* & related genera.

51 Spores with warts which darken in solutions containing iodine.
. . . *Leucopaxillus.*
Spores not so colouring in solutions containing iodine.
. . . *Tricholoma* & related genera.

52 Gills thick and with rather blunt edges. . . . *Cantharellula* & *Hygrophoropsis.*
Gills thin and with distinct sharp edge. . . . 53

53 Gills attached to and descending down the stipe (decurrent); cap often depressed at the centre and sterile cells absent from the gills and the surface of the cap.
. . . *Clitocybe* & *Omphalina.*
Gills attached to the stem but not descending down the stipe (adnate to adnexed), or if descending then distinct sterile cells on the gills, cap and stipe. . . . 54

54 Cap-edge straight and usually striate when young; cap thin and somewhat conical and gills descending down the stipe or not.
. . . *Mycena* & related genera.
Cap-edge incurved; non-striate and cap rather fleshy; gills not descending down the stipe. . . . 55

55 Stipe dark and woolly at least in the lower half and the cap viscid; fruit-bodies growing in clusters on tree trunks. . . . *Flammulina.*
Stipe not dark and woolly. . . . 56

56 Cap viscid and stipe usually rooting; fruit-body growing directly on wood or attached to wood by long strands or cords of mycelium (rhizomorphs).
. . . *Oudemansiella.*
If cap viscid and fruit-body neither attached to wood by cords of mycelium nor stipe with a rooting base. . . . *Collybia* & related genera.

57 Stipe central and gills often interconnected by veins; can be dried and later

revived purely by moistening. . . . *Marasmius* & related genera.
Stipe not attached to the centre of the cap and fruit-body, although persistent, not easily revived to natural shape after once being dried. . . . 58

58 Spore-print blue-black with solutions containing iodine. . . . 59
Spore-print yellowish in solutions of iodine. . . . 60

59 Gills toothed or notched along edges. . . . *Lentinellus*.
Gills even along their edges and not toothed. . . . *Panellus*.

60 Gills appearing as if split down their middle. . . . *Schizophyllum*.
Gills not splitting. . . . 61

61 Gills notched or toothed along their edges. . . . *Lentinus*.
Gills even along their edges and not toothed. . . . *Panus*.

62 Spore-print yellowish, purplish-black or pink. . . . 63
Spore-print some shade of brown, but without purplish flush. . . . (66)

63 Spore-print yellowish or pinkish. . . . 64
Spore-print purplish-brown or blackish. . . . 65

64 Spore-print yellowish. . . . *Gyroporus*.
Spore-print pinkish. . . . *Tylopilus*.

65 Spore-print purplish-brown. . . . *Porphyrellus*.
Spore-print blackish and spores ornamented. . . . *Strobilomyces*.

66 Cap glutinous and stem with or without girdling veil (ring); sterile cells (cystidia) within tubes clustered together. . . . *Suillus*.
Cap at most viscid and then only in wet weather and sterile cells within tubes individually sited. . . . 67

67 Stipe-surface covered with distinct black or dark brown, or white then darkening scales; spore-print clay-brown with or without a flush of cinnamon-pinkish brown. . . . *Leccinum*.
Stipe-surface covered completely or in part with a network or pattern of faint lines, or pale yellow or red-rust but never black dots; spore-print olivaceous-buff. . . . *Boletus* & related genera.

68 Spore-bearing layer (hymenium) quite smooth, or spread over veins, or shallow pores; fruit-body top-shaped, fan-shaped or club-shaped, or spread over the substrate (resupinate). . . . 69
Spore-bearing layer lining the inner surface of tubes or borne on warts or spines. . . . (84)

69 Fruit-body club-shaped, coral-shaped or distinctly funnel-shaped, fan-like or resembling an agaric. . . . 70
Fruit-body resupinate or with poorly developed cap. . . . (78)

70 Fruit-body coral-like or club-shaped with clubs grouped or branched. . . . 71
Fruit-body resembling an agaric or funnel-shaped to fan-shaped. . . . (76)

71 Fruit-body large, branched with flattened and curled lobes and so resembling a cauliflower. . . . *Sparassis*.
Fruit-body of single or grouped clubs, or if branched then not resembling a cauliflower, the lobes being cylindrical or only slightly flattened and hardly bent. . . . 72

72 Fruit-body small arising from a seed-like structure or growing attached to dead herbaceous plant remains. . . . 73
Fruit-body medium to large, simple or branched and usually growing on the

ground; one large species grows on wood. . . . 74

73 Fruit-body arising from a seed-like body embedded in the plant tissue or found loose in the soil. . . . *Typhula*.
Fruit-body on dead plant remains but seed-like structure absent. . . *Pistillaria*.

74 Fruit-body much branched; spores ornamented.
. . . *Ramaria*. (see also *Thelephora* below).
Fruit-body simple or if with well-developed branches then spores smooth. . . . 75

75 Fruit-body branched irregularly with many to few branches, grey, white or dull-coloured; spores large, subglobose and smooth. . . . *Clavulina*.
Fruit-body club-shaped or if branched then brightly coloured and spores not large and subglobose. . . . *Clavaria, Clavulinopsis* & *Clavariadelphus*.

76 Fruit-body resembling an agaric with spores borne on fold-like, often forked and shallow ridges and veins, and often brightly coloured.
. . . *Cantharellus*. (compare very carefully with *Craterellus* below).
Fruit-body funnel-shaped or fan-shaped. . . . 77

77 Fruit-body often dull-coloured or grey with smooth or slightly veined outer surface. . . . *Craterellus*.
Fruit-body wrinkled, irregular or smooth and powdery, lilaceous to chocolate-brown in colour.
. . . *Thelephora*. (in N. America compare carefully with *Polyozellus* which resembles a cluster of irregular funnels and *Craterellus* above).

78 Fruit-body sessile or resupinate and fleshy; spores borne on veins united to form shallow pores. . . . 79
Fruit-body resupinate or bracket-like and spore-surface veined or rugulose but lacking distinct pores. . . . 80

79 Spores colourless. . . . *Merulius*
Spores brown. . . . *Serpula*.

80 Spore-bearing layer containing long brown spines. . . . *Hymenochaete*.
Fruit-body lacking spines although often having encrusted sterile cells. . . . 81

81 Surface of fruit-body more or less radiately veined. . . . *Phlebia*.
Surface of fruit-body not radiately veined. . . . 82

82 Spores brown. . . . *Coniophora*.
Spores colourless. . . . 83

83 Flesh distinctly formed and fruit-body with or without a reflexed cap.
. . . *Stereum* & related genera.
Flesh poorly differentiated and fruit-body lacking a cap.
. . . Members of the *Corticiaceae* (including *Peniophora* & *Hyphodontia*).

84 Spores borne on teeth or spines. . . . 85
Spore-bearing layer lining tubes or elongate pores. . . . (89)

85 Fruit-body with central stipe; agaric-like but not attached to cones. . . . 86
Fruit-body encrusting or bracket-like or with lateral stipe if resembling an agaric. . . . 87

86 Fruit-body fleshy; spores smooth. . . . *Hydnum* & related genera.
Fruit-body rubbery or tough; spores rough. . . . *Hydnellum* & related genera.

87 Fruit-body growing attached to cones and cap with lateral stipe.
. . . *Auriscalpium*,
Fruit-body not on cones and distinct stipe lacking. . . . 88

88 Spores borne on a series of radially arranged notches resembling gills. . . . *Lentinellus*.
Spores borne on a resupinate layer of spines. . . . *Mycoacia* & related genera.
89 Tubes free one from another; resembling a piece of flesh or liver. . . . *Fistulina*.
Tubes united to form a distinct tissue; resembling wood, leather or cork. . . . 90
90 Fruit-body perennial and exhibiting more than one layer of tubes. . . . 91
Fruit-body annual although it can persist in a dried depauperate form for several months. . . . (94)
91 Spores brown. . . . 92
Spores colourless. . . . 93
92 Large brown cells present in the tubes; spores simple. . . . *Phellinus* & *Cryptoderma*.
Brown sterile cells absent from tubes; spores complex. . . . *Ganoderma*.
93 Large woody fruit-body with crust-like top. . . . *Fomes*.
Medium-sized to small; fleshy tough fruit-body with downy or crust-like top. . . . *Oxyporus, Fomitopsis* & *Heterbasidion*.
94 Spores borne in labyrinth-like elongate pores, cap either poorly developed or absent, and only resupinate pore-surface present. . . . 95
Spores borne in distinct pores on well-developed woody fruit-bodies. . . . (98)
95 Spores borne in labyrinth-like pores. . . . *Daedalea* & *Daedaleopsis*.
Spores borne in elongate pores like very thick gills, or fruit-body completely resupinate. . . . 96
96 Spore-layer in elongate pores. . . . *Lenzites* (white) & *Gloeophyllum* (brown).
Spore-layer consisting of a resupinate pore-layer. . . . 97
97 Pore-layer totally resupinate; flesh very poorly developed. . . . *Fibuloporia* & related genera.
Fruit-body resupinate or developing ill-formed caps at the margin; flesh well-developed and quite tough. . . . *Datronia, Gloeoporus* & *Bjerkandera*.
98 Fruit-body with a distinct stipe. . . . 99
Fruit-body sessile or with a poorly developed stipe, or if merely with a basal swelling then pores darkening or bruising on handling. . . . 100
99 Pores dark-coloured but spores pale-coloured in mass. . . . *Coltricia*. (also see *Phaeolus* below).
Pores white or creamy, foot often darkened black and pores hyaline. . . . *Polyporus*.
100 Pores brightly coloured, red, lilaceous or orange to apricot colour. . . . 101
Pores never as brightly coloured, cream, white, grey or in some shade of brown. . . . 102
101 Pores red to orange-red. . . . *Pycnoporus*.
Pores lilac to violacaeous, or lilaceous-orange to apricot colour. . . . *Haplopilus* (orange to apricot). . . . *Hirschioporus* (lilaceous).
102 Pore-surface brown or dark-grey and spores often colourless. . . . 103
Pore-surface white or creamy, or yellow; spores hyaline. . . . 105
103 Pore-surface firm and grey. . . . *Bjerkandera*.
Pore-surface greenish-yellow, bruising brown or yellow-brown and darkening

with age. . . . 104

104 Fruit-body lacking a stem, rust-brown, breaking easily, cheesy in texture and with a silky sheen. . . . *Inonotus.*
Fruit-body with a broad basal hump, fibrillose spongy with yellow margin to cap. . . . *Phaeolus.*

105 Tubes forming a layer quite distinct from the flesh; fruit-body fleshy and tough. . . . 106
Tubes not forming a layer distinct from the flesh; fruit-body woody or corky. . . .(110)

106 Pore-surface bright yellow; upper surface yellow or orange. . . . *Laetiporus.*
Pore-surface white; upper surface usually dull coloured or white. . . . 107

107 Fruit-body medium to large, shell-shaped, whitish-brown or silvery-grey on top; on birch. . . . *Piptoporus.*
Fruit-body often frond-like, infrequently shell-shaped and if on birch then small. . . . 108

108 Fruit-body fan-shaped or frond-shaped, composed of innumerable more or less complete caps joined together at their base or to half way.
. . . *Grifola & Meripilus.*
Fruit-body neither fan-shaped nor frond-shaped and compound. . . . 109

109 Fruit-body wholly pale-coloured, white, cream, ivory etc. . . . *Tyromyces.*
Fruit-body except pores usually some shade of brown. . . . *Polyporus.*

110 Cap thick corky or woody and pores medium or large.
. . . *Trametes & Pseudotramets.*
Cap thin but leathery and pores small. . . . *Coriolus.*

111 Fruit-body club-shaped or coral-like. . . . *Calocera.*
Fruit-body top-shaped or with irregular bumps. . . . 112

112 Fruit-body top-shaped. . . . *Ditiola.*
Fruit-body cushion-like or brain-like or with irregular bumps.
. . . *Dacrymyces.*

113 Fruit-body lacking a cap and more or less forming a gelatinous coating on plant debris. . . . *Helicobasidium.*
Fruit-body with more or less distinct cap; gelatinous but tough. . . . 114

114 Fruit-body ear-like or cup-shaped; upper surface with grey hairs and lower surface lilaceous-brown or Burgundy-coloured. . . . *Hirneola.*
Fruit-body at first cup-shaped but then spreading; upper surface grey and hairy, and lower surface purplish. . . . *Auricularia.*

115 Fruit-body with distinct stipe and spines on lower surface.. . . *Pseudohydnum.*
Fruit-body lacking a well-developed stipe, the latter either reduced to a small lobe or entirely absent. . . . 116

116 Fruit-body flattened or disc-shaped, often with warts or veins on the surface; spores more or less sausage-shaped. . . . *Exidia.*
Fruit-body brain-like or with irregular bumps, sometimes lobed or irregular and encrusting. . . . 117

117 Fruit-body brain-like or with bumps or bosses; spores rounded to ovoid.
. . . *Tremella.*
Fruit-body encrusting woody or herbaceous material; spores ellipsoid.
. . . *Sebacina.*

B—*Key to major groups based on characters of fruit-body and spores (only large and obvious genera included)*

118 Asci borne on a distinctly stalked fruit-body. . . . 119
Asci borne on an irregularly lobed, rounded, or club-shaped fruit-body, or the latter cup-shaped, but never stalked. . . .(123)

119 Cap cup-shaped. . . . 120
Cap honeycomb-like, saddle-shaped or irregular. . . . 121

120 Fruit-body grey. . . . *Cyathipodia* (see also 130).
Fruit-body red within. . . . *Sarcoscypha*.

121 Cap irregularly chambered to honeycomb-like.
. . . *Morcella* and allies (Morels)
Cap saddle-shaped or irregular. . . . 122

122 Stipe stout, furrowed, ribbed or chambered. . . . *Helvella*.
Stipe slender with even surface. . . . *Leptopodia*.

123 Fruit-body growing beneath soil-surface.
. . . *Tuber, Elaphomyces* and allies. (True & False truffles).
Fruit-body not growing beneath soil-surface. . . . 124

124 Fruit-body black and carbonaceous either within, externally or throughout. . . . 125
Fruit-body brightly coloured, or if brown then soft and pliable. . . .(127)

125 Fruit-body hemispherical with distinct concentric zones of growth when cut. . . . *Daldinia*.
Fruit-body variously shaped or if hemispherical then without zonation. . . . 126

126 Fruit-body club-shaped, cylindrical or spindle-shaped.
. . . *Xylosphaera*. (Dead man's fingers: Stag's horn fungus).
Fruit-body hemispherical or cushion-shaped.
. . . *Ustulina* & *Hypoxylon*. (if growing on pore-fungi see *Hypocrea*).

127 Fruit-body distinctly cup-shaped or ear-shaped. . . . 128
Fruit-body irregularly lobed, undulating. . . . *Rhizina*.

128 Fruit-body with a split down one side. . . . *Otidea*.
Fruit-body cup-shaped or at most with a wavy margin. . . . 129

129 Fruit-body orange or red; spores ornamented with ridges and reticulations.
(margin with short brown hairs). . . . *Melastiza*.
(margin with eyelash-like hairs). . . . *Scutellinia*.
(margin naked like orange peel). . . . *Aleuria*.
Fruit-body duller in colour, yellow, brown, violaceous but never orange or red; spores smooth or minutely warted or faintly netted. . . . 130

130 Spore-bearing layer becoming bluish-green in solutions containing iodine; (if with stalk then rudimentary). . . . *Peziza*.
Spore-bearing layer not blueing with iodine solutions; cup with broad, ribbed or furrowed stalk-like base. . . . *Paxina*.

Systematic List of Genera—Agarics & Boleti

Family 1. *CANTHARELLACEAE*

1 Cantharellus
2 Craterellus
3 Leptoglossum
4 Gomphus
= Neurophyllum
5 Plicatura
also Polyozellus

Family 2. *BOLETACEAE*

1 Boletus
includes Tubiporus
Phlebopus
Xerocomus
Pulveroboletus
Suillus
= Ixocomus
Leccinum
2 Tylopilus
3 Porphyrellus
4 Gyroporus
5 Gyrodon
6 Boletinus
7 Strobilomyces
8 Phylloporus
9 Paxillus

Family 3. *GOMPHIDIACEAE*

1 Gomphidius

Family 4. *HYGROPHORACEAE*

1 Hygrophorus
Subgenus i Hygrophorus
= Limacium
Subgenus ii Camarophyllus
Subgenus iii Hygrocybe

Family 5. *PLEUROTACEAE*

1 Pleurotus
includes Pleurocybella
2 Hohenbuehelia
3 Resupinatus
4 Pleurotellus
5 Phyllotopsis
6 Crepidotus
7 Geopetalum
8 Lentinus
9 Lentinellus
10 Panus
11 Panellus
12 Schizophyllum

Family 6. *TRICHOLOMATACEAE*

Tribe (a) Tricholomeae

1 Tricholoma
2 Tricholomopsis
3 Lyophyllum
4 Melanoleuca
= Melaleuca
5 Squamanita

Tribe (b) Clitocybeae

6 Clitocybe
= Omphalia
7 Armillaria
8 Leucopaxillus
9 Cantharellula
10 Hygrophoropsis
11 Laccaria

Tribe (c) Collybieae

12 Collybia
Subgenus i Collybia
Subgenus ii Tephrophana
13 Asterophora
= Nyctalis
14 Oudemansiella
= Mucidula
includes Xerula
15 Flammulina
16 Macrocystidia
17 Clitocybula
18 Dermoloma
19 Pseudohiatula
20 Baeospora
21 Mycena
22 Fayodia
23 Myxomphalia
24 Omphalina
= Omphalia
25 Marasmius
includes Androsaceus
Marasmiellus
26 Micromphale
27 Crinipellis
28 Xeromphalina

Family 7. *CLITOPILACEAE*

1 Clitopilus
2 Lepista
= Rhodopaxillus
3 Rhodocybe
includes Clitopilopsis
4 Rhodotus

Family 8. *RHODOPHYLLACEAE*

1 Entoloma
2 Nolanea
3 Leptonia
4 Eccilia
5 Claudopus

Family 9. *CORTINARIACEAE*

1 Cortinarius
  Subgenus i Myxacium
  Subgenus ii Phlegmacium
  Subgenus iii Sericeocybe
  Subgenus iv Cortinarius
    = Inoloma
  Subgenus v Dermocybe
  Subgenus vi Telamonia
    includes Hydrocybe
2 Phaeocollybia
3 Leucocortinarius
  includes Cortinellus
4 Rozites
5 Phaeolepiota
6 Flocculina
7 Phaeomarasmius
8 Tubaria
9 Gymnopilus
  = Fulvidula
  includes Flammula
10 Galerina
  includes Galera
11 Pholiota
  = Dryophila
12 Hebeloma
  includes Myxocybe
    Hylophila
13 Naucoria
  includes Alnicola
    Simocybe
    Hylophila
14 Inocybe
  Subgenus i Inocybe
    = Eu-Inocybe
  Subgenus ii Clypeus
    = genus Astrosporina and genus Clypeus

Family 10. *BOLBITIACEAE*

1 Bolbitius
2 Pluteolus
3 Conocybe
  includes Pholiotina
    Galerella
    Galera
4 Agrocybe
  includes Togaria

Family 11. *STROPHARIACEAE*

1 Stropharia
2 Hypholoma
  = Naematolma
3 Psilocybe
4 Deconica

Family 12. *COPRINACEAE*

1 Coprinus
  includes Pseudocoprinus
    Coprinarius
2 Psathyrella
  = Drosophila
3 Lacrymaria
4 Panaeolus
  includes Coprinarius
    Anellaria
5 Panaeolina

Family 13. *AGARICACEAE*

Tribe (a) Agariceae

1 Agaricus
  = Pratella
  = Psalliota
  includes Chitonia
2 Melanophyllum
  includes Chlorospora
    Glaucospora

Tribe (b) Lepioteae

3 Lepiota
  includes Leucocoprinus
    Macrolepiota
    Leucoagaricus
4 Leucocoprinus
  = Hiatula
5 Cystoderma
6 Drosella
  = Lepiotella

Family 14. *VOLVARIACEAE*

1 Volvariella
  = Volvaria
2 Pluteus

Family 15. *AMANITACEAE*

1 Amanita
  includes Amanitopsis
    Lepidella
    Aspidella
2 Limacella

Family 16. *RUSSULACEAE*

1 Russula
2 Lactarius

Identification keys

Genera of lamellate and tubulate fungi; poisonous fungi from other groups

Key to classification into the principal groups

1a Fungi mostly large, with soft flesh, ephemeral, not forming perennial fruit-bodies. Here also tough, annual, stipitate fungi with lamellae or pores on the underside of the pileus, and small lamellate fungi
  2a Fungi with lamellae, tubes, or ridges on the underside of the pileus. Hymenium with basidia . . . . . **Key I: Basidiomycetes with stipitate-pileate fruit bodies and/or lamellae, tubes, or ridges on the underside of the pileus**
  2b Fungi not with lamellae, tubes, or ridges on the underside of the pileus
    3a Fungi with basidia. Fruit-bodies often clavate, ramificate, globose, lageniform; if stipitate-pileate, then with teeth on the underside of the pileus . . . . . **Key XVIII: Remainder of the Basidiomycetes excluding lamellate, tubulate and plicate fungi**
    3b Fungi with asci. Fruit-bodies crateriform, claviform, liguliform, globose, or stipitate/capitate . . . . . **Key XIX: Ascomycetes**
1b Fungi very small and/or with very tough, perennial fruit-bodies. Here all tough, dimidiate, fruit-bodies with pores or tough fungi with a smooth hymenophore . . . . . **Fungi not dealt with here**

I Basidiomycetes (forming short-lived fruit-bodies) with stipitate-pileate fruit-bodies and/or with lamellae, tubes, or ridges on the underside of the pileus

1a Fungi with pores or tubes on the underside of the fruit-body
  2a Fungi with soft flesh, readily decaying. Tubes mostly readily detached from the base of the pileus . . . . . **"Boletus"**; key XVII,
  2b Fungi tough, in the dry state hard, mostly not rapidly decaying. Tubes firmly attached to the base of the pileus . . . . . **"Polyporus"**; not dealt with further
1b Fungi with lamellae on the underside of the fruit-body
  3a Lamellae split lengthwise in two halves. Depending on the humidity, lamellae halves involute or straight. Fruit-body tough, dimidiate, not stipitate, on top matted tomentose, whitish grey . . . . . **Schizophyllum**
  3b Lamellae not split lengthwise
    4a Fruit-body mostly already tough in the fresh state; drying rapidly or not. Dried fungi swelling again in water. Spore mass white or ochre. Here also fungi growing on wood with lamellae having a coarsely serrate edge and a white spore mass
      5a Stipe thick and short; more or less lateral or absent. Fruit-body with fairly thick flesh in the pileus and stipe
        6a Edge of lamellae largely entire . . . . . **"Panus"**; key VIII,
        6b Edge of lamellae ragged, serrate, or dentate . . . . . **"Lentinus"**; key VII,
      5b Stipe more or less slender and centric. Fruit-body more or less small and with thin flesh . . . . . **"Marasmius"**; key VI,

4b Fresh fungi not woody and tough, but readily decaying. Dried fungi usually not swelling again in water. In doubtful cases, carry on the identification from here

7a Lamellae ridged, only slightly raised and obtuse, sometimes much reduced; edges of the lamellae not acute and therefore sometimes indistinct

8a Fungi fruiting on the decaying remains of lamellate fungi, surface of the pileus decomposing and becoming partly pulverulent. Lamellae not anastomosing . . . . . **Asterophora**

8b Fungi not parasitizing other lamellate fungi or with other characters. Lamellae ridged or plicate, repeatedly furcate and anastomosing, often decurrent . . . . . **"Cantharellus"**; key V,

7b Lamellae not ridged or plicate. Edges of the lamellae acute, not obtuse

9a Fungi exuding latex and large- to medium-sized. When laticiferous and at the same time smaller, then the stipe at least 4mm thick. Spores with amyloid ornamentation . . . . . • **Lactarius**,

9b Fungi without latex. Or, when small and laticiferous, then the stipe less than 4mm thick. When small and laticiferous and the spores have amyloid ornamentation, see above under Lactarius

10a Fungi almost exclusively with lamellae that run from the margin of the pileus to the stipe, i.e. largely without lamellulae, and/or are brittle. Flesh easily broken. Often with a distinctively coloured pileus, but sometimes white, black, or blackening. Spores with amyloid ornamentation. Flesh on microscopical examination showing sphaerocysts . . . . . • **Russula**,

10b Fungi usually with lamellae that have intercalated lamellulae and that are neither brittle nor splitting. No sphaerocysts in the flesh (with the exception of the pileal surface)

11a Lamellae deliquescing

12a Entire fungus, but especially the lamellae, soon deliquescing to a black ink . . . . . • **Coprinus**,

12b Fungus with brown deliquescing lamellae. Pileus in the most frequent species bright yellow, otherwise white, grey-lilac, pinkish to violettish and then with a reticulately veined surface or not and on the stumps of deciduous trees . . . . . **Bolbitius**

11b Lamellae not deliquescing and not having the characteristics mentioned under 12a and 12b

13a Lamellae soft, gradually becoming black because of the maturing spores and at the same time more or less viscid, and decurrent. Spores elongated fusiform and pigmented. Either with a viscid-glutinous veil or the base of the stipe amyloid. In the most common species base of the stipe yellow . . . . . **"Gomphidius"**; key XI,

13b Not with the foregoing combination of characters. Fungi mostly not amyloid and not with the base of the stipe yellow; if seemingly so, then spores not simultaneously pigmented and fusiform

14a Fungi with spider's web-like veil (= cortina) between the margin of the pileus and the stipe. Cortina in older specimens often only an indistinct fibrous zone on the stipe which is coloured brown, rust-brown, etc. by the falling spores. Lamellae some sort of brown, ochre, or dark colour, not white or whitish; stipe and/or pileus viscid to glutinous, or pileus distinctly hygrophanous, or stipe bulbous. In doubtful cases, see also . . **"Cortinarius"**; key XII,

14b Fungi without cortina

15a Large to moderately large fungi with a universal veil that is present as a basal volva or in the form of detersile scales or conical papillae on the surface of the pileus. Lamellae white or whitish, free. When a manchette is also present on the stipe, then not as a loose, movable ring. Spores sometimes amyloid, but never pseudoamyloid . . . . . • **Amanita**,

15b Fungi with other characters

16a Lamellae readily detached from the fleshy base of the pileus and the margin of the pileus involute or long remaining so . . . . . **"Paxillus"**; key X,

16b Not simultaneously with lamellae readily detached from the base of the pileus and the margin of the pileus involute. In doubtful cases, continue the identification procedure here

17a Lamellae thick and at the same time waxy (the faces usually transversely rugose, like dripping wax). Spore mass white. Fungi partly with bright red and/or yellow or green colours or partly with viscid surfaces. Basidia usually longer than five times the spore length and longer than 45 µm . . . . . **"Hygrophorus"**; key IV,

17b Either the lamellae not waxy and thick or the spore mass not white. Basidia less than five times the spore length (at least in the case of ellipsoidal spores) . . . **"Agaricus"**; key II, overleaf

II Key to "Agaricus" based on the colour of the spore mass

1a Spore mass white or whitish; in rare cases dingy yellow, but not ochre . . . . . **"Leucospori"**; key III,
1b Spore mass deeper in colour, dingy flesh-coloured to black
2a Spore mass pink, reddish, reddish ochre, or dingy flesh-coloured . . . . . **"Hyporrhodii"**; key IX,
2b Spore mass ochre, brown, purple, or black
3a Spore mass rust-coloured, ochre, ochre-brown, brown . . . . . **"Dermini"**; key XIII,
3b Spore mass purple-brown to black
4a Spore mass purple-brown or purple-black, but not pure deep black . . . . . **"Pratelli"**; key XV,
4b Spore mass pure black . . . . . **"Coprinarii"**; key XVI,

III Key to the "Leucospori"

1a Stipe in most specimens essentially centric
2a Veil present and distinct as a universal and/or partial veil. If veil indistinct in the form of flocci on the pileus and stipe, then the lamellae free and/or the cortical layer comprising globose cells
3a Stipe clearly differentiated from the pileus, more or less cleanly separable from the pileus and/or the cortical layer comprising globose cells and hence the surface appearing granulose, farinose, etc.
4a Universal veil either on the surface of the pileus in the form of warts or scales or at the base of the stipe as a saccate volva. If a manchette is present on the stipe, this not in the form of a free, movable annulus. Cortical layer not comprising globose cells. Spores not pseudoamyloid, sometimes amyloid . . . . . • **Amanita**,
4b Remains of the universal veil firmly attached to the surface of the pileus and therefore not detersile; moreover, not forming a saccate volva. Partial veil mostly present as an annulus. Cortical layer sometimes comprising globose cells. Spores often pseudoamyloid, sometimes also amyloid . . . . . **"Lepiota"**; key IIIa,
3b Stipe firmly joined to the pileus and therefore not readily separated from it. Stipe with an annulus, volva absent. Cortical layer not comprising globose cells . . . . . **"Armillaria"**; key IIIb,
2b Veil completely absent or present only as a spider's web-like cortina. Outside layer of the cortical layer not comprising globose cells, but at most clavate elements (and then the cortical layer hymeniform)
5a Lamellae not decurrent. Or if decurrent, then the pileus also campanulate and the fungus small
6a Large to moderately large, more or less fleshy species
7a Stipe fleshy, not corticate. Lamellae sinuate-adnate . . . . . **"Tricholoma"**; key IIIc,
7b Stipe fibrous-fleshy and corticate. Lamellae adnexed-adnate. Pileus mostly plano-convex with involute margin when young . . **"Collybia"**; key IIId,
6b Tiny to small, at most moderately large, thin-fleshed to membranous species
8a Pileus plano-convex; margin of pileus involute when young, later often straight; old specimens sometimes depressed in the centre. Lamellae adnexed to somewhat sinuate . . . . . **"Collybia"**; key IIId,
8b Pileus campanulate, conical, or applanate, when young with a straight margin that is often close to the stipe and pointing downwards. Lamellae variously attached to the stipe; when decurrent, the pileus never with a depressed centre . . . . . **"Mycena"**; key IIIe,
5b Lamellae decurrent. Pileus not campanulate, but plano-convex or infundibuliform or umbilicate in the centre
9a Stipe more or less elastic and inside with fibrous flesh. Pileus more or less fleshy . . . . . **"Clitocybe"**; key IIIf,
9b Stipe stiffly corneous. Pileus more or less thinly membranous, umbilicate and often pellucid-striate. In doubtful cases, continue here . . . . . **"Omphalia"**; key IIIg,
1b Stipe absent, lateral, or usually very excentric; mostly growing on wood . . . . . **"Pleurotus"**; key IIIh,

IIIa Key to "Lepiota"

1a Lamellae free, thus neither narrow adnate nor sinuate-adnate

2a Pileus dry, not viscid-glutinous; spores mostly pseudoamyloid

3a Very large fungi, pileus 5 to 20cm and more. Annulus on the stipe freely movable. Spores with a distinct germ pore, never spindle- or projectile-shaped; larger than 10µm; clamp-connexions present in the trama of the pileus and stipe or absent. Pileus and stipe dry . . . • **Macrolepiota**,

3b Small to moderate-sized fungi. Spores less than 10µm or spindle- or projectile-shaped

4a Spores with germ pore. Hyphal septa without clamp-connexions

5a Marginal zone of the pileus finely striate. Pileus small, more or less floccose. When the fungus is large, then reddening distinctly. Annulus not movable. Spores sometimes longer than 9µm. . . . . **Leucocoprinus**

5b Marginal zone of the pileus not striate. Fungi in part moderately large, like Agaricus species; if with reddening flesh, then with a movable annulus. Spores smaller than 9µm . . . . . **Leucoagaricus**

4b Spores without germ pore. Hyphal septa in some cases with clamp-connexions

6a Pileus with farinose-corneous surface. Cortical layer composed of balloon-shaped to globose elements. Spores often not pseudoamyloid . . . . . **Cystolepiota**

6b Pileus naked, squamose or sericeous-fibrillose. Cortical layer not composed of balloon-shaped or globose elements

7a Hyphal septa without clamp-connexions. Surface of the pileus naked, fibrillose-sericeous, at most towards the margin with scarcely visible flocci, white . . . . . **Sericeomyces**

7b Hyphal septa mostly with clamp-connexions. Surface of the pileus squamose to furfuraceous-squamose, variously coloured, sometimes also white . . . . . • **Lepiota**,

2b Pileus and sometimes also the stipe viscid or glutinous. Spores not amyloid

8a Pileus ochraceous ivory. Smell more or less unpleasant. Spores small, 4.5 to 5×2 to 2.5µm. Cortical layer hymeniform. Cystidia present . . . . . **Chamaemyces**

8b Pileus yellow, brown, red-brown to vinaceous, sometimes also white. Spores broader and/or longer, globose to broadly ellipsoidal. Trama of the lamellae when young distinctly bilateral. Cortical layer not hymeniform. Cystidia absent . . . . . **Limacella**

1b Lamellae narrowly adnate. Pileus with pulverulent to corneous surface. Cortical layer comprising globose cells. Spores amyloid or not amyloid . . . . . **Cystoderma**

IIIb Key to "Armillaria"

1a Lamellae more or less decurrent, certainly not sinuate. Pileus not simultaneously white and viscid-glutinous

2a Pileus with a thin, pellucid-striate or striate marginal zone. Stipe about 1.5 to 2.5cm broad, with a single annulus. Clamp-connexions at the septa of the tramal hyphae mostly absent, but sometimes present at the foot of the basidia. Spores ellipsoidal, not amyloid. Often on wood, frequently fasciculate; less often, a connection with the wood substrate not recognizable or occasionally fungi (rare species) not on wood . . . . . • **Armillaria**,

2b Compact thick- or hard-fleshed fungi with the margin of the pileus long remaining involute and not pellucid-striate. Stipe massive, with a double annulus. Clamp-connexions at the septa of the tramal hyphae present. Spores narrow, elongate, 11 to 13×5 to 6µm, amyloid. Among grass and at the edges of forests, in mountain meadows, etc. . . . . **Catathelasma**

1b Lamellae not decurrent but sinuate, or pileus white

3a Pileus white or whitish

4a Pileus with viscid-glutinous surface. Stipe almost corneous. Growing on wood (mostly beech). Basidia not siderophilous. Spores 14 to 18×12 to 16µm, glabrous, not amyloid . . . . . **Oudemansiella**

4b Pileus dry. Spores with other dimensions, partly with amyloid tubercles. Partly with siderophilous basidia

5a Stipe white. Spores 7 to 8.5×4 to 5µm, not with amyloid tubercles, but tuberculate to spinose. Basidia siderophilous . . . . . **Calocybe** in part (constricta)

5b Stipe appearing as if punctate because of black flocci. Spores 8.5 to 10(to 11)×4.5 to 5.5µm, with amyloid tubercles. Basidia not siderophilous . . . . . **Melanoleuca** partly (verrucipes)

3b Pileus distinctly coloured

6a Pileus straw-yellow to greenish yellow. Stipe below the annulus covered with small, concolorous scales. Fairly large fungi, pileus 7 to 10cm. Spores amyloid, 8 to 9×5.5 to 6µm . . . . . **Floccularia**

6b Pileus grey, ochre, brown, reddish brown, or orange. Spores not amyloid

7a Stipe at the base with a marginate bulb. Veil more like a cortina than a membranous annulus. Pileus brown. Spore mass very pale ochraceous whitish. Septa of the tramal hyphae with clamp-connexions . . . . . **Leucocortinarius**

7b Stipe at the base not with a marginate bulb. Pileus dark grey, brown, reddish brown, to orange. Septa of the tramal hyphae without clamp-connexions . . . . . • **Tricholoma** partly,

IIIc Key to "Tricholoma"

1a Spores amyloid
- **2a** Spores with amyloid tubercles
  - **3a** Fungi distinctly soft-fleshed and at the same time often with a more or less slender stipe. Hyphal septa without clamp-connexions. Hymenium sometimes with distinctly lanceolate, tubular, or ventricose-rostrate cystidia . . . . . **Melanoleuca**
  - **3b** Fungi mostly firm-fleshed. Hyphal septa with clamp-connexions. Cystidia absent
- **2b** Spores not with amyloid tubercles; the smooth wall, however, entirely amyloid
  - **4a** Pileus squamose-squamulose or conical to conico-umbonate. Hyphal septa with clamp-connexions. Cortical layer not hymeniform . . **Porpoloma**
  - **4b** Pileus not squamose-squamulose, mostly convex-campanulate. Hyphal septa with or without clamp-connexions. Cortical layer more or less hymeniform . . . . . **Dermoloma**

1b Spores not amyloid
- **5a** Hyphal septa without clamp-connexions. Conspicuous, large cheilocystidia absent . . . . . • **Tricholoma**,
- **5b** Hyphal septa with clamp-connexions
  - **6a** Fungi with lemon-yellow lamellae, growing on or near wood. Pileus of the most common species densely covered with vinaceous scales. Conspicuous, large cheilocystidia present . . . . . **Tricholomopsis**
  - **6b** Fungi not with lemon-yellow lamellae or not growing on or near wood. Pileus not with squamose surface
    - **7a** Spore wall with fine tubercles, sometimes indistinctly punctate (staining with Cotton Blue; oil-immersion objective). Fruit-body often violet or lilac at least on the lamellae or smell aromatic (orris root, iris oil, orange flowers). Spores 6 to 9(to 10) × 3 to 5 µm. Lamellae easily detached from the base of the pileus with the finger-nail . . . . . • **Lepista** partly, (e.g. nuda, irina)
    - **7b** Spore wall smooth and smell other than above. Lamellae not readily detached from the base of the pileus
      - **8a** Pileus broadly convex with squamose, grey to dun surface, 4 to 12 cm. Smell and taste farinaceous (Care! Poisonous fungus!). Spores 8 to 10 × 6 to 7 µm . . . . . • **Tricholoma** partly, (pardinum)
      - **8b** Not with the foregoing combination of characters
        - **9a** Smell mostly not farinaceous, rather like soap or laundry. Pileus 5 to 10 cm, white to dun, with distant whitish to waxy yellowish grey lamellae. Fruit-body when old becoming more or less coppery red or flesh-coloured, at least on the stipe. Spores 5 to 6 × 3.5 to 4 µm. In woods . . . . . • **Tricholoma** partly, (saponaceum)
        - **9b** Not with the foregoing combination of characters. If the fungi redden, basidia siderophilous (as with most species of the following genera, but not with most species of the preceding genera)
          - **10a** Moderately large to small fungi, never fasciculate, never inclining to reddish, blue, or black. Stipe 1 to 6 mm broad, often somewhat cartilaginous or longitudinally fibrillose. Apex often floccose. Pileus grey, brownish grey, brown, sometimes pellucid-striate. Smell frequently farinaceous rancid
            - **11a** Cortical layer largely hymeniform. Fungi of grassy localities with broadly convex to almost triangular lamellae, a short whitish stipe, and often a rugose, grey to brownish, 1.5 to 3 cm broad pileus. Spores 4.5 to 5(to 6) × 3 to 3.5 µm . . . . . **Dermoloma** partly (cuneifolium)
            - **11b** Cortical layer of elongated hyphae. Fungi growing in forests or on moors or burnt ground and also with other character . . . . . **Tephrocybe**
          - **10b** Mostly large fungi, more or less fleshy, not with membranous, pellucid-striate marginal zone. Pileus sometimes either white or brightly coloured; fungi fasciculate or not
            - **12a** Fruit-bodies densely caespitose to fasciculate or lamellae where bruised becoming reddish, blue, or black and violet with iron(III) chloride or lamellae bright yellow and the pileus then slate-blue to blue-lilac. Mostly, pileus white, grey, or brown. Spores smooth, sometimes triangular, rhomboid, or globose . . . . . • **Lyophyllum**
            - **12b** Fruit-bodies not densely caespitose to fasciculate. Pileus fleshy, white, yellow, pink, violettish, or dun to bistre. Lamellae without the above-mentioned colours, not becoming violet with iron(III) chloride, often very light-coloured in contrast with the pileus; if yellow, then concolorous with the pileus. A very common species often forming fairy rings in spring; its white fruit-bodies have a farinaceous smell. Spores sometimes ornamented, mostly smooth . . . . . **Calocybe**

IIId Key to "Collybia"

1a Spores amyloid

2a Spores with amyloid ornamentation. Clamp-connexions absent at the hyphal septa . . . . . **Melanoleuca** partly

2b Spores smooth, without amyloid ornamentation or if seemingly so, then globose and in pairs on the basidia. Clamp-connexions present at the hyphal septa

3a Spores 6 to 9 µm, subglobose, with an amyloid wall in which non-amyloid tubercles or spines are enclosed. Basidia two-spored . . . **Fayodia** partly (anthracobia, gracilipes)

3b Spore wall uniformly amyloid. Basidia four-spored

4a Spores globose, 6 to 6.5(to 7) µm. Growing on wood. With 2 to 5 cm large, radially fibrillose to fissured, convex, umbilicate pileus . . . . . **Clitocybula**

4b Spores not globose

5a Spores small, 3 to 4.5 × 1 to 2.5 µm. Fruit-bodies on decaying wood in spring or on spruce and pine cones in spring or autumn. Lamellae very crowded . . . . . **Baeospora**

5b Spores larger 6 to 9.5 × 3.5 to 6 µm. Fungi with other than the afore-mentioned characters . . . . . **Hydropus**

1b Spores not amyloid

6a Spores tuberculate or spinose

7a Pleurocystidia absent. Spores echinate, often globose, often considerably larger than 8 µm. Fungi sometimes distinctly lilac-violet or flesh-coloured, sometimes with surface of the pileus squamose. Lamellae thickish . . . . . **Laccaria**

7b Pleurocystidia present. Spores spinulose or tuberculate, 5.5 to 8 × 3.5 to 5.5 µm. Pileus yellowish dun, beige-buff, brownish grey; surface glabrous. Lamellae white, pale grey, or flesh-coloured, thin . . . . . **Fayodia** partly (leucophylla, pseudoclusilis)

6b Spores not tuberculate and not densely covered with hyaline spines

8a Large to moderately large fungi, diameter of the pileus more than 3 cm

9a Stipe dark brown velutinous, often narrowing towards the base, fasciculate. Pileus glutinous, mostly rust-yellow, less often white. Usually growing on wood; fruit-bodies appearing in late autumn and in mild winters. Spores 8 to 9 × 4.5 to 6 µm . . **Flammulina**

9b Not simultaneously with stipe dark brown to black velutinous and pileus glutinous, rust-yellow, white

10a Stipe radicating on the underground remains of wood, glabrous or velutinous to setose. Pileus glutinous or pilose-setose. Spores 8 to 15 × 7 to 11 µm, often subglobose . . . . . **Xerula**

10b Stipe not radicating or not simultaneously with the afore-mentioned characters

11a Pileus radially striate and also the base of the stipe with white rhizoids. On decaying wood. Spores 7 to 10 × 5.5 to 7.5 µm . . . . . • **Megacollybia**, see p. 254

11b Not with the above combination of characters

12a Lamellae blackening on bruising. Spores 8 to 9 × 4 to 4.5 µm . . . . . **Lyophyllum** partly (semitale)

12b Lamellae not blackening on bruising

13a Hyphal septa without clamp-connexions. Non-annulate species related to the Honey Mushroom with brown, yellowish brown, light brown colours. Centre of the pileus squamulose. Rare; in climatically favoured wine-growing areas, fasciculate on stumps and roots, principally of oak. Spores 8 to 10 × 5 to 7 µm (cf. also **23a** Callistosporium) . . . . . **Armillaria** partly (tabescens)

13b Hyphal septa with clamp-connexions

14a Fungi with sulphur-yellow lamellae, growing on or near wood. Pileus of the most common species densely vinaceous squamose. With unusually large cheilocystidia . . . . . **Tricholomopsis**

14b Fungi not with sulphur-yellow lamellae or not growing on wood or surface of the pileus not squamose

15a Cortical layer hymeniform, comprising clavate to subglobose elements . . . . . **Marasmius** partly (e.g. oreades)

15b Cortical layer with elongated, filamentous, often interwoven, elements

16a Basidia with siderophilous granulation, fungi partly with farinaceous smell, partly fruiting on burnt ground . . . . . **Tephrocybe**

16b Basidia without siderophilous granulation. Fungi without a farinaceous smell; partly with a burning pungent taste, partly with a garlic-like or stinking smell, partly densely fasciculate; partly with a longitudinally striate stipe; partly with a stipe floccose to furfuraceous throughout its length

17a Lamellae at least in places broadly adnate to uncinate. In part, fungi white or whitish, with a non-striate stipe. In part, spores fusiform-elongate . . . . . • **Clitocybe** partly, (species with feebly or indistinctly decurrent lamellae)

17b Lamellae adnexed. If fungi white, then with a striate stipe . . . . . • **Collybia**,

8b Small fungi, diameter of the pileus less than 3 cm

18a Small whitish species, mostly on decaying remains of fungi or arising from sclerotia. Spores 4 to 6 × 2 to 3 µm . . . . . **Collybia** partly (e.g. cirrhata)

18b Not concurrently small, white, on decaying lamellate fungi, or arising from sclerotia

19a Spores pseudoamyloid. Pileus when young deep brown-lilac, when old beige-lilac. Lamellae dark lilac-brown, when old brown. Spores 3.2 to 4 × 2.8 to 3.2 µm . . . . . **Pseudobaeospora**

19b Spores not pseudoamyloid

20a Cortical layer comprising elongated, filamentous elements

21a Cortical layer comprising long, filamentous, mostly acicular elements, whose thick cell walls are strongly pseudoamyloid. Spores 7 to 9.5 × 5 to 6.5 µm. Pileus 0.5 to 1.2 cm, whitish with brownish scales and fibrils, applanate, with small brown papillae. Stipe rust-coloured, sulcate, tomentose. On wood remains and twigs, and on grass remains . . . . . **Crinipellis**

21b Cortical layer not with pseudoamyloid elements. Not with the above-mentioned combination of characters

22a Stipe dark brown velutinous, often narrowing towards the base. Pileus glutinous, mostly rust-yellow, less often white. Mostly on wood; fruit-bodies appearing in late autumn and in mild winters . . . . . **Flammulina**

22b Stipe not simultaneously dark brown to black velutinous and pileus glutinous, rust-yellow, white

23a Clamp-connexions absent at the hyphal septa. Spores 5 to 7.5 × 3.2 to 4.5 µm, with yellowish contents (necro-pigment). Pileus greenish olive or brownish yellow, brownish olive, olivaceous mixed with honey-coloured tints . . . . . **Callistosporium**

23b Clamp-connexions present at the hyphal septa

24a Stipe either dark and/or filamentous or somewhat thicker and narrowed towards the base, often pruinose. Cortical layer sometimes with diverticulate hyphae (ramealis structure) . . . . . **"Marasmius"**, key VI

24b Stipe neither filamentous thin, nor at the same time dark coloured and pruinose. If the cortical layer with diverticulate hyphae, then the base of the stipe with a dense mycelial tomentum

25a Basidia not siderophilous. Stipe floccose to furfuraceous throughout its length, or fungi with a burning, pungent taste, or with a garlic-like or stinking smell, or densely fasciculate; or with diverticulate hyphae in the cortical layer. Never with a farinaceous smell . . . . . • **Collybia**,

25b Basidia siderophilous. Fungi in part smelling and tasting farinaceous. The other characters mentioned above not present . . . . . **Tephrocybe**

20b Cortical layer hymeniform, consisting of clavate or globose elements; these sometimes with digitate projections

26a On the cones of coniferous trees in spring. Clamp-connexions at the hyphal septa absent. With conspicuous muricate cheilo- and pleurocystidia. Spores 5.3 to 8 × 2.5 to 4 µm . . . . . **Strobilurus**

26b Not on the cones of coniferous trees in spring . . . . . **"Marasmius"**; key VI

IIIe Key to "Mycena"

1a Spores amyloid

2a Trama pseudoamyloid and/or pleuro- and often cheilocystidia like broom-cells

3a Cortical layer of elongated elements . . . . . • **Mycena**,

3b Cortical layer of broad, more or less globose elements . . . . . **Hydropus**

2b Trama not pseudoamyloid. Cystidia absent or not like broom-cells

4a Very small white fungi with veined lamellae. Spores 7 to 9 × 3 to 5 µm . . . . . **Delicatula**

4b Fungi with other characters: not white or whitish, larger, etc.

5a Cortical layer of longish, more or less radially parallel, hyphae. Spores 8.5 to 11.5 × 4.5 to 6 µm. With tubular cheilo- and pleurocystidia (40 to 65 × 10 to 15 and 70 to 80 × 10 to 20 µm, respectively). Cf. also Fayodia, key IIId, ... **Hydropus**

5b Cortical layer hymeniform or at least consisting of broad, short, erect elements

6a Edge of lamellae jagged . . . . . **Dermoloma**

6b Edge of lamellae not jagged . . . . . **Hydropus** partly

1b Spores pseudoamyloid or not amyloid
7a Spores pseudoamyloid, 3.2 to 4 × 2.8 to 3.2 µm. Pileus and lamellae more or less brownish . . . . . **Pseudobaeospora**
7b Spores not pseudoamyloid
8a Spores verrucose-tuberculate, subglobose. Cheilocystidia present, lanceolate or branched at the tip. Cortical layer comprising elongated hyphae, which are rough because of numerous protrusions, and having dermatocystidia. Cf. also Fayodia, key IIId, ... **Mycenella**
8b Spores not verrucose-tuberculate; furthermore, not with the above-listed characters
9a Cortical layer consisting of elongated, more or less parallel elements
10a Minute to very small fungi
11a In the pileus and in part also in the stipe white to faint ochraceous and at the same time the trama never pseudoamyloid
12a Spores 11 to 16.5 × 4 to 5.5 µm. If 7 to 8 × 3.3 to 4.5 µm, then the lower part of the stipe distinctly rust-brown to reddish brown. Base of the stipe without a mycelium . . . . . **Marasmiellus**
12b Spores globose (and smaller) or (when e.g. up to 11 µm long) the lower part of the stipe not distinctly brown or (when spores are more than 11 µm) the fungus fruiting at the base of comfrey (Symphytum) plants or the base of the stipe with a distinct mycelium . . . . . **Hemimycena**
11b Fruit-bodies mostly deeper coloured than white to ochraceous and/or trama pseudoamyloid
13a Stipe only slightly longer than the diameter of the pileus, furfuraceous-floccose or pruinose. On trunks, stumps, and branches. Spores 8 to 12 × 2.5 to 4.5 µm . . . . . **Marasmiellus** partly (ramealis, amadelphus)
13b Stipe distinctly longer than the diameter of the pileus, or not on the remains of wood, or the dimensions of the spores otherwise . . . . . **Mycena** partly (e.g. acicula)
10b Fungi on average larger. Pileus often more than 1 to 1.5 cm. Stipe not filamentous thin
14a Spores oblong ellipsoidal, not globose
15a Cheilo- and pleurocystidia present
16a Spores narrowly cylindrical, slightly allantoid, 6.5 to 10 × 2.5 to 4 µm. Pleurocystidia numerous and large, 57 to 65 µm. Hyphae of the cortical layer glabrous . . . . . **Hydropus** partly (subalpinus)
16b Spores oblong ellipsoidal, not allantoid, 6 to 9 × 3 to 4 or 10 to 12 × 3.5 to 5.5 µm. Hyphae of the cortical layer with brush-like branching. Fruit-bodies sometimes with a delicate reddish colour . . . . . **Mycena** partly (adonis, flavoalba)
15b Pleuro- and sometimes also cheilocystidia absent
17a Hyphae pseudoamyloid or not. Spores 5 to 7.5 × 2.5 to 4.5 µm; if spores longer, then 4.5 to 5.7 µm broad . . . . . **Mycena** partly (atropapillata, roseipallens)
17b Hyphae not pseudoamyloid; spore size otherwise
18a Spores 8.5 to 11.5 × 3 to 4.5 µm . . . . . **Hemimycena** partly (cucullata)
18b Spores 4.5 to 5(to 6) × 3 to 3.5 µm . . . . . see 19a **Dermoloma** partly
14b Spores broadly ellipsoidal, 5.7 to 7 µm, or globose. Hyphae from the trama of the pileus and stipe pseudoamyloid. In the pileus (cortical layer) under a zone of narrow hyphae a hypoderm of large, rather broad hyphae which have a diameter of about 40 µm . . . . . **Hydropus** partly (floccipes)
9b Cortical layer hymeniform, comprising globose, clavate, or at least broad, short, erect elements
19a Lamellae broadly convex, almost triangular; sinuate-adnate; whitish. Pileus 1.5 to 3 cm, dingy grey to brownish, often rugose. Spores 4.5 to 5(to 6) × 3 to 3.5 µm. Meadows, pastures, grassy places . . . . . **Dermoloma** partly (cuneifolium)
19b Not with the above combination of characters . . . . . **"Marasmius"**; key VI,

IIIf Key to "Clitocybe"

1a Spores amyloid
2a Clamp-connexions absent at the hyphal septa. Fungi brownish to brownish black. Spores 7 to 10 × 5 to 7 µm . . . . . **Pseudoclitocybe**
2b Clamp-connexions present at the hyphal septa
3a Large fungi with pileus that is 10 to 30 cm and white . . . . . **Leucopaxillus** partly (candidus, giganteus)
3b Smaller fungi with pileus that is 2 to 5 cm, hygrophanous, and buff-ochre to light brownish red. Spores 6.5 to 8 × 3.5 to 5 µm. Cf. also Fayodia and Myxomphalia; key IIIg, ... **Pseudoomphalina**
1b Spores not amyloid
4a Spore wall spinose or verruculose, sometimes indistinctly punctate (stain with Cotton Blue; oil-immersion objective)
5a Lamellae distant and thickish. Spores subglobose, 7 to 13 µm, covered with fairly long, e.g. 0.8 to 1 µm, hyaline spines. Some species distinctly lilac-violet or flesh-coloured, some species with a squamose surface, moderately large to small . . . . . **Laccaria**
5b Lamellae not distant and thickish. Spores otherwise
6a Pileus up to 3 cm. Lamellae with pleurocystidia. Cf. Fayodia and Tephrocybe; key IIIg, p. 226
6b Pileus either larger or lamellae without pleurocystidia. Spores punctate to verruculose. Spore mass mostly creamy yellowish (flesh-coloured to pink; see key IX), rarely white . . . . . • **Lepista**

4b Spore wall completely smooth (oil-immersion objective)

7a Pileus on white ground with appressed blackish scales. Fungi tough, fasciculate, on the stumps of deciduous trees, radicating towards the base. Spores 7 to 8(to 10) × 3 to 3.5 µm . . . . . **Panus** partly (tigrinus)

7b Not with the above combination of characters

8a Fruit-body more or less bright orange, on wood. If on the ground, then lamellae usually furcate towards the marginal zone of the pileus. Spores in part globose, in part pseudoamyloid

9a Lamellae not usually furcate. On wood. Spores globose, not pseudoamyloid, 4.5 to 7 × 4 to 6.5 µm . . . . . • **Omphalotus**

9b Lamellae furcate. Mostly on the ground. Spores pseudoamyloid, 4.8 to 8 × 2.5 to 5 µm . . . . . **Hygrophoropsis**

8b Fruit-body with other colours or if with orange-yellow tints then neither growing on wood nor at the same time with pseudoamyloid spores

10a Less common fungi smelling of fruit bonbons, with flesh-coloured to ochre-pink and clearly furcate lamellae. Spores 3.5 to 5.5 × 2.5 to 3 µm . . . . . **Hygrophoropsis** partly (morganii)

10b Fungi with other characters

11a Hyphal septa without clamp-connexions

12a Non-annulate relatives of the Honey Mushroom. Pileus 5 to 6(to 7) cm, squamose, brownish yellow to honey-coloured. Spores 8 to 10 × 5 to 7 µm. Fasciculate, in warm parts (wine-growing regions) on stumps and roots, especially of oak . . . . . **Armillaria** partly (tabescens)

12b Not with the combination of characters indicated. Fungi smaller . . . . . **"Omphalia"**; key IIIg, below

11b Hyphal septa with clamp-connexions

13a Fungi blackening or fasciculate on the ground. If the fungus is white, then the lamellae give a violet reaction with iron(III) chloride. Basidia mostly siderophilous . . . . . • **Lyophyllum** partly,

13b Fungi not distinctly fasciculate. Lamellae not blackening on bruising and not giving a violet reaction with iron(III) chloride. Basidia not siderophilous

14a Fungi large and fleshy

15a Fungi with sulphur-yellow lamellae, growing on or near wood. Pileus always more or less densely squamulose, on the commonest species vinaceous. With conspicuously large cheilocystidia . . . . . **Tricholomopsis**

15b Fungi either not with sulphur-yellow lamellae or not growing on wood or the surface of pileus not squamose

16a Pileus 7 to 15 cm, convex to applanate, with a grey tinge. Lamellae pale, sinuate, readily detached from the base of the pileus (with the finger-nail). Spores 6 to 7 × 3 to 4 µm, cyanophilous. Spore mass cream . . . . . • **Lepista** partly,

16b Fungi not with the above combination of characters. Pileus infundibuliform. If the pileus is convex to applanate, then either brownish yellow, dingy grey, brownish, or spores 6 to 10 × 3 to 4 µm or spore mass white or lamellae not easily detached from the base of the pileus. Spores (mostly) acyanophilous . . . . . • **Clitocybe**

14b Fungi small to moderately large . . . . . **"Omphalia"**; key IIIg, below

IIIg Key to "Omphalia"

1a Fungi small to tiny and white

2a Hyphal septa without clamp-connexions

3a Pileus lobate to multi-pileate. Spores 5.3 to 6.5 × 3 to 3.5 µm . . . . . **Leptoglossum** partly (polycephalum)

3b Pileus at the margin repand, not lobate or multi-pileate. Spores (4.5 to)5 to 5.5 × 3 to 3.5 µm . . . . . **Gerronema** partly (albidum)

2b Hyphal septa with clamp-connexions

4a Spores amyloid. 7.5 to 8(to 9) × 4 to 5 µm. On mouldy wood, litter, and humus . . . . . **Delicatula** partly (integrella)

4b Spores not amyloid

5a Spores 20 to 22 × 5 to 6 µm. Basidia with two sterigmata. On dead stems of Carex . . . . . **Gloiocephala**

5b Spores below 16 µm, mostly only up to 11 µm long

6a Fungi lichenized; at the base of the stipe either with a squamose layer of lichen (Coriscium) or with green globose algae (Botrydina). Spores 7 to 10 × 4.5 to 7 µm . . . . . **Omphalina** partly

6b Fungi not lichenized and/or the spore size otherwise . . . . . **Hemimycena**

1b Fungi larger and/or not white

7a Spores amyloid

8a Clamp-connexions absent at the hyphal septa. Spores 7 to 10 × 5 to 7 µm . . . . . **Pseudoclitocybe**

8b Clamp-connexions present at the hyphal septa (occasionally absent in the pellicle)

9a Spores globose, 6 to 10 µm, spore membrane amyloid with non-amyloid projections. Basidia two-spored. Cheilocystidia often well developed . . . . . **Fayodia** partly

9b Not with the above combination of characters: spores neither globose nor at the same time reaching the size indicated

10a Pellicle of the bistre pileus elastic. Cheilo- and pleurocystidia present, tubular, 40 to 65 × 10 to 15 µm and 70 to 80 × 10 to 20 µm, respectively. Spores 5 to 6.5 × 4 µm . . . . . **Myxomphalia**

10b Pellicle not elastic

11a Fruiting late in the year on the stems of reeds (Phragmites). Spores 10.5 to 14(to 16) × 5.4 to 6.4 µm. Trama pseudoamyloid . . . . . **Mycena** partly (e.g. belliae)

11b Not fruiting on the stems of reeds (Phragmites). Trama pseudoamyloid or not

12a Pileus 2 to 4 cm. Stipe 2 to 4 mm broad. Spores 6.5 to 10 × 3.5 to 5 µm. Not growing on decaying wood . . . . . **Pseudoomphalina**

12b Pileus 0.5 to 2 cm. Spores 4 to 7 × 2.8 to 4.5 µm. Mostly on decaying wood

13a Pileus grey. Spores 6 to 7 × 3.5 to 4.5 µm. Mostly on decaying stumps of fir trees. Lamellae white, edge pruinose and often brownish . . . . . **Hydropus** partly (marginellus)

13b Pileus yellow to rust-brown. Spores 4 to 7 × 2.8 to 4 µm. Commonest species of the genus gregarious on decaying conifer wood with a rust-yellow to rust-brown pellucid-striate pileus and intervenose lamellae . . **Xeromphalina**

7b Spores not amyloid

14a Spores tuberculate, spinose, to verrucose

15a Clamp-connexions absent at the hyphal septa. Trama of the lamellae slightly bilateral. Spores tuberculate, echinate, 6 to 7 × 5 to 6 µm . . . . . **Omphaliaster**

15b Clamp-connexions present at the hyphal septa. Trama of the lamellae not bilateral. Spores verrucose to spinose

16a Lamellae thickish and at the same time distant. Spores globose to ellipsoidal, covered with fine hyaline spines, 7 to 12 µm . . . . . **Laccaria**

16b Lamellae neither thickish nor distant. Spores mostly smaller or not covered with spines but rather verrucose

17a On burnt places and/or basidia siderophilous. Pileus mostly with dark brown to bistre colours. Smell usually farinaceous. Spores mostly 5 to 8 µm . . . . . **Tephrocybe**

17b Not on burnt places and basidia not siderophilous. Pileus with yellowish or brown colours, sometimes with grey hues. Smell not farinaceous. Spores 5.5 to 8 × 3.5 to 5.5 µm . . . . . **Fayodia** partly (leucophylla, pseudoclusilis)

14b Spores smooth, i.e. not at all verrucose (staining with Cotton Blue; oil-immersion objective)

18a Tiny to very small species and clamp-connexions present at the hyphal septa and the pileus up to about 1.5 cm with a relatively long stipe that is several times the diameter of the pileus

19a Always connected to mosses and peat moss. Fruit-bodies orange, yellow, to fawn. Stipe concolorous or pale brownish and violet at the apex. Spores fairly small, 4 to 5.5 × 2 to 3 µm . . . . . **Gerronema** partly (subgenus Rickenella)

19b Not obligately connected to mosses, on woody plant parts. Spores 6.5 to 10.5 × 4 to 5 µm . . . . **Mycena** partly (speirea)

18b Either larger species or clamp-connexions at the hyphal septa absent and stipe usually not conspicuously long

20a Clamp-connexions at the hyphal septa absent

21a Entire fungus bright orange, orange-yellow, yellow, or tawny . . . . . **Gerronema** partly (subgenera Romagnesia and Haasiella partly)

21b Fungi with other colours, e.g. dark brown, dun, light brown . . . . . **Omphalina** partly (e.g. rustica, grisella)

20b Clamp-connexions at the hyphal septa present

22a Entire fungus orange, orange-yellow, salmon-orange. Spore wall 0.3 to 0.8 µm thick . . . . . **Gerronema** partly (subgenus Haasiella)

22b Fungi with other colours, lamellae at most yellow or yellowish

23a Pileus 2 to 8 cm, grey to dun, darker innately radially fibrillose. Lamellae whitish, yellowish white, to yellow, distant. Stipe 3 to 6 × 0.2 to 0.7 cm, whitish, yellowish, or grey, more or less appressed fibrillose. Taste mild to somewhat bitter. Spores (6 to)7 to 9(to 11) × 3 to 6(to 7) µm. On remains of wood, decaying deciduous and coniferous tree stumps, occasionally on pine cones . . . . . **Gerronema** partly (strombodes)

23b Not with the above combination of characters. If lamellae yellowish, then the pileus with greenish grey colours and/or smaller

24a With thick-walled pleuro- and cheilocystidia (metuloids). Lamellae very narrow. Spores 9.5 to 11 × 4.8 to 5.5 µm . . . . . **Hohenbuehelia** partly

24b Without metuloids

25a Spores mostly rather large, not less than 6 µm, partly attaining a length of more than 10 µm. Fungi with a deeply umbilicate pileus that is often grey or black, sometimes also whitish; on decaying wood or growing among peat moss . . . . . **Omphalina**

25b Spores shorter than 10µm, often smaller than 6µm and/or habit, colour, and habitat characters not applicable
26a Basidia siderophilous . . . . . **Tephrocybe**
26b Basidia not siderophilous . . . . . • **Clitocybe**

IIIh Key to "Pleurotus"

1a Spore mass ochre, sometimes very pale . . . . . **"Dermini"**; key XIII
1b Spore mass white or whitish. Here also species with a pink or flesh-coloured spore mass and absent, lateral, or highly excentric stipe
2a Completely astipitate. Lamellae bright orange or longitudinally split
3a Lamellae with bright orange coloration. Spores reniform, 4 to 5×2µm . . . . . **Phyllotopsis**
3b Lamellae reddish grey to violettish grey, from the edge longitudinally split into two halves. Spores sub-allantoid, 3 to 4×1 to 1.5µm . . . . . **Schizophyllum**
2b Either not completely astipitate or lamellae neither bright orange nor longitudinally split
4a Spores reniform or allantoid
5a Fruit-body spherical with a veil, 1 to 2cm, later plano-scutellate. Lamellae dark ochre-yellow to brownish. Growing on withered branches of beech trees. Cheilocystidia often thick-walled, not dendriform. Spores 3 to 4×1 to 2µm, weakly amyloid . **Tectella**
5b Not with the above-mentioned macroscopic characters. Spores amyloid, 3 to 10×1 to 4µm. Cheilocystidia on the gelatinous edges of the lamellae dendriform, sometimes only claviform but then very large . . . . . **Panellus**
4b Spores not allantoid
6a Hymenium with thick-walled metuloids
7a Metuloids pseudoamyloid; cf. also Chaetocalathus . . . . . **Geopetalum**
7b Metuloids not pseudoamyloid
8a Cheilocystidia absent or thin-walled. Pleurocystidia metuloid. Tramal hyphae thin-walled. Cortical layer in some cases with an inner gelatinous layer that is covered on the outside by a trichoderm. Spore length variable, partly over 8µm, partly under 7µm . . . . . **Hohenbuehelia**
8b Metuloid cheilo- and pleurocystidia present; these blunt-ended. Tramal hyphae thick-walled. Cortical layer without a gelatinous layer, in some cases with setose fasciculate hyphae instead. Spores 7 to 8×3 to 4µm . . . . . **Panus** partly (rudis, conchatus)
6b Hymenium without thick-walled metuloids
9a Spores verrucose, tuberculate, or in longitudinal view with 6 to 8 grooves and thus in end view with an angular outline
10a Spores amyloid and also with fine amyloid verrucosities. Spore mass white . . . . . **Lentinellus**
10b Spores not amyloid. Spore mass pink to flesh-coloured
11a Spores verrucose, globose, 6 to 8µm. Surface of pileus veined with a reddish pink tinge. Cortical layer hymeniform . **Rhodotus**
11b Spores not verrucose and globose at the same time, the remaining characters otherwise
12a Spores with large tubercles, 7 to 13×5 to 10µm, not fusiform . . . . . • **Entoloma** partly,
12b Spores viewed end-on with an angular outline, in side view with 6 to 8 ridges, 6 to 14×3 to 6µm, fusiform . . . **Clitopilus**
9b Spores glabrous
13a Spores globose to sub-globose
14a Fruit-body large or with a long stipe
15a Pileus orange to dark brown. Lamellae very thick (transverse section!). Spores 4.5 to 7×4 to 6.5µm . . . . . • **Omphalotus**
15b Pileus not conspicuously orange to brown
16a Spores 6 to 8×5.5 to 6µm. Stipe short and lateral or absent. Fruit-body white . . . . . **Pleurocybella**
16b Spores smaller or stipe longer
17a Lamellae decurrent at the 3.5 to 5cm long stipe. Pileus whitish or slightly grey, sericeous-fibrillose. Spores 4 to 5.5×3.5 to 4.5µm . . . . . **Clitocybe** partly (lignatilis)
17b Lamellae adnexed to sinuate. Stipe 5 to 8cm. Pileus whitish ochre. Spores distinctly globose, 5 to 8µm. Basidia in part siderophilous . . . . . **Lyophyllum** partly (ulmarium)
14b Fruit-body small, 0.5 to 1(to 2)cm, on higher plants, remains of plants, mosses, pieces of wood, etc.
18a On living mosses
19a Spores 3 to 5.5µm. Hymenium smooth. Fruit-body whitish . . . . . **Mniopetalum**
19b Spores 6.5 to 10µm. Fruit-body grey to dun . . . . . **Leptoglossum** partly
18b On wood, etc.
20a Fungus white. Spores 6×5.5 to 6µm . . . . . **Cheimonophyllum**
20b Fungus not pure white, at least grey or dun, often deeper to dark brown. Fruit-body very small, with the surface of the pileus sitting on the substratum . . . . . **Resupinatus**

13b Spores not globose

21a Clamp-connexions absent at the hyphal septa. Spores 3 to 5µm broad. Often growing among moss . . **Leptoglossum**

21b Clamp-connexions present

22a Spores 3 to 6×2 to 3µm and fruit-body sometimes very small

23a Fruit-body astipitate or laterally substipitate

24a Taste bitter. Growing on wood. Spores 3 to 6×2 to 3µm, amyloid . . . . **Panellus** partly (stypticus)

24b Taste mild. Growing on moss. Spores 3 to 4×2µm, not amyloid . **Mniopetalum** partly (bryophilum)

23b Fruit-body excentrically to laterally stipitate. Lamellae in the most common species orange, distinctly furcate . . . . . **Hygrophoropsis**

22b Spores longer than 6µm or broader than 3µm and/or fruit-body large

25a Edge of the lamellae coarsely serrate or fungus tough. Trama near the edge of the lamellae regular . . . . . **Lentinus**

25b Margin of the lamellae not coarsely serrate. When fungus tough, then spores up to 8µm long and/or trama of the lamellae irregular

26a Fungus with sulphur-yellow lamellae. Pileus in the commonest species densely vinaceous squamose. Spores 5.5 to 8×3.5 to 6µm . . . . . **Tricholomopsis**

26b Fungus not with the above combination of characters

27a Fungus pure white. Lamellae with iron(III) chloride soon turning violet, very crowded, subdecurrent. Spores 6 to 7×3.5 to 4µm . . . . . • **Lyophyllum** partly (connatum)

27b Not with the above combination of characters. Lamellae with iron(III) chloride not turning violet. Spores larger

28a Spores 8 to 12×3 to 5µm. Hyphae of the cortex of the stipe not amyloid. Trama of the lamellae irregular, subhymenium developed . . . . . **Pleurotus**

28b Spores 7 to 8×3 to 3.5µm. Hyphae of the cortex of the stipe sometimes amyloid. Trama of the lamellae irregular, subhymenium indistinct . . . . . **Panus** partly (suavissimus, tigrinus)

IV Key to "Hygrophorus"

1a Growing on wood . . . . . **"Omphalia"**; key IIIg

1b Not growing on wood

2a Spores echinate or spinose. Fruit-body dun, dark brown, and pileus umbilicate, or flesh-coloured, pink to violet

3a Fruit-body dun, dark brown, and with an umbilicate pileus. Clamp-connexions absent from the hyphal septa. Spores spinose to tuberculate . . . . . **Omphaliaster**

3b Fruit-body pink, flesh-coloured to violet. Lamellae conspicuously thickish and distant. Clamp-connexions present at the hyphal septa. Spores spinose . . . . . **Laccaria**

2b Spores smooth, not spinose or tuberculate

4a Fruit-body with conspicuous bright and deep colours: red, yellow, orange, green, or pink. Sometimes red and becoming black. Ethanol extracts a yellow pigment (muscaflavin). Trama of the lamellae regular. Species of meadows and pastures, almost always outside woods . . . . . • **Hygrocybe**

4b Fruit-body without the above colours or the trama of the lamellae bilateral or species occurring in forests

5a Fungi with a nitrous smell and/or the lamellae at least reddening on bruising. Trama of the lamellae regular. Spores 7 to 12×4.5 to 6µm. In meadows and pastures, almost always outside woods . . . . . **Hygrocybe** partly (e.g. ingrata), see p. 252

5b Fungi with other characters. If lamellae reddening somewhat, then fungi with a farinaceous smell

6a Spores amyloid. Smell and taste mostly farinaceous

7a Cortical layer hymeniform or with individual vesiculose elements. Neither pileus conico-umbonate nor lamellae reddening . . . . . **Dermoloma** partly

7b Cortical layer generally consisting of elongated elements. Either pileus conico-umbonate or lamellae reddening somewhat . . . . . **Porpoloma**

6b Spores not amyloid. Smell and taste mostly not farinaceous

8a Cortical layer hymeniform or with individual vesiculose elements. Spores 4 to 6×3 to 5µm

9a Clamp-connexions present at the hyphal septa. Smell often farinaceous. Mostly in meadows and pastures . . . . . **Dermoloma**

9b Clamp-connexions absent at the hyphal septa. Smell unpleasant like coal gas, absent, or otherwise, but not farinaceous. Occasionally also in woods . . . . . **Hygrotrama**

8b Cortical layer comprising filamentous, interwoven, or radially parallel hyphae. Spores with dimensions other than those indicated

10a Spores 3 to 5×2.5 to 4µm. Pileus 0.5 to 1 to 2.5cm, tawny to sepia umber-brown. Cortical layer with scattered subglobose cells. In meadows and pastures . . . . . **Hygrotrama** partly (schulzeri)

10b Spore size and/or remaining characters different
11a In woods or at least near trees; at the same time, the trama of the lamellae bilateral . . . . . **Hygrophorus**
11b In meadows and pastures; at the same time, the trama of the lamellae not bilateral
12a Fungi viscid and grey. If beige, grey, or brownish and pileus not viscid, then lamellae more or less sinuate and with a regular trama . . . . . **Hygrocybe** partly (unguinosa, fornicata)
12b Fungi not viscid. Lamellae more or less decurrent and with an irregular interwoven trama especially towards the marginal zone of the pileus . . . . . **Camarophyllus**

V Key to "Cantharellus"

1a Species tiny, mostly white-coloured with a centric stipitate pileus not exceeding 1 to 2 cm. Not parasitic on lamellate fungi
2a Spores amyloid, trama not pseudo-amyloid (if trama pseudo-amyloid, cf. Mycena) . . . . . **Delicatula**
2b Spores not amyloid
3a Spores 20 to 22 × 5 to 6 µm. Basidia with two sterigmata. Cortical layer hymeniform. On dead stems of Carex . . . . **Gloiocephala**
3b Not with the above-mentioned combination of characters
4a Hyphal septa without clamp-connexions. Spores 4.5 to 6.5 × 3 to 3.5 µm
5a Pileus lobate to multi-pileate. Spores 5.3 to 6.5 × 3 to 3.5 µm . . . . . **Leptoglossum** partly (polycephalum)
5b Pileus not lobate to multi-pileate, with repand marginal zone. Spores (4.5 to)5 to 5.5 × 3 to 3.5 µm . . . . . **Gerronema** partly (albidum)
4b Hyphal septa with clamp-connexions and/or spore size different
6a Cortical layer hymeniform . . . . . **Marasmius** partly (e.g. epiphyllus)
6b Cortical layer comprising filamentous hyphae, often densely covered with small protuberances . . . . . **Hemimycena**
1b Species either larger or not white-coloured or laterally stipitate or astipitate. A group of fungi on the remains of decaying lamellate fungi
7a Species on the remains of decaying lamellate fungi, especially on certain Russula species. 12 to 15 µm long chlamydospores formed on the surface of the pileus . . . . . **Asterophora**
7b Species not on the remains of decaying lamellate fungi
8a Fruit-body with a lateral stipe or without a stipe
9a Fruit-body resupinate on branches of beech; astipitate . . . . . **Plicatura**
9b Fruit-body on mosses
10a Fungi grey, dun, brownish. Spores 6.5 to 10 µm . . . . . **Leptoglossum**
10b Fungi white or whitish. Spores 3 to 5.5 µm . . . . . **Mniopetalum**
8b Fruit-body with a centric or only slightly excentric stipe
11a Fruit-body leathery and tough, on wood or burnt ground. A group with metuloids (thick-walled cystidia)
12a On burnt ground. Pileus black, bistre, to umber. Hymenium with metuloids. Spores 7 to 11 × 4 to 5 µm . . . **Geopetalum**
12b Not with the above combination of characters . . . . . **"Panus"**, key VIII
11b If fruit-body somewhat tough, then growing on the ground and always without metuloids
13a Hymenophore distinctly ridged, like the Chantarelle. With more or less firm flesh or with grey, dun, to black colours in places. Basidia extremely long, 6.5 to 10 × longer than broad, about 45 to 100 µm
14a Spores rough (punctate). Fruit-body compact napiform with furcate-reticulate ridges on the underside . . . **Gomphus**
14b Spores smooth. Fruit-body stipitate-pileate
15a Ridged hymenophore grey, dun, or in the dry state dingy cream, reddish to ochraceous and/or hyphal septa without clamp-connexions
16a Pileus 1 to 3 cm, the marginal zone curled and deeply crenate . . . . . **Pseudocraterellus**
16b Pileus 3 to 5 cm, not particularly deeply crenate . . . . . **Cantharellus** partly (cinereus)
15b Ridged hymenophore yellow, orange, or at least the stipe yellow or the fruit-body pale in all its parts. Hyphal septa with clamp-connexions . . . . . **Cantharellus**
13b Hymenophore more lamelloid. Basidia smaller and more compact
17a Spores fusiform and amyloid, 7 to 11 × 2.7 to 3.5 µm. Hyphal septa with clamp-connexions . . . . . **Cantharellula**
17b Spores not simultaneously fusiform and amyloid. Hyphal septa with or without clamp-connexions
18a Pileus and/or lamellae of the fruit-body distinctly tinged with orange or pink, then however smelling distinctly like orange flowers. Hyphal septa with clamp-connexions. Spores in the commonest species pseudoamyloid, 5.5 to 7 × 4 to 4.7 µm . . . . . **Hygrophoropsis**
18b Not with the foregoing characters. Hyphal septa without clamp-connexions . . . . **"Omphalia"**; key IIIg,

VI Key to "Marasmius"

1a Spore mass deep ochre, rust-brown, to almost umber. Spores on microscopical examination appearing pigmented. Pileus squamose. Cortical layer comprising elongated, thick-walled, brown-pigmented hyphae (if comprising globose or, nevertheless, broad elements, cf. Flammulaster) . . . . . **Phaeomarasmius**

1b Spore mass whitish. Spores on microscopical examination hyaline

2a On conifer cones. Spores amyloid

3a Fruiting in spring. Cortical layer comprising globose elements . . . . . **Strobilurus**

3b Fruiting in autumn. Cortical layer comprising radially parallel hyphae . . . . . **Baeospora**

2b Not on conifer cones. Spores amyloid or not

4a With a leek-like taste. Stipe without a strong basal mycelium. Commonest species with a small pileus (0.5 to 1.2 cm), and a velutinous dark brown stipe sitting on pine needles. In species with a larger pileus, the stipe distinctly short as compared with the diameter of the pileus and sitting on wood or growing on litter. Hyphae from the trama of the pileus and the cortical layer gelatinous or very loosely interwoven. Cortical layer not hymeniform. Spores not amyloid . . . . . **Micromphale**

4b When with a leek-like taste and smell, then either the cortical layer hymeniform or the stipe with a strong basal mycelium

5a Stipe black, horse-hair thin, in part shiny, entirely naked (hand lens). Spores 6.5 to 9×3 to $4\,\mu m$ . . . . . **Marasmius** partly (androsaceus, splachnoides)

5b Stipe not simultaneously black and horse-hair thin

6a Cortical layer hymeniform

7a Stipe longer than 1 cm. Pileus variously coloured; when white or whitish, then the lamellae forming a collar or the spores shorter than $10\,\mu m$. Smell may be leek-like and unpleasant . . . . . **Marasmius**

7b Stipe very short, 0.2 to 0.6 cm. Pileus white, about 0.5 cm broad; lamellae not forming a collar and spores 10 to $22 \times 5\,\mu m$. On remains of Carex (sedge) . . . . . **Gloiocephala** (caricis)

6b Cortical layer of filamentous, interwoven, or radially parallel hyphae

8a Spores amyloid. Fungi yellowish orange. Commonest species of the group on decaying conifer wood and with intervenose lamellae at the base of the pileus . . . . . **Xeromphalina**

8b Spores not amyloid. Fungi not with bright yellowish orange-ochraceous hues

9a Pileus with a dense tomentum. Hyphae of the cortical layer pseudo-amyloid, thick-walled, and mostly with pointed ends. On remains of grass . . . . . **Crinipellis**

9b Pileus not with a dense tomentum. Hyphae of the cortical layer not pseudoamyloid

10a Pileus and stipe with the exception of the stipe base white or whitish; fungi growing on stumps, branches, and trunks of trees . . . . . **Marasmiellus**

10b Fungi with other colours . . . . . **"Collybia"**; key IIId

VII Key to "Lentinus"

1a Spores with amyloid ornamentation, small, maximally 5 to 6(to 7) μm, globose . . . . . **Lentinellus**

1b Spores not amyloid

2a Trama of the lamellae (at least when young) regular and the subhymenium distinct . . . . . **Lentinus**

2b Trama of the lamellae irregular and the subhymenium indistinct . . . . . **"Panus"**; key VIII, below

VIII Key to "Panus"

1a Fungi not stipitate, with the underside of the pileus uppermost (resupinate) on the substratum. Spores amyloid

2a Lamellae dark ochraceous. Spores 3 to 4×1 to $2\,\mu m$. On withered branches from beech trees . . . . . **Tectella**

2b Lamellae flesh-coloured or violettish. Spores 6 to 10×2 to $4\,\mu m$. Mostly on wood from fir trees . . . . **Panellus** partly (violaceofulvus)

1b Fungi distinctly stipitate

3a Stipe lateral, spores amyloid . . . . . **Panellus** partly

3b Stipe centric or excentric, spores not amyloid

4a Lamellae ridge-like. Fungus growing on burnt ground. Hymenium with pseudo-amyloid metuloids . . . . . **Geopetalum**

4b Without the above characters

5a Among mosses (e.g. Aulacomnium) in lowland moors and transition moors. Thick-walled cheilo- and pleurocystidia (metuloids) present. Smell and taste farinaceous . . . . . **Hohenbuehelia** partly

5b On wood. Metuloids present or absent. Smell and taste not farinaceous

6a Trama of the lamellae irregular and the subhymenium indistinct . . . . . **Panus**

6b Trama of the lamellae regular, at least when young, and the subhymenium distinct . . . . . **Lentinus**

IX Key to the "Hyporrhodii"

1a Lamellae free. Stipe usually distinct from the pileus

2a Veil completely absent. Lamellae often with more or less dirty reddish pink hues. Spores not amyloid or pseudo-amyloid . . . . . • **Pluteus**

2b Veil mostly present as a volva or only as an annulus. When completely without a veil, then spores pseudo-amyloid

3a Veil as a volva at the base of the stipe. Lamellae with a red tinge . . . . . **Volvariella**

3b Veil as an annulus or absent. Lamellae sometimes whitish

4a Clamp-connexions absent at the hyphal septa. Spores smaller than 9 µm, with a germ pore . . . . . **Leucoagaricus**

4b Clamp-connexions present at the hyphal septa

5a Spores with a germ pore and never fusiform or truncate, mostly more than 10 µm. With an annulus separating from the stipe and then movable . . . . . • **Macrolepiota**

5b Spores without a germ pore, often fusiform, truncate, in part less than 10 µm. With or without an annulus on the stipe

6a Lamellae carmine, wine-red, to wine-brown or with greenish tinge . . . . . **Melanophyllum**

6b Lamellae differently coloured . . . . . **Lepiota**

1b Lamellae sinuate-adnate or decurrent

7a Lamellae unusually thick and distant. Fruit-body flesh-coloured, violettish to lilac. Spores mostly with hyaline spines . . . . . **Laccaria**

7b Not with the above characters

8a Pileus orange-pink, flesh-coloured, apricot, yellow-orange. Spores not angular-tuberculate, but sometimes verrucose

9a Surface of pileus conspicuously favoid. Lamellae flesh-coloured. Fruit-body with an excentric stipe, on wood. Cortical layer hymeniform. Spores globose, verrucose . . . . . **Rhodotus**

9b Surface of pileus not favoid. Lamellae orange. Fruit-body with a centric stipe, on soil and wood. Cortical layer not hymeniform. Spores not globose or verrucose

10a Spores 5.6 to 8 × 3.8 to 6 µm. . . . . **Gerronema** (subgenus Haasiella)

10b Spores 9 to 12 × 6 to 8 µm . . . . . **Omphalina** partly (demissa)

8b Not with the above-mentioned characters or spores angular-tuberculate

11a Spores angular and verrucose or with at least six to eight grooves in side view and hence with an angular outline in end-on view

12a Lamellae not decurrent . . . . . • **Entoloma** partly

12b Lamellae decurrent

13a Spores also angular and tuberculate in side view. Clamp-connexions present or absent. Without a farinaceous smell . . **Entoloma** partly

13b Spores ellipsoidal to fusiform with six to eight grooves in side view, angular in end-on view. Clamp-connexions absent. Commonest species of the genus white with pink to flesh-coloured lamellae and with distinct farinaceous smell . . . . . **Clitopilus**

11b Spores at most verruculose to punctate or completely smooth, at any rate not angular and tuberculate or longitudinally sulcate

14a Pileus conchate, on wood, without or with a short lateral stipe

15a Lamellae rust-yellow to almost orange. Spores small, reniform, 4 to 5 × 2 µm. . . . . **Phyllotopsis**

15b Spores with other dimensions

16a Spores 6 to 8 × 2.5 to 5 µm, smooth . . . . . **Pleurotellus**

16b Spores with other dimensions, smooth or rough . . . . . **Crepidotus**

14b Pileus with centric stipe

17a Clamp-connexions absent at the hyphal septa . . . . . **Rhodocybe**

17b Clamp-connexions present at the hyphal septa

18a Spores with verruculose to punctate ornamentation (if necessary, stain with Cotton Blue and examine with an oil-immersion objective)

19a Spores small, 4 to 5(to 6.4) µm, subglobose. Pileus small, 2 to 4.5 cm . . . . . **Ripartites**

19b Spores mostly larger, 6 to 9 µm, mostly not globose. Or pileus larger, e.g. 4 to 10 cm . • **Lepista**

18b Spores smooth. Attachment of lamellae varying

20a Fungus with an unpleasant fish-oil smell and with conspicuous lanceolate pleurocystidia . . . **Macrocystidia**

20b Fungus without an unpleasant fish-oil smell and without pleurocystidia

21a Lamellae adnate to sub-decurrent . . . . . • **Clitocybe** partly, (phyllophila, etc.)

21b Lamellae sinuate or straight . . . . . • **Collybia** partly

X Key to "Paxillus"

1a Spore mass ochre-brown to clay-brown
- **2a** Pileus whitish, flesh-coloured to yellowish orange, small, 2 to 4.5 cm. Spores subglobose, verrucose, small . . . . . **Ripartites**
- **2b** Pileus deep ochre-brown to reddish brown, large, usually more than 5 cm
  - **3a** Hymenophore bright yellow, lamellate with numerous anastomoses. Spores fusiform, 9.5 to 14 × 3 to 5 µm . . . . . **Phylloporus**
  - **3b** Lamellae yellowish ochre, but not bright yellow, with less extensive anastomoses. Spores ellipsoidal, 4 to 10 × 3 to 6 µm. Stipe in one species black velutinous, in the other common species the flesh inclines to brown. One species with a conchate fruit-body is astipitate or substipitate . . . . . • **Paxillus**

1b Spore mass white, whitish, pale ochraceous, or pink
- **4a** Lamellae decurrent. Spores sulcate or smooth
  - **5a** Fungi with a distinct farinaceous smell. Spore mass pink. Spores in side view with six to eight grooves, in end-on view angular . . . . **Clitopilus**
  - **5b** Fungi without a farinaceous smell. Spore mass white to cream-coloured. Spores without grooves and in end-on view not angular
    - **6a** Lamellae bright orange, ridge-like, strongly furcate . . . . . see **Hygrophoropsis**
    - **6b** Lamellae neither bright orange nor ridge-like nor strongly furcate . . . . . • **Lepista**
- **4b** Lamellae sinuate. Spores smooth or with verruculose to punctate ornamentation
  - **7a** Spores with amyloid verrucosities or smooth and amyloid. Spore mass white, and when tested macroscopically amyloid . . . . . **Leucopaxillus**
  - **7b** Spores not with amyloid verrucosities and not amyloid
    - **8a** Clamp-connexions absent at the hyphal septa . . . . . **Rhodopaxillus**
    - **8b** Clamp-connexions present at the hyphal septa . . . . . • **Lepista**

XI Key to "Gomphidius" in the broadest sense

1a Base of the stipe not amyloid, but often with chrome-yellow flesh. Lamellae when young whitish to grey. Veil and hence also the surface of the pileus viscid. Flesh in the pileus whitish to pink . . . . . **Gomphidius**

1b Base of the stipe amyloid, not with chrome-yellow flesh. Lamellae when young orange-pink. Veil fibrous and surface of the pileus dry to glutinous. Flesh in the pileus orange . . . . . **Chroogomphus**

XII Key to "Cortinarius"

1a Spore mass whitish or whitish ochraceous, always very pale
- **2a** Pileus reddish brown to flesh-coloured. Stipe with a marginate bulb, 5 to 10 × 1 to 1.5 cm. Spores relatively thick-walled, 7 to 9 × 4 to 5 µm. Clamp-connexions present at the hyphal septa . . . . . **Leucocortinarius**
- **2b** Pileus with other colours. Stipe not with a marginate bulb. Clamp-connexions absent at the hyphal septa . . . . . • **Tricholoma** partly

1b Spore mass deeper coloured, at least brown, but also purple-brown, etc.
- **3a** Spores smooth or angular and tuberculate, but not verrucose-punctate
  - **4a** Thick-walled cystidia (metuloids; often muricate) present in the hymenium. Spores angular and tuberculate, or not. Surface of the pileus often radially fibrillose or striped. Spore mass dingy brown to tobacco-brown . . . . . • **Inocybe**
  - **4b** Thick-walled cystidia absent from the hymenium or spore mass purple-brown
    - **5a** Pileus convex, lanose-squamose, squarrose, or strongly radially fibrillose-striate. Lamellae yellow to olive. Spores 8 to 12 (to 16.5) × 4.5 to 7.5 µm, without a germ pore, smooth, not angular and tuberculate. Cheilocystidia very crowded, not muricate; pleurocystidia absent. Spore mass dingy brown to tobacco-brown . . . . . • **Inocybe** partly
    - **5b** Fungi not with the above characters
      - **6a** Spore mass rust-coloured to brown . . . . . **"Dermini"**; key XIII
      - **6b** Spore mass purple-brown to purple-black . . . . . **"Pratelli"**; key XV

3b Spores verrucose to punctate (oil-immersion objective)

7a Spore mass purple-brown to purple-black . . . . . **"Pratelli"**; key XV

7b Spore mass rust-coloured to brown

8a Spore mass brownish ochre with rust-coloured tones

9a Not growing on wood (mostly also not on decaying wood.) Stipe may be conspicuously ventricose and the pileus often viscid; if with other characters, then no yellow or red pigments extractable to any great extent with potassium hydroxide. Fungi often large and massive . . . . . **Cortinarius** subgenera, see 10a to 15b

10a Pileus and stipe viscid (at least in wet weather) . . . . . **Cortinarius** subgenus **Myxacium**

10b Only the pileus viscid or the fruit-body completely dry

11a Pileus viscid and stipe dry . . . . . • **Cortinarius** subgenus **Phlegmacium**,

11b Pileus and stipe dry

12a Fruit-bodies small with ochraceous pileus and bitter . . . . . **Cortinarius** subgenus **Myxacium**

12b Fruit-bodies larger with different colours or not bitter

13a Fruit-bodies large, dark violet and tomentose-squamose. With large cheilo- and pleurocystidia . . . . . **Cortinarius** subgenus **Cortinarius**

13b Fruit-bodies not simultaneously dark violet and tomentose-squamose

14a Hyphae of the cortical layer thick, often more than 6 to 10µm. Fruit-body olivaceous, green, olivaceous brown, yellow, tawny, orange-brown, to red. Pileus often fibrillose, squamose, or tomentose. Spores mostly globose or broadly ellipsoidal . . . . . • **Cortinarius** subgenus **Leprocybe**,

14b Hyphae of the cortical layer thinner and often with other macroscopical characters

15a Pileus hygrophanous or fungus thick-fleshed and then the pileus and the flesh turning black with potassium hydroxide . . . . . **Cortinarius** subgenus **Telamonia**

15b Pileus not hygrophanous, sericeous, micaceous, or squamose. Spores often globose. Pileus and flesh not turning black with potassium hydroxide . . • **Cortinarius** subgenus **Sericeocybe**,

9b Growing on wood or on the ground, in both cases with yellow, red, or violet pigments soluble in potassium hydroxide or, if without such pigments, then spores with a smooth spot (= plage) above the apiculus. Fungi moderately large to small. In doubtful cases, continue here

16a Spores with a plage above the apiculus or, if without a plage, then hyphae of the trama without clamp-connexions at the septa. Stipe more or less cartilaginous or very thin. Fruit-body mostly small and with a campanulate or expanded pileus whose marginal zone is not involute. Taste may be farinaceous. On the ground, among moss, or on decaying wood. Potassium hydroxide does not dissolve any yellow or red pigment . . . . . • **Galerina** partly,

16b Spores without a plage above the apiculus. Hyphal septa with clamp-connexions. Marginal zone of the pileus involute when young. Taste not farinaceous

17a Potassium hydroxide dissolves a yellow, red, or violet pigment. With bright yellow, ochre-yellow, orange-foxy, orange-brown, orange, or red colours in the lamellae; as mixed colours or entirely so

18a Taste of the most frequent species bitter. Fungi growing mostly on wood. Cheilocystidia numerous, staining green in Cotton Blue. Spores partly small and globose, e.g. 4 to 5.5×3.5 to 4.5µm, partly also larger and ellipsoidal to amygdaliform . . . . . • **Gymnopilus**,

18b Taste not bitter. Growing on the ground. Spores not simultaneously small and globose. Cheilocystidia not staining green in Cotton Blue

19a Spores globose to broadly ellipsoidal . . . . . • **Cortinarius** subgenus **Leprocybe** partly,

19b Spores ellipsoidal . . . . . • **Dermocybe**,

17b Fungi with other colours, at least in the lamellae. Potassium hydroxide does not dissolve any yellow or red pigment **"Dermini"**; key XIII,

8b Spore mass without rust-coloured tones

20a Mostly robust species with a stipe thicker than 3mm not having a cartilaginous fracture. Stipe thinner in only a few species and then the cortical layer without dermatocystidia and comprising radially parallel hyphae. Pileus mostly with a pale, more or less off-white, tinge, at least at the marginal zone . . . . . • **Hebeloma**,

20b Small species with a stipe thinner than 3mm having a cartilaginous fracture. Cortical layer comprising more or less short, partly also globose, elements and with dermatocystidia . . . . . **Naucoria**

XIII Key to the "Dermini"

1a Stipe excentric or absent

2a Clamp-connexions at the hyphal septa absent

3a Spores 6 to 8 × 3 to 4.5 µm. Cheilocystidia absent . . . . . **Pleurotellus**

3b Spores 7 to 12 × 5 to 6 µm. Cheilocystidia present. The most frequent species of the genus with a gelatinous cortical layer . . . . . **Crepidotus** partly (mollis, pubescens)

2b Clamp-connexions at the hyphal septa present

4a Fruit-body laterally or excentrically stipitate. Spores not globose

5a Pileus 0.5 to 1 cm, cinnamon rust-coloured, short-haired; dried fruit-body swelling in water. Spores 12 to 16(to 18) × 7 to 9 µm . . **Phaeomarasmius** partly (rimulincola)

5b Spores 7 to 10 µm long

6a Spores 8 to 10 × 5.5 to 7 µm . . . . . **Simocybe** partly (rubi)

6b Spores 7 to 7.5 × 3 to 3.5 µm . . . . . **Crepidotus** partly (phillipsii)

4b Fruit-body laterally substipitate or astipitate. Spores verrucose-punctate or smooth, in part globose . . . . . **Crepidotus**

1b Stipe more or less centric

7a Lamellae free, carmine, wine-red to wine-brown or with some kind of greenish tinge. Cortical layer comprising globose cells . . . . . **Melanophyllum**

7b Lamellae not free

8a Hymenium with thick-walled, mostly muricate cystidia. Spores smooth or angular and tuberculate. Surface of the pileus often radially fibrillose or striped . . . . . • **Inocybe**

8b Hymenium without thick-walled cystidia (metuloids)

9a Pileus convex to conical, lanose-tomentose-squamose, squarrose, or distinctly radially fibrillose-striate, occasionally becoming red . . . . . • **Inocybe** partly, see p. 252

9b Fungi not with the above characters, especially the surface of the pileus not radially fibrillose, striped, or striate. If squamose, then the stipe with an annulus and/or the spore mass with a rust-coloured tinge or the spores verrucose

10a Stout species with a stipe bearing an annulus and thicker than 4 mm . . . . . **"Pholiota"**; key XIV, p. 237

10b Species with a stipe not bearing a membranous annulus or less than 4 mm thick

11a Spores coarsely verrucose to verruculose (oil-immersion objective)

12a Spore mass without a rust-coloured tinge, mostly tobacco-brown. Spores always without a plage above the apiculus

13a Lamellae decurrent and the fruit-body more or less Clitocybe-like. Brownish lamellae contrasting with a mostly whitish, flesh-coloured, or yellowish orange pileus. Spores subglobose, verrucose, small, 3.5 to 5(to 6.4) µm . . **Ripartites**

13b Lamellae not decurrent. Spores larger and not globose

14a Stout species. Stipe thicker than 3 mm, without a cartilaginous fracture; only in a few species is the stipe thinner and then dermatocystidia are absent from the cortical layer, as with all the species of this genus; cortical layer comprising radially parallel hyphae. Pileus mostly with pale, more or less off-white tints, at least so at the marginal zone . . . . . • **Hebeloma**

14b Small species. Stipe thinner than 3 mm, with a cartilaginous fracture. Cortical layer comprising more or less short, and partly also globose, elements and with dermatocystidia . . . . . **Naucoria**

12b Spore mass brownish ochre with rust-coloured tinges

15a Potassium hydroxide dissolves yellow, red, or violet pigment. Cheilocystidia sometimes green in Cotton Blue, sometimes unaffected or absent. Small- to moderate-sized fungi. Spores without a germ pore

16a Taste of the most common species bitter. Fungi on wood with bright yellow, ochre-yellow, foxy orange, or orange-brown colours. Cheilocystidia numerous, staining green in Cotton Blue. Spores partly small and globose, e.g. 4 to 5.5 × 3.5 to 4.5 µm, partly also larger and ellipsoidal to amygdaliform . . . . . • **Gymnopilus**

16b Taste not bitter. Fungi on the ground, less often joined to the remains of wood, with olivaceous, green, yellowish, bright yellow, orange, orange-brown, or red colours, at least in the lamellae. Cheilocystidia, if present, not staining green in Cotton Blue. Spores not simultaneously small and globose

17a Spores globose to obtusely ellipsoidal . . . . . • **Cortinarius** partly, see **(Leprocybe)**

17b Spores ellipsoidal . . . . . • **Dermocybe**

15b Potassium hydroxide does not dissolve yellow, red, or violet pigment, or in the rare cases that it does, then the fungus is massive and large or the spores have a germ pore. The cheilocystidia never staining green in Cotton Blue

18a Fungus cartilaginous. Stipe mostly deeply rooted in the forest soil. Pileus mostly conical. Hyphal septa mostly without clamp-connexions. Small- to moderate-sized fungi . . . . . **Phaeocollybia**

18b Fungus not cartilaginous, or with characters other than those indicated above. Occasionally very large

19a Spores with a plage above the apiculus or, when without a plage, then the hyphal septa without clamp-connexions. Fruit-body mostly small with a campanulate or expanded pileus whose marginal zone is usually not involute. Stipe more or less cartilaginous or very thin. Several species on wood, others among moss or on the ground . . . . . • **Galerina**,

19b Spores without a plage above the apiculus (oil-immersion objective). Hyphal septa with clamp-connexions and/or fruit-body large. Pileus with an involute marginal zone when young. Either the stipe distinctly bulbous or the stipe and/or pileus viscid or the pileus hygrophanous. Not on wood (mostly also not on decaying wood) . . . . . see **Cortinarius**, (there also a key to the subgenera)

11b Spores appearing smooth (oil-immersion objective)

20a Fungi with a distinctly unpleasant fish-oil smell (along with cucumber components). Hymenial cystidia large, lanceolate, 60 to 100 × 12 to 24 µm, also with caulo- and dermatocystidia of similar shape. Spores 8 to 9 × 3 **Macrocystidia**

20b Fungi not with a conspicuous fish-oil smell and not with lanceolate hymenial cystidia of the indicated dimensions

21a Cortical layer comprising subglobose to clavate cells

22a Basidia capitulate to globose at the apex

23a Spores 10 to 15 × (5 to)6 to 9 µm. Lamellae with a fully developed trama and deliquescent . . . . . **Bolbitius**

23b Spores 8 to 10 × 5 to 6 µm. Lamellae with a greatly reduced trama; subhymenium thick . . **Galerella**

22b Basidia normally clavate towards the apex

24a Cheilocystidia narrow-necked and abruptly capitulate

25a Trama of the lamellae reduced; subhymenium strongly developed and occupying almost the whole section of the lamellae . . . . . • **Conocybe**,

25b Trama of the lamellae not as above, not reduced in relation to the subhymenium . . . . . • **Pholiotina** partly, see p. 255 (intermedia, pygmaeoaffinis)

24b Cheilocystidia not abruptly capitulate

26a Surface of the pileus velutinous or squamose. Small species with the pileus measuring less than 2 cm

27a Surface of the pileus velutinous. Lamellae dirty brownish with grey or olivaceous components. Spores 6 to 9 × 4 to 5 µm . . . . . **Simocybe** partly (centunculus, reducta)

27b Surface of the pileus squamose. Lamellae yellow, brown, ochre, whitish to reddish . . . . . **Flammulaster**

26b Surface of the pileus neither velutinous nor squamose. Small to large species

28a Pileus hemispherical to expanded convex, marginal zone not striate. With cheilo- and pleurocystidia . . . . . **Agrocybe**

28b Pileus campanulate, conico-campanulate, marginal zone striate. With cheilocystidia, but without pleurocystidia . . . . . • **Pholiotina**

21b Cortical layer comprising more or less elongated hyphae

29a Stipe more than 3 mm thick, more or less fleshy. Diameter of the pileus more than 2 cm, often fairly large

30a Pileus hygrophanous, with a pellucid-striate marginal zone. Commonest species of the genus with a stipe that below the annulus is squamulose and coated with dark brown encrusted hyphae. Spores 6 to 7.5 × 3 to 4.6 µm, with a germ pore. Chrysocystidia absent . . . . . **Kuehneromyces**

30b Pileus not hygrophanous and not with a pellucid-striate marginal zone. Chrysocystidia present or absent. Occasionally yellow pigment dissolving in potassium hydroxide

31a Cortical layer of radially parallel hyphae, below which is a hypoderm of short cylindrical and broad elements whose walls are somewhat thickened and in potassium hydroxide yellow pigmented. Spores mostly with a distinct germ pore. Chrysocystidia present. Potassium hydroxide mostly dissolves a yellow pigment . . . . . • **Hypholoma**

31b Cortical layer and deeper layers comprising more or less elongated hyphae, i.e. hypoderm absent

32a Lamellae sinuate. Stipe farinose-pruinose. Fungi growing on the ground. Spores without a germ pore. Chryso- and pleurocystidia absent. Potassium hydroxide does not dissolve any yellow pigment. Pileus mostly with pale, more or less off-white, colours; at least so at the marginal zone . . . . . • **Hebeloma**

32b Lamellae not sinuate. Stipe not farinose-pruinose. Fungi growing on wood or on the ground. Spores often with a germ pore. In some cases, chryso- or pleurocystidia present. Potassium hydroxide mostly dissolves a yellow pigment. Fungi mostly more brightly coloured than indicated above . . . . . • **Pholiota**

29b Stipe less than 3 mm thick, more or less cartilaginous. Diameter of the pileus mostly less than 2 cm

33a Potassium hydroxide mostly dissolves a yellow pigment. Chrysocystidia present

34a Cortical layer of radially parallel hyphae, below which a hypoderm of short, cylindrical, broad elements . . . . . • **Hypholoma**,

34b Cortical layer and deeper layers comprising more or less elongated hyphae . . . . . • **Pholiota**,

33b Potassium hydroxide does not dissolve any yellow pigment. Chrysocystidia absent

35a Lamellae broadly adnate or decurrent and/or spores with a germ pore

36a Spores longer than 11 µm

37a Spores in 10% potassium hydroxide thin-walled, with 0.5 to 0.8 µm thick walls, 11 to 22 µm long. Germ pore in some cases indistinct . . . . . **Phaeogalera** partly (macrospora, stagnina, zetlandica)

37b Spores in 10% potassium hydroxide thick-walled, with about (1.0 to)1.2 to 1.5 µm thick walls, at most 16 to 19 µm long, with a distinctly visible (apical tubular) germ pore . . . . . • **Psilocybe** partly,

36b Spores shorter than 11 µm

38a Spores 7.5 to 10.5 × 4 to 5 µm (rarely larger), with a clearly visible germ pore at the often truncated end, in some cases distinctly lentiform, i.e. broader in front view than in side view. Not on manure or among peat moss, but rather in coniferous forests and there partly in wet places. Smell farinaceous or rancid. Pileus campanulate or plano-convex, umbonate, pellucid-striate, very hygrophanous, with a removable viscid-slimy pellicle. Cheilocystidia ventricose-fusiform with a slender narrowing neck . . . . . **Phaeogalera** partly (medullosa)

38b Fungi not with the above combination of characters

39a Spores with a germ pore and lentiform, broader in front view than in side view . . . • **Psilocybe**

39b Spores without a germ pore and not lentiform . . . . . **Tubaria**

35b Lamellae narrowly adnexed to sinuate. Spores without a germ pore

40a Pileus even when young with a straight, pellucid-striate marginal zone; campanulate, in some cases later somewhat expanded . . . . . • **Galerina**

40b Pileus when young with an inflexed to involute, in some cases not pellucid-striate, marginal zone

41a Spores more than 10 µm and/or cheilocystidia rostrate and closely packed

42a Spores more than 10 µm long, more than 7 µm broad. Hyphae of the cortical layer elongated, relatively thick-walled and (observed in potassium hydroxide) with dark brown pigment in the walls, more or less erect; in some cases also with epimembranous encrusted pigment . . . . . **Phaeomarasmius** partly

42b Spores smaller than 10 µm, cheilocystidia rostrate and closely packed . . . . . **Naucoria** partly

41b Spores less than 10 µm and cheilocystidia not rostrate . . . . . **Simocybe**

XIV Key to "Pholiota"

1a Surface of pileus conspicuously pruinose on an ochraceous ground. Tramal hyphae with amyloid deposits. Spores 10.5 to 14 × (6.5 to)7 to 9 µm . . . . . **Rozites**

1b Pileus not pruinose. Tramal hyphae without amyloid deposits

2a Spores verrucose, without a germ pore

3a Fungus with an aromatic bitter-almond smell and stipe deeply radicating and coarsely squamose. Pileus clay-buff, glutinous. Spores 8 to 10 × 5.5 to 6 µm . . . . . **Hebeloma** partly (radicosum)

3b Fungus not with a bitter-almond smell and stipe not deeply radicating. Pileus not glutinous

4a Pileus clay-grey, innately fibrillose marbled. Spores broadly rounded at the apex, not amygdaliform or limoniform, 8 to 10 × 5 to 6 µm. Cystidia absent. On the ground in deciduous forests . . . . . **Cortinarius** partly (torvus)

4b Pileus bright yellow, orange-yellow, innately fibrillose to fibrillose-squamose. Spores 8 to 10 × 5 to 6 µm. Cheilocystidia capitulate, in Cotton Blue greenish. Stumps of deciduous trees . . . . . • **Gymnopilus** partly, see p. 251 (spectabilis)

2b Spores smooth, with or without a germ pore

5a Surface of pileus micaceous-furfuraceous, granulose; the subglobose elements of the cortical layer with tuberculate protuberances. Spores 10.5 to 13 × 5.5 to 6.5 µm . . . . . **Phaeolepiota**

5b Surface of pileus not micaceous-furfuraceous, not granulose

6a Fungus with a hygrophanous pileus having a pellucid-striate marginal zone. Stipe below the annulus squamulose and with a coating of dark brown encrusted hyphae. Chrysocystidia absent: Spores with a germ pore, 6 to 7 × 3 to 4.5 µm. Fasciculate, on wood from deciduous and coniferous trees (see Galerina, p. 250) . . . . . **Kuehneromyces** partly (mutabilis)

6b Fungi with other characters

7a Cortical layer hymeniform. Surface of the pileus smooth . . . . . **Agrocybe**

7b Cortical layer consisting of elongated, mostly radially parallel, hyphae . . . . . • **Pholiota**

XV Key to the "Pratelli"

1a Lamellae free. Stipe distinct from the pileus and mostly with an annulus

2a Small species with a dark coloured, floccose pileus. Spores verruculose . . . . . **Melanophyllum**

2b Larger species or pileus not floccose and spores smooth. Flesh often becoming yellow or red. Stipe with an annulus . . . . . • **Agaricus**

1b Lamellae not free, but adnate to decurrent

3a Stipe with annulus

4a Surface of the pileus viscid-glutinous and/or hymenium with chrysocystidia . . . . . • **Stropharia**

4b Surface of the pileus dry. Hymenium without chrysocystidia . . . . . **Psathyrella** partly (spintrigera, leucotephra, etc.)

3b Stipe without an annulus

5a Cortical layer made up of globose elements, at most with a very thin layer of elongated, radially parallel hyphae over a layer of globose elements. Chrysocystidia absent

6a Spores verrucose, 12 to 16 × 7 to 9 µm . . . . . • **Panaeolina**

6b Spores smooth or shorter than 12 µm and less than 7 µm broad . . . . . **Psathyrella**

5b Cortical layer made up of a thicker layer of elongated, radially parallel hyphae

7a Spores longer than 11 µm or lentiform, i.e. broader in front view than in side view. Chrysocystidia absent. Cortical layer even in the deeper layers comprising elongated, radially parallel hyphae. Marginal zone of the pileus straight when young . . . . . • **Psilocybe**

7b Spores shorter than 11 µm or hymenium with chrysocystidia. Further, cortical layer with a hypoderm of short, cylindrical, broad elements below a layer of elongated, radially parallel hyphae. Spores never lentiform. Marginal zone of the pileus involute when young . . . . . • **Hypholoma**

XVI Key to the "Coprinarii"

1a Pileus campanulate, pale clay to brownish red, and the stipe with an annulus. Spores 15 to 22 × 9 to 13 µm. Growing on manure . . **Anellaria**

1b Fungi not simultaneously with a campanulate pileus, the stipe with an annulus, and spores with the above dimensions

2a Fungi with a conspicuously sulcate to plicate pileus and/or lamellae deliquescing to an ink-like fluid . . . . . • **Coprinus**

2b Fungi with other characters

3a Spores verrucose

4a Spores 12 to 16 × 7 to 9 µm . . . . . • **Panaeolina**

4b Spores 8 to 12 × 5 to 7 µm . . . . . **Psathyrella**

3b Spores smooth (oil-immersion objective)

5a Spores fusiform, smooth, 14 to 23 × 5 to 9 µm. Base of the stipe sometimes amyloid, sometimes chrome-yellow

5b Spores not fusiform and with other dimensions . . . . . **"Gomphidius"**; key XI

6a Cortical layer comprising elongated, radially parallel hyphae as far as the deeper parts of the trama. Stipe mostly with an annulus. Chrysocystidia present . . . . . • **Stropharia**

6b Cortical layer comprising globose cells or hymeniform, in part overlaid with a thin layer of elongated hyphae

7a Lamellae flecked when mature, as spores do not mature simultaneously. Pileus campanulate, hemispherical to expanded convex. Spores in concentrated sulphuric acid not decolorized (microscopic examination), not smaller than 9 µm . . . . . • **Panaeolus**

7b Lamellae evenly coloured when mature, since spores mature simultaneously. Pileus typically not campanulate. Spores in concentrated sulphuric acid decolorized, usually towards lilac, in part less than 9 µm long . . . . . **Psathyrella**

XVII Key to "Boletus"

1a Pileus and/or flesh (trama) with dark brown, porphyry-brown, grey, or black colours. Spore mass mostly dark brown. Spores in part ridged to tuberculate, not fusiform or if so then the spore mass brown to reddish brown

2a Pileus black with large squarrose, almost imbricate, scales. Hymenophore white to grey; with pressure, like the flesh becoming at first red then black. Spores globose, with reticulate ridges, very dark purple, 10 to 13 × 9 to 10 µm . . . . . **Strobilomyces**

2b Pileus not squamose, or spores otherwise

3a Stipe short and/or broadening towards the apex and often forming more than one pileus. Flesh (trama) yellow to rust-brown. Pileus reddish brown with a marginal zone long remaining yellow. On the ground on roots or stumps of conifers. Spores 5 to 8 × 3.5 to 4.5 µm, smooth-walled . . . . . **Phaeolus**

3b Stipe long. Flesh at first white or whitish

4a Entire fruit-body more or less uniformly olive, umber, to porphyry-brown, velutinous. Hymenophore when young grey. Flesh becoming pale pink, grey, yellowish, greenish, to slightly blue. Spores oblong ellipsoidal, 10.5 to 20 × 5 to 10.5 µm . . **Porphyrellus**

4b Pileus grey to brownish, pores white to light grey; tubes 1 to 8 mm long. Flesh at first white, reddening or becoming somewhat darker in the air. Spores irregularly verrucose, 4.5 to 6 × 4 to 5 µm . . . . . **Boletopsis**

1b Pileus differently coloured and not coarsely squamose; trama not brown. Spore mass white, pinkish, yellowish, olive-green, olive-brown, to almost dark umber. Spores neither globose nor verrucose nor with reticulate ridges. Species with a dark brown to reddish brown pileus do not have a pure brown to reddish brown spore mass

5a Hymenophore when young whitish

6a Tubes 1 to 5(to 10) mm long. Spores either shorter than 11 µm or not narrowing to fusiform at the ends

7a Growing on the ground. Spores 4 to 9(to 11) × 4.5 to 6.5 µm . . . . . • **Albatrellus**

7b Growing on wood. Spores 10 to 16 × 4 to 6 µm, narrowly ellipsoidal, somewhat curved . . . . . **Polyporus**, partly (squamosus)

6b Tubes more than 10 mm long and easily detached from the base of the pileus. Spores often longish, fusiform

8a Hymenophore when mature becoming ochraceous, yellowish, or greenish olive

9a Stipe mostly soon hollow. Spore mass pale yellow. Flesh unchanged in colour or turning deep cornflower-blue . . **Gyroporus**

9b Stipe solid. Spore mass olive-grey to olive-green. Flesh becoming at most slightly blue

10a Stipe with a fine reticulum, at least at the apex. Pileus light to dark brown . . . . . • **Boletus**

10b Stipe without a reticulum. Pileus chestnut to chocolate-brown . . . . . • **Xerocomus** partly (badius)

8b Hymenophore when mature pink or grey or remaining whitish

11a Stipe with small white, reddish, brown, or blackish scales. Cortical layer forming a marginella. Hymenophore round the stipe strongly depressed, often becoming grey. Flesh mild . . . . . • **Leccinum**

11b Stipe with a coarse reticulum, sometimes broadly clavate. Hymenophore becoming pink. Flesh bitter. Spore mass pink flesh-coloured . . . . . **Tylopilus**

5b Hymenophore when young yellow, olivaceous, greenish, orange, brown, to red

12a Tubes and pores very wide, hymenophore decurrent. Tubes short. Pileus tomentose, with a veil. Stipe often hollow. Spores less than 12 µm long . . . . . **Boletinus**

12b Tubes and pores narrow or fungi with other character combinations

13a Pileus when wet more or less glutinous

14a Stipe somewhat viscid-glutinous. Pileus pink. Hymenophore golden-yellow. Spore mass olive-brown . . . **Pulveroboletus**

14b Stipe dry, at most the annulus viscid

15a Hymenophore decurrent. Tubes at most 4 mm long, yellow. When pressed becoming blue. Near alder. Clamp-connexions present at the hyphal septa. Spores 4 to 6.5(to 8) × 3 to 5 µm, roundish ellipsoidal . . . . . **Gyrodon**

15b Hymenophore not decurrent or clamp-connexions not present at the hyphal septa. Spores fusiform

16a Hymenophore cinnamon-orange to carmine or raspberry-red. Stipe always without an annulus. Base of the stipe and the mycelium deep sulphur-yellow. Taste of the commonest species peppery . . . . . **Chalciporus**

16b Hymenophore and base of the stipe with other colours. Stipe often with an annulus, sometimes with glandular dots at the apex. Taste not pungent. In potassium hydroxide hymenial cystidia with a brown content . . . . . **Suillus**

13b Pileus mostly dry even in wet weather. Stipe not glutinous-viscid and taste not pungent and not associated with alder and clamp-connexions not present at the hyphal septa and in potassium hydroxide hymenial cystidia not with a brown content

17a Hymenophore sub-decurrent, tubes short. With bright sulphur- to chrome-yellow colours in the flesh and hymenophore, becoming blue on bruising. On wood, decaying roots, or joined to such substrates through the mycelium . **Pulveroboletus**

17b Hymenophore sinuate or adnate (straight). Mostly not joined to wood

18a Stipe somewhat squamose, rimose, floccose. Hymenophore and stipe more or less yellow . . . . . **Leccinum** partly (nigrescens)

18b Stipe not squamose

19a Stipe thick, often ventricose-bulbous, floccose or not, with a reticulum or not. If pileus dark brown, then the stipe at least at its apex with a fine reticulum. Trama of tubes with strongly diverging lateral strata. No fungi with a chestnut to chocolate-brown pileus without a reticulum on the stipe belong here . . . . . • **Boletus**

19b Stipe not particularly thick, not ventricose-bulbous, not floccose, not with a regular reticulate pattern. Fungi with a brown, more or less shiny, pileus also belong here

20a Flesh, hymenophore, and stipe on bruising becoming blue to blue-black . . **Boletus** partly (pulverulentus)
20b Where pressed not or only to a moderate extent turning blue
21a Hymenophore when young olive. Pileus tawny-brown to tawny with fine tomentose scales, as if punctate. Fungi nowhere carmine . . . . . **Suillus** partly (variegatus)
21b Hymenophore when young whitish yellow, yellow, greenish yellow, or carmine. If pileus tomentose-squamose, then not tawny-brown to tawny. Fungi flushed or not with carmine . . . . . • **Xerocomus**

XVIII Other Basidiomycetes, excluding lamellate, tubulate, and ridged fungi

1a Fungi without pores, spines, lamellae, etc.
2a Fruit-body globose-ventricose, closed, or with a pre-formed opening; often with powdery content when mature
3a Fruit-body underground or when mature projecting slightly above the ground . . . . . – (hypogeous Gasteromycetes; some edible, some of no culinary importance) –
3b Fruit-body above-ground
4a Fruit-body not with exoperidium tearing to form a star-shaped opening and not with globose or lentiform peridioles inside. Spores not embedded in a viscid, stinking mass, but when mature spore mass pulveraceous
5a Spores globose, with verrucose or reticulate ornamentation, 8 to 15 µm, and spore mass inside the fruit-body when mature black to brown. Fruit-body without a preformed opening; opening irregularly
6a Fruit-body inside when young with roughly pea-sized chambers that become pulveraceous when mature . . . . . – (Pisolithus; when young and not pulveraceous, edible) –
6b Fruit-body not chambered inside
7a With thorny fibres among the spores (capillitium) . . . . . – (Mycenastrum) –
7b Without fibres among the spores; thus without capillitium or this indistinct and not thorny . . . . . (+) **Scleroderma**; all species toxic or suspected of being so
5b Fungi with different characters . . . . . – (Remaining Gasteromycetes) –
4b Fungi with other characters. Fruit-body with exoperidium tearing to form a star-shaped opening or not, with globose or lentiform peridioles inside or not. Spores embedded in a viscid, stinking mass or not . . . . . – (Various groups of Gasteromycetes) –
2b Fungi not globose-ventricose, etc.
8a Fruit-body coralloid to barbate
9a Basidia bifurcate . . . . . – (Calocera) –
9b Basidia undivided, not bifurcate
10a Fruit-body with foliaceous and often undulate-corrugate branches . . . . . – (Sparassis) –
10b Fruit-body with roundish branches
11a Spores yellowish to brown
12a Spores tuberculate-verrucose, not ellipsoidal . . . . . – (Thelephora; inedible) –
12b Spores ellipsoidal . . . . . (+) **Ramaria pallida** and (+) **Ramaria formosa**, besides non-toxic and untried species
11b Spores colourless hyaline . . . . . – (Remaining genera with coralloid to barbate fruit-bodies) –
8b Fruit-body stipitate-pileate, dimidiate, etc., but not coralloid . . . . . – (Various other groups of Basidiomycetes) –
1b Fungi with pores, spines, lamellae, etc., thus with a differentiated hymenophore
13a Fungi with stipitate-pileate or dimidiate fruit-bodies
14a Fungi stipitate-pileate and with spines or teeth on the underside of the pileus. Flesh azonate, hardly tough
15a Spores tuberculate-verrucose, not amyloid. Fungi growing on the ground
16a Fruit-body dry, without a 'Maggi' smell. Spores brown
17a Hyphal septa with clamp-connexions
18a Pileus with light to dark brown scales; not coloured bright orange-brown and not becoming green on drying . . . **(R) Sarcodon imbricatum**
18b With other characters . . . . . – (Other Sarcodon species) –
17b Hyphal septa without clamp-connexions . . . . . – (Remaining Sarcodon species) –
16b Fruit-body dry, with a 'Maggi' smell. Spores colourless hyaline . . . . . – (Bankera) –
15b Spores smooth or, if verrucose, then amyloid . . . . . – (Remaining stipitate-pileate species with a spinose hymenophore) –
14b Fungi dimidiate, not stipitate-pileate, or if so then flesh zonate and tough
19a Fungus when young with very soft flesh and with a large, flat, imbricate pileus. Upper surface of fresh and young specimens reddish yellow; pores sulphur-yellow, clamp-connexions absent from hyphal septa. Spores colourless hyaline, 5 to 7.5 × 3.5 to 5 µm, ellipsoidal. On wood from deciduous trees and larch . . . . . **(R) Laetiporus sulphureus** (Sulphur Polypore, Chicken of the Woods)
19b Fungi with other characters. Fruit-body often woody and tough . . . . . – (Remaining Basidiomycetes that for the most part are not usable or have not been tried) –
13b Fungi with fruit-bodies that are not stipitate-pileate or dimidiate . . . . . – (Remaining Basidiomycetes that for the most part are not usable or have not been tried) –

1a Fruit-body bulbous and living underground (hypogeous)
2a Spores globose
3a Asci soon breaking down; content of the fruit-body consisting of a pulveraceous spore mass . . . . . – (Elaphomyces, inedible) –
3b Asci not breaking down
4a Asci grouped in enclosed sacs; fertile sacs with asci separated by sterile tissue
5a Spores with reticulate ornamentation
6a Asci containing five to eight spores. Spores 18 µm . . . . . (+) **Mattirolomyces terfezioides**
6b Asci containing two to four spores . . . . . – (e.g. Delastria rosea; edibility unknown) –
5b Spores spinose, verrucose, etc., but not with reticulate ornamentation . . . . . – (Other members of the Terfeziaceae, mostly edible but some toxic when raw; e.g. **(R) Choiromyces maeandriformis** with 16 to 22(to 26) µm spores) –
4b Asci not enclosed in sacs . . . . . – (Other species of unknown edibility, most of which are not found in Western and Central Europe, e.g. Paradoxa monospora with a 50 to 60 µm spore in each ascus) –
2b Spores not globose, but ellipsoidal, etc. . . . . – (Here, among others, the edible species of the genus Tuber) –
1b Fruit-body not bulbous and not living entirely underground
7a Fruit-body urceolate, cupulate and also sometimes stipitate, crateriform
8a Fruit-body 2.5 to 13 cm broad and 5 to 6 cm high, ventricose-urceolate, at the top opening into five to ten more or less triangular lobes. Inside mostly coloured some kind of violettish to violet. Spores colourless hyaline, smooth, (11.5 to)13 to 15(to 20) × (5 to)7 to 8(to 9) µm, containing one or two oil droplets, not amyloid . . . . . (+) **Sarcosphaera crassa**
8b Fruit-body with other characters and/or smaller . . . . . – (Species largely untried, and hence of unknown edibility, e.g. of the genus Peziza) –
7b Fruit-body divided into stipe and head or pileus, or fruit-body clavate, ligulate, etc., but not urceolate to cupulate
9a Fruit-body stipitate; above with a head- or pileus-like part that is clearly differentiated from the stipe. Stipe rather long; pileus higher than 1.5 cm
10a Spores with large oil droplets inside. Pileus not alveolate or campanulate over the stipe
11a Fruit-body mostly with a brown pileus: deep reddish brown, olive reddish brown, tawny, dark sepia, hazel. Stipe mostly unevenly rugose, neither slender and smooth nor costate-sulcate. Surface of the pileus cerebrose or lobate
12a Spores globose (7 to)8 to 10(to 12) µm . . . . . (+) **Pseudorhizina sphaerospora**
12b Spores ellipsoidal
13a Spores with an obtusely rounded apex. Pileus cerebrose or undulate-lobate to mitrate. Stipe hollow without internal folds. Fructifying in spring or autumn
14a Pileus with cerebrose surface, reddish to bistre, less often tawny. Fructifying in spring. Spores 18 to 22(to 25) × 9 to 12(to 14) µm . . . . . (++) **Gyromitra esculenta**
14b Pileus lobate, seemingly mitrate, lobes irregularly undulate, but not cerebrose. Fructifying in autumn
15a Pileus and stipe with a violettish tinge. Fruit-body mostly somewhat smaller than in the following species. Spores 22 to 33(to 37.5) × 7.5 to 12 µm. Paraphyses cylindrical, at the apex somewhat clavate to capitate . (+) **Gyromitra ambigua**
15b Pileus and mostly also the stipe without violettish tints. Stipe pale brown to greyish lilac. Spores (17 to) 20 to 23(to 26) × 7 to 10 µm. Paraphyses from a narrow base becoming fairly strongly clavate or capitate . . (?) **Gyromitra infula**
13b Spores with an acute apex or ending in twin tubercles. Pileus undulate-lobate, surface not cerebrose. With folds projecting into the hollow of the stipe. Fructifying in spring
16a Mature spores fusiform, 33 to 38(to 40) × 11 to 13 µm; at each end a button-shaped rounded appendage; surface with fine reticulations. Pileus ochre-brown, when old chocolate-brown. Chiefly in coniferous forests, often on stumps; rare in purely deciduous forests . . . . . (?) **Gyromitra gigas**
16b Mature spores more ellipsoidal, 30 to 33 × 13 to 15 µm, each end with (1 to)2 to 3(to 4) verrucose to spinose appendages; surface with fine reticulations. Pileus foxy reddish, when old violettish brown. In deciduous forests on calcareous soils; rare; in the warmer parts of Germany . . . . . (?) **Gyromitra fastigiata**

11b Fruit-body mostly with a differently coloured pileus. Stipe costate-sulcate or smooth and slender. Pileus crateriform, lobate, or sellaeform

17a Stipe glabrous

18a Fruit-body cupulate at the top . . . . . – (Leptopodia) –

18b Fruit-body with a sellaeform or lobate pileus . . . . . – (Cyathipodia) –

17b Stipe rugose and distinctly longitudinally costate . . . . . – (Helvella) –

10b Spores without oil droplets inside; fresh spores exhibit small accumulations of oil droplets at the ends on the outside

19a Pileus as if fitting over the top of the stipe, with free overlapping marginal zone

20a Pileus free, only attached to the stipe at the apex

21a Surface of the pileus essentially smooth or undulate to coarsely plicate. Spores 20 to 24 × 12 to 14 µm . . . . . – (Verpa) –

21b Surface of the pileus closely plicate; the folds sinuous, obtuse. Spores 60 to 80 × 17 to 22 µm . . . . . – (Ptychoverpa) –

20b Pileus with a free marginal zone as far as ½ to ¼ of its length. Alveolar depressions arranged in rows. Spores 22 to 30 × 13 to 17 µm . . . . . – (Mitrophora) –

19b Pileus going over into the stipe, the marginal zone being at best minimally free. Surface of the pileus alveolate; alveolae separated by ridges with sterile sides. Spores 17 to 26 × 10 to 16 µm . . . . . **(? R) Morchella**

9b Fruit-body not with a head- or pileus-like part or the stipe indistinct or short or the pileus less than 1.5 cm or the upper part of the fruit-body more ligulate than pileate . . . . . – (Remaining Ascomycetes) –

Keys for identifying poisonous fungi in the genera of lamellate and tubulate fungi

Agaricus

Most Agaricus species belong to the well-tried edible fungi. Nevertheless, wild species may have a high content of heavy metals.

1a Fungi immediately after rubbing becoming intense chrome-yellow especially at the base (bulb) of stipe; other parts also taking on this colour; yellow flushes disappearing after some time. Smell ink-like (also of phenol, iodoform), less often almost absent. Pellicle of pileus and flesh not giving a deep orange chrome-yellow reaction with alkali and aniline (Schäffer reaction: negative)
- **2a** Pileus white or whitish. Spores 5 to 7 × 3 to 4 µm . . . . . (+) **A.xanthoderma**
- **2b** Pileus with small fine to coarser brown, grey or blackish scales
  - **3a** Pileus with small reddish brown scales on a cinnamon-brown ground. Spores 4.5 to 6 × 3.0 to 3.5 µm . . . . . (+) **A.phaeolepidotus**
  - **3b** Pileus with small, grey, blackish, to dirty brown scales. Spores 4 to 5(to 7) × 3.5 to 4.2 µm . . . . . (+) **A.placomyces**

1b Fungi when cut or rubbed reddening or scarcely changing colour or with flesh that becomes yellow but not intense chrome-yellow. Smell not of phenol; many species smell of aniseed. Spores larger or not. Schäffer reaction often positive . . . . . – (Non-toxic or untried species) –

Amanita

The following fungi, although toxic when raw, are considered to be edible when cooked: A.vaginata, A.fulva, A.spissa, and A.rubescens. Only species that are unequivocally identified and known to be edible should be used! For Wieland's amanitin test

1a A saccate, persistent, free volva at the base of the stipe. Manchette present or absent. Amanitin test often positive
- **2a** Stipe without a manchette. Marginal zone of the pileus distinctly striate. Spores amyloid, globose. Amanitin test negative . . . . . **(R) A.fulva** (Tawny Grisette), **A.vaginata** (Grisette), and related forms; edible when cooked
- **2b** Stipe with a manchette that is evanescent or not, the remains of which lie on the stipe
  - **3a** Pileus not orange-red and lamellae, stipe, and flesh not yellow. Spores amyloid, globose, or broadly ellipsoidal. Amanitin test positive
    - **4a** Pileus dingy grey-green or yellow-green, expanded convex. Smell sweetish and honey-like to obnoxious. Spores 8 to 11 µm. Particularly under oak trees . . . . . (++) **A.phalloides**
    - **4b** Pileus white
      - **5a** Pileus white, otherwise as 4a, in deciduous forests . . . . . (++) **A.verna**
      - **5b** Pileus hemispherical to subconical, when old flatter, pure white. Manchette evanescent. Stipe squamose to fibrous. Spores 7 to 10 µm. In coniferous, less often deciduous, forests . . . . . (++) **A.virosa**
  - **3b** Pileus orange-red and lamellae, stipe, and flesh yellow. Spores not amyloid. Amanitin test negative . . . . . – (A.caesarea; edible fungus) –

1b Persistent, free, saccate volva absent; bulb of the stipe at most sharply marginate. Manchette present

6a Spores not amyloid, marginal zone of the pileus almost always striate

7a Pileus red with white (in part, disappearing) verrucosities or tawny to dark brown. If with brown colours in the pileus, then the flesh under the skin of the pileus yellow

8a Pileus red . . . . . (++) **A.muscaria**

8b Pileus tawny to dark brown, the flesh under the skin of the pileus yellow, tawny, yellowish green . . . . . (++) **A.regalis**

7b Pileus neither red nor when brown the flesh under the skin of the pileus yellow

9a Pileus yellow, whitish, flesh salmon-coloured, pink, or wine-brown

10a Pileus convex, not umbonate at the apex, marginal zone sub-striate, creamy lemon-coloured, wax- or ochre-yellow . . . . . (+?) **A.gemmata**

10b Pileus campanulate, then expanded, marginal zone striate over a greater length, white, flesh to salmon-coloured, pink, or wine-brown, ochraceous . . . . . (?) **A.eliae**

9b Pileus brown, dun, marginal zone striate. Stipe as if stuck into the bulb. Manchette not striate. Spores 10 to 12 × 7 to 8 µm . . . . (++) **A.pantherina**

6b Spores amyloid, marginal zone of the pileus not striate or only shortly and indistinctly so

11a Spores subglobose

12a Pileus pale yellow . . . . . (?) **A.citrina**

12b Pileus dun . . . . . (?) **A.porphyria**

11b Spores oblong ellipsoidal

13a Flesh where eaten by maggots becoming flesh-pink to brownish vinaceous, especially so at the bulb of the stipe. Pileus brown with a flesh tinge, less often violettish; also yellowish straw to yellowish lemon or only delicately flesh-coloured

14a Flesh under the skin of the pileus pale flesh-coloured to pink . . . . . (R) **A.rubescens**, edible when cooked

14b Flesh under the skin of the pileus yellowish . . . . . (?) **A.aspera** (= A.francheti)

13b Flesh where eaten by maggots not becoming reddish

15a Pileus white, flesh to salmon-coloured, pink, or brownish vinaceous, ochraceous, 5 to 9 cm, campanulate, then expanded and umbonate; marginal zone striate over a considerable length. Flesh under the skin of the pileus pinkish. Spores sometimes faintly amyloid, (9 to)11 to 14(to 15) × 6.5 to 8.5 µm . . . . . (?) **A.eliae**

15b With different characters

16a Pileus grey, dun, manchette distinctly striate. Spores 8 to 12 × (5.5 to)7 to 10 µm . . . . . (R) **A.spissa**

16b With different characters . . . . . Species suspected of being toxic, e.g. (?) **A.echinocephala,** (?) **A.strobiliformis**

Armillaria

The Honey Mushroom, (**R**) **Armillaria mellea**, is a collective species. In general, fungi belonging to this group should not be eaten when raw.

Boletus

1a Taste bitter

2a Pores whitish to pink . . . . . – (Tylopilus felleus, inedible) –

2b Pores already yellowish when young

3a Stipe rather intense red with a red or white reticulate pattern. Pileus grey, dun, or ochre-brown. Spores 10 to 14 × 4 to 6 µm. Deciduous and coniferous forests . . . . . (R) **B.calopus** (even after boiling, not edible)

3b Stipe bulbous, radicating, normally without any red, more or less yellowish lemon. Pileus pale greyish or brownish grey. Spores 9 to 16 × 4 to 6 µm. Deciduous forests . . . . . – (B.radicans, not edible) –

1b Taste mild

4a Pores at most when young yellow, then more or less red, red-orange, orange, orange-yellow (later becoming somewhat more intense red)

5a Stipe without a reticulate pattern, carmine flocculose on a yellowish ground. Pileus more or less dark brown, velutinous, 5 to 20 cm, dry. Pores red (brown). Flesh lemon-yellow, when cut immediately dark blue. Spores 11 to 19 × 4.5 to 7 µm. In deciduous and coniferous forests (**R**) **B.erythropus** and closely related species with slightly different characters but similar toxicity: (**R**) **B.lupinus**, (**R**) **B.queletii**, with flesh taking on a weak blue colour, pores more orange-red, pileus brown to olive or orange-red, less often dark red

5b Stipe with a distinct reticulate pattern

6a Reticulate pattern coarse and stretched. Stipe mostly more or less orange, base vinaceous. Pileus olive-brown, orange-brown, to dark brown, also reddish. Flesh pale, in the base of the stipe often vinaceous, taking on a weak blue colour. Pores reddish brown. Spores 9 to 17 × 5 to 7 µm. Deciduous and coniferous forests . . . . . (A?, R) **B.luridus**

6b Reticulate pattern fine-meshed, not stretched

7a Smell even when young unpleasant, when old somewhat carrion-like. Pileus silver-grey to olive-grey, when old also ochraceous, 10 to 30cm. Stipe yellow with a carmine zone in the centre. Flesh pale, almost whitish, taking on a faint blue colour, in the stipe also reddening. Pores carmine, less often reddish brown. Spores 10 to 16×5 to 7µm. Deciduous forests on chalk . . . . . (+) **B.satanas**

7b Pileus not silver-grey, smell hardly unpleasant. Pileus whitish, bright pink, when old with a yellowish or brownish yellow tinge. Stipe with a purple-red reticulate pattern on a bright golden-yellow ground, base purple to blood-red. Flesh lemon-yellow, becoming somewhat blue. Pores when young lemon- to golden-yellow, then purple-red. Spores 10 to 16×4.0 to 5.5µm. Beech and oak forests (+) **B.rhodoxanthus**. Closely related species presumably with similar toxic activity have slightly diverging characters. Finally, with a deep red pileus: **B.rhodopurpureus**. – Pileus with just a hint of pink, more dirty pallid, grey to coffee-brown: **B.splendidus**. – Pores of all the above-mentioned species (except B.rhodoxanthus) more or less purple-red from the beginning. – **B.torosus** has golden-yellow, then orange to blood-red, pores. Pileus flecked with apple-green, yellowish, buff, vinaceous

4b Pores white or yellowish, not orange or becoming red . . . . . – (Edible Boletus species) –

Clitocybe

Many species are poisonous, suspected of being poisonous, or unwholesome. Few species are considered to be edible.

1a Fungi white, mostly not hygrophanous. Many species belonging here are poisonous, suspected of being so, or readily confused with poisonous species!

2a Fruit-body mostly with an excentric stipe, smell strongly farinaceous or absent . . . – (C.lignatilis, C.josserandii; edibility unknown) –

2b Fruit-body with a centric stipe

3a Pileus infundibuliform, often umbonate, moderately large (3 to 8cm) or very large (5 to 30cm). Spore mass white, spores 6 to 8.5×4 to 6µm

4a Large fungi, stipe 2 to 15×2 to 4cm. Pileus 5 to 30cm . . . . . – (C.geotropa; C.maxima; edible fungi of modest worth) –

4b Moderately large fungi, stipe 3 to 3.5×0.6 to 1.8cm. Pileus 4 to 12cm . . . – (C.catinus; C.subsalmonea; edibility unknown) –

3b Pileus either not infundibuliform or not large, or spores smaller and not broadly ellipsoidal. Spore mass sometimes with a flesh-coloured tinge

5a Surface of the pileus with a glazed-pruinose appearance (as if varnished). Lamellae mostly very crowded. Here also larger species with a flesh-coloured spore mass

6a Larger species, pileus reaching 7 to 8 to 11cm, but also some smaller ones, where bruised soon watery dun. Spore mass with a flesh-coloured tinge. Spores 4 to 5×2.5 to 3.5(to 4)µm. Coniferous and deciduous forests

7a Lamellae moderately distant, creamy white, adnate to adnate with a decurrent tooth. Pileus 5 to 11cm, convex, then depressed, pure white or the centre somewhat yellowish, ochraceous. Stipe 5 to 8×1 to 2cm. Smell absent or faint. Spores 4.5 to 5.5×3 to 4µm . . . . . (++) **C.phyllophila**

7b Lamellae distinctly crowded and narrow (to 3mm), whitish, when old off-whitish. Pileus 2 to 8cm, expanded convex, sometimes slightly umbonate. Marginal zone involute; colour white, where bruised watery dun, when old also brownish. Stipe 4 to 8×0.3 to 1.3cm. Smell strong, with somewhat farinaceous components. Spores 4 to 5×2.5 to 3.5(to 4)µm . . . (++) **C.cerussata**

6b Smaller species, pileus scarcely exceeding 4cm. Spore mass white

8a Outside woods in meadows and pastures, less often beneath trees

9a Smell and taste farinaceous or with more or less farinaceous components. Stipe 4 to 6×0.4 to 0.7cm. Pileus 2 to 4(to 6)cm, white to off-white, when old striated or flecked with grey to dingy yellowish buff. Mostly not very distinctly pruinose, visible (hand lens) as a more or less appressed to innate coating. Lamellae almost horizontally adnate. Spores 5 to 6×3 to 4µm . . . . . (++) **C.dealbata**

9b Smell and taste not farinaceous and not with farinaceous components. Stipe 1.5 to 3×0.3 to 0.5(to 0.7)cm. Pileus 1 to 4(to 6)cm, mostly more strongly white pruinose, which even in young specimens allows the reddish buff ground to be seen as flecks, streaks, or concentric lines. Lamellae uncinate. Spores 4 to 5.5×2.5 to 3µm . . . . . (++) **C.rivulosa**

8b In woods or low scrub (*Dryas* heath) in the Alps, thickets of Green Alder (*Alnus viridis*)

10a In woods from lowlands to mountains. Lamellae uncinate, almost straight adnate. Pileus 1 to 3(to 5)cm, convex-depressed. Marginal zone long remaining involute, when dry pure white, when wet off-white. Stipe 2 to 4×0.15 to 0.4(to 0.5)cm, often crooked; base strigose and geniculate. On leaf or needle litter. Smell mostly absent. Spores 4.5 to 5(to 7.8)×3 to 4µm. Cortical layer not comprising knobbly-sinuate hyphae . . . . . (++) **C.candicans**

10b In the alpine and subalpine zones (cf. also the species indicated under 18a)

11a Among Mountain Avens (*Dryas octopetala*) in the alpine zone. Spores 4 to 5×3 to 3.5µm . (++) **C.dryadicola**

11b Under Green Alder (*Alnus viridis*) in the Alps. Cortical layer of thin, branched, in part knobbly-sinuate hyphae . (?) **C.alnetorum**

5b Surface of the pileus different, lamellae crowded or not

12a Smell not of aniseed, pileus infundibuliform to napiform, not umbonate

13a Lamellae distant and strongly decurrent. Pileus white, somewhat yellowing, 2 to 5cm, napiform. Taste mild to slightly bitterish. Stipe 3 to 4×0.4 to 0.5cm. Spores 5 to 6×3 to 3.5µm. Like *Camarophyllus niveus* . . . . . (++) **C.ericetorum** (Snowy Meadow Cap)

13b Lamellae not distant and sub-decurrent. Pileus 2 to 5cm, somewhat hygrophanous, off-white creamy grey and marginal zone when moist pellucid-striate, infundibuliform. Lamellae very crowded, more or less adnate to uncinate. Stipe at the base dingy creamy grey, 2 to 6×0.2 to 0.4cm. Spores (3 to)4 to 5×3µm . . . . . (++) **C.angustissima**

12b Smell of aniseed. Pileus umbonate . . . . . – (Other species mostly suspected of being poisonous, e.g. C.anisata) –

1b Pileus hygrophanous or not white

14a Large, fleshy fungus with grey, dun, convex, 7 to 15cm, pruinose pileus. Lamellae pale, uncinate, readily separating from the base of the pileus. Stipe bulbous at the base, solid, 6 to 10×1.5 to 3.0cm, whitish. Spores 6 to 7×3 to 4µm. Spore mass cream. Woods, gregarious . . . . . **(R) Lepista nebularis**

14b Fungi not at the same time large, fleshy, grey in the pileus, and with readily separating lamellae

15a Fungi with an aniseed-like or sweetish smell. Pileus mostly hygrophanous, when wet not pure white, mostly grey or brownish

16a Pileus greenish grey, 3 to 4cm. Stipe somewhat clavate with a tomentose base. Spores 6 to 7×3 to 4µm . . . . . – (C.odora; usable mixed with other fungi) –

16b Pileus ochraceous, flesh-brown to grey

17a Spores 8 to 10×4 to 5µm . . . . . – (C.obsoleta; edibility unknown) –

17b Spores less than 8µm long, e.g. 6 to 8×3 to 4µm. Pileus dun, buff-ochre. Marginal zone slightly striate, when dry pale. Stipe scarcely longer than the breadth of the pileus. Lamellae off-whitish grey . . . . . (++) **C. fragrans**, incl. **C.suaveolens** and closely related species of doubtful wholesomeness

15b Fungi not with an aniseed-like or sweetish smell

18a In alpine meadows among Mountain Avens (*Dryas octopetala*) and alpine pastures. Not with a farinaceous smell or fruity, pleasant smell, and not with a deeply umbilicate and, at the same time, dark dun to dark olivaceous brown pileus

19a Pileus rather deeply coloured

20a Spore mass more or less dark ochraceous. Spores 5.5×3 to 4µm. Pileus 1 to 4(to 7)cm, plano-convex, applanate, finally somewhat concave with a flat marginal zone, dun with a beige-chocolate tinge; when young whitish pruinose. Lamellae yellowish grey. Smell not unpleasant (if unpleasant, C.festivoides) . . . . . (++) **C.festiva**

20b Spore mass white or whitish

21a Spores 4.5 to 5.5×4 to 4.5µm. Pileus at first brown, then yellowish brown, faintly pruinose. Lamellae light coloured, not white, dingy brown with light yellowish cream areas . . . . . (++) **C.serotina**

21b Spores 5 to 6×3 to 4µm. Pileus when soaked fairly dark, dun, chocolate-coloured, pruinose. Lamellae light-coloured, off-white . . . . . (++) **C.nuoljae**

19b Pileus fairly light-coloured

22a Spore mass creamy beige. Pileus hygrophanous, light-coloured and dirty beige, sometimes somewhat flesh-coloured, dry whitish cream. Marginal zone of pileus often with a white tomentose edge. Lamellae dirty cream, not white. Spores 5 to 6×3.5 to 4µm. Remaining alpine species with a light-coloured pileus, see under 10b . . . . . (++) **C.marginella**

22b Spore mass white or whitish. Pileus hygrophanous, with a light colour when soaked: pale horn colour and at the same time a translucent yellowish tint. Lamellae off-whitish beige. Spores 4.5 to 5.5×3.5 to 4.5µm. Other alpine species with a light-coloured pileus, see under 10b . . . . . (++) **C.gracilipes**

18b Not in alpine meadows, or remaining characters different

23a Lamellae white to light beige, stipe without conspicuous mycelial rhizoids and not fruiting in spring. Pileus with ochraceous or brownish flesh-coloured tint; marginal zone faintly pellucid-striate and minimally white pruinose. Spore mass beige. Spores 4.5 to 6×3 to 3.5(to 4)µm. Particularly in coniferous forests (cf. 22a, C.marginella) . . . . . (++) **C.diatreta**

23b Fungi not with the foregoing characters. Lamellae mostly grey, greyish, or brownish. If lamellae white to light beige, then pileus pale buff, beige, or greyish

24a Taste very bitter. Pileus about 4cm depressed-infundibuliform, dun. Marginal zone somewhat striate. Lamellae narrow, grey, adnate with a decurrent tooth, somewhat furcate. Stipe more or less short, darker than the pileus. Spores 5×3.5µm. Deciduous forests . . . . . (++) **C.fritilliformis**

24b Taste at most slightly bitter

25a Pileus with predominantly ochraceous tinges

26a Pileus beige, ochraceous, brown. If spores subglobose or obtusely ellipsoidal, e.g. 5 to 7×5.6 to 6.2µm, or (4.5 to)5 to 7(to 8)µm, then certain edible species may be concerned, e.g. . . . – (C.geotropa, C.gibba) –

26b Fungi with different characters . . . . – (Species of unknown edibility or suspected of being poisonous) –

25b Pileus with grey tints

27a Pileus brown-grey, grey, 4 to 7(to 10)cm. Lamellae yellowish cream, light ochre. Stipe distinctly clavate and also soft and compressible, 3.5 to 10×0.6 to 1.2cm. Spores 6 to 8.5(to 11)×3.6 to 4.3µm . . . . . **(A, R) C.clavipes**

27b Fungi with different characters . . . . . – (Species of unknown edibility, some of which are suspected of being poisonous) –

Collybia

1a Fungi with a distinct leek smell or stinking, or with a leek-like or pungent taste
2á Stipe whitish, throughout its length pruinose to tomentose, below white strigose-tomentose, towards the apex also with a light flesh-coloured reflex, not or hardly fasciculate, sometimes gregarious on leaf litter, 5 to 9 × 0.8 to 0.9 cm. Pileus up to 7 cm, hygrophanous, pale reddish brown. Lamellae crowded. Smell and taste of rotting cabbage (lentinic acid), not burningly pungent. Spores 6 to 8 × 3 to 3.5 µm. Beech woods . . . . . **(?) C.hariolorum**
2b With different characters . . . . . Species that are inedible or of unknown toxicity
1b Fungi neither stinking nor with a leek-like or burning pungent taste
3a Stipe 8 to 12 × 1 to 2 cm distinctly striate and costate, often twisted, stiff, on trunks of oak and beech trees radicating or sub-radicating, dark reddish brown. Pileus 4 to 7(to 10) cm, uniformly coloured, soon fading, brown. Lamellae thickish and distant, flesh-coloured, often spotted with red. Spores 4 to 6 × 3 to 4.5 µm . . . . . **(?) C.fusipes** (Spindle Shank)
3b Fungi with different characters . . . . . Largely inedible and of unknown or doubtful worth – (Moderately good edible fungi: C.butyracea, C.erythropus, C.dryophila, C.exsculpta) –

Conocybe

Conocybe species (indigenous ones as well?) can give rise to symptoms of hallucinogenic intoxication (Psilocybin syndrome).

Coprinus

1a Surface of the pileus with remains of a fibrous or micaceous veil and at the same time fungi either around wood stumps or with a large pileus, e.g. 5 to 10 × 3 to 6 cm, or with spores that are rough or greatly broadened at the apex
2a Veil on the surface of the pileus micaceous
3a Flocci of the veil comprising only globose cells. Stipe with setae. Pileus rust-yellow to foxy, striate, with a pale ochraceous to brownish micaceous coating. Stipe whitish, 5 to 10 × 0.3 to 0.5 cm. Spores 7.5 to 10 × 4.5 to 5.2 × 6.2 µm, greatly broadened at the apex. If spores broadest in the middle, cf. C.truncorum, which, as regards its toxicity, is probably not different from C.micaceus . . . . . **(A?) C.micaceus**
3b Veil of globose cells with mixed-in elongated elements . . . . . – (Related species possibly with similar toxicity) –
2b Veil not micaceous
4a Stipe without an annulus. Pileus 3 to 7 cm. Only when young, the veil weakly developed on the surface of the pileus, later the pileus naked. Spores rough and (10 to)11 to 14.5 × (6 to)7 to 8 µm or smooth and 7.5 to 12.5(to 14) × 6 to 8 µm
5a Spores rough, amygdaliform to limoniform, (10 to)11 to 14.5 × (6 to)7 to 8 µm. Pileus 4 to 7(to 10) cm, apex violettish, when young covered with silvery hairs, when old naked. Stipe white, towards the base white floccose. Base almost fuscoid radicating, 6 to 15(to 17) × 0.7 to 1(to 1.5) cm. At the bottom of the trunks of deciduous trees . . . . . **(A) C.alopecia**
5b Spores smooth
6a Pileus (centre) and at least the lower half of the stipe with a distinct apricot colour, grey reddish brown, or olive reddish brown scales. Spores 8 to 12(to 13) × 5 to 6 µm . . . . . **(A?) C.romagnesianus**
6b Veil less conspicuous and differently coloured
7a Pileus grey to dun, towards the apex with detersile brownish scales, marginal zone striate-plicate, 3 to 7 cm. Lamellae white, then black. Stipe paler than the pileus, the base sometimes ringed with a volva-like structure
8a Spores 7 to 11 × 5 to 6.5 µm . . . . . **(A) C.atramentarius**
8b Spores (7.5 to)8 to 10 × 4 to 5.2 µm . . . . . **(A?) C.acuminatus**
7b Fungi with different characters . . . . . – (Closely related species suspected of being toxic) –
4b Stipe with an annulus and/or the other characters mentioned absent
9a Pileus closed, 5 to 12 cm high, cylindric to globose, white tomentose-squamose, the apex sometimes ochraceous. Lamellae white, then purplish pink, finally black deliquescing. Stipe 15 × 1 to 1.5 cm, the annulus often evanescent. Spores 12 to 16 µm. Grassy and manured places . . . . . – (C.comatus, edible fungus) –
9b With different characters, e.g. smaller, etc. . . . . – (Fungi with unknown effects) –
1b Pileus either already naked when young or velutinous pruinose or with a whitish, farinose, detersile veil . . . . . – (Species that are suspect or about whose edibility nothing is known) –

Cortinarius

Nothing is known about the suitability of most species for culinary purposes. Besides a relatively small number of tested edible fungi from the subgenera Phlegmacium and Myxacium, there are a few species that are extremely toxic. Recently, it has been shown unequivocally that poisonous fungi occur in the subgenera Leprocybe (Take care! Deadly poisonous species!) and Phlegmacium. The edibility of species from the subgenera Telamonia and Sericeocybe is largely unknown. The subgenus Cortinarius has two rare species that are valueless as edible fungi and should therefore be left alone. In the subgenus Myxacium, the bitter-tasting species are unusable.

Cortinarius, subgenus Leprocybe

1a Fruit-body not with a predominantly greenish olive to yellowish olive tinge (and such colours absent particularly from the lamellae and stipe) and not with predominantly brown or red ground colours. Pileus rather with yellow, tawny, orange-brown to foxy colours and the flesh yellow, tawny, orange-brown. If flesh white, then not so in all parts and never yellowing

2a Spores obtusely ellipsoidal. Fluorescence of the fruit-body extract more or less blue. Fruit-body with an orange-brown or cinnamon-brown colour, at least on the pileus and lamellae. Lamellae thickish and distant

3a Stipe yellow, brass- to golden-yellow, without discernible traces of a veil, sometimes narrowing towards the base. Pileus orange foxy, 3 to 8.5 cm, appressed fibrillose, then naked. Lamellae bright cinnamon-brown, thickish, distant. Smell when cut sometimes radish-like. Spores amygdaliform, 8.5 to 12 × 5.5 to 7 µm. Deciduous and mixed forests, preferring warmth . . . . . (++) **C.orellanus**

3b Stipe and pileus often almost concolorous, orange-brown, orange foxy, reddish brown, often with one or more ochraceous to lemon-yellowish velar rings

4a Species of deciduous forests . . . . . – (Species related to the next one, and presumably with similar toxicity) –

4b Species of acid, often marshy, coniferous forests. Pileus 3 to 8 cm, usually cuspidate, less often bluntly convex, reddish brown to orange, slightly tomentose. Lamellae uniformly coloured, thick, broad, distant. Stipe uniformly coloured, without or with lemon-yellowish velar rings, 5 to 12(to 15) × 0.6 to 1.5(to 2.0) cm. Flesh reddish quince-yellow. Sometimes with radish smell. Spores 9 to 12 × 6.5 to 9 µm . . . . . (++) **C.orellanoides**

2b Spores globose to ellipsoidal. Fluorescence of the fruit-body extract mostly yellow to deep yellow. Fruit-body with an ochre-yellow, bright yellow, tawny, or orange-brown colour. Stipe slender and long, or not. Spores 7.5 to 9 × 5.5 to 6.5(to 7) µm . . . . . (+) **C.gentilis**; cf. also (?) **C.callisteus** and (?) **C.limonius**

1b Fruit-body greenish olive, yellowish olive, or predominantly brown, red, or with colours other than those given above . . . . . – (Various inedible species) –

Cortinarius, subgenus Phlegmacium

1a Flesh of the cut fruit-body bright yellow or greenish yellow; coloured right through, i.e. not whitish in the centre or in the pileus

2a Stipe with a marginate bulb

3a Smell of aniseed. Pileus in the centre coppery reddish brown, towards the margin grey-green, greenish yellow, or violettish; less often entirely yellow. Lamellae and stipe yellow with a greenish tinge. Spores 9 to 13 × 5 to 7 µm. Coniferous forests on calcareous soils, especially in spruce forests of mountains and middle ranges . . . . – (C.odorifer, considered an edible fungus of moderate value) –

3b Smell not aniseed-like

4a Pileus largely copper-brown, only towards the margin greenish yellow to yellow. Lamellae olive-yellow to greenish. Stipe blue-green (glaucous), chrome-yellow fibres on bulb. Spores (8 to)9 to 12 × (5 to)6 to 7 µm. Deciduous and coniferous forests . . . . . – (C.glaucescens; edibility unknown) –

4b Pileus predominantly green, very dark green, or bright yellow, tawny, to bronze-foxy. Apex sometimes with umber or purple-brown to rust-brown flecks

5a Slender species with the pileus up to 5 cm and at the same time with small, 8 to 9 × 4.5 to 5 µm, spores. Stipe 0.5 to 1 cm thick. Lamellae when young greenish yellow to olivaceous. Deciduous forest . . . . . – (C.citrinus; cf. also C.prasinus; edibility unknown, perhaps poisonous) –

5b Either fungus more robust or spores (8 to)9 to 12 × 5 to 7 µm

6a Pileus with a greenish tinge: yellow-green to dark olive-green, very dark green; surface mostly innately fibrillose

7a Lamellae greenish yellow to olivaceous. Beech woods on chalk. Marginal zone of the pileus yellow-green, dark olive-green, towards the apex olive-brown, 4 to 8 cm. Spores (8 to)9 to 10 × 5 to 6 µm . . . . . – (C.pseudosulphureus; edibility unknown, perhaps poisonous) –

7b Lamellae when young bright chrome-yellow. Beneath fir trees on calcareous soils. Pileus very dark green, 4 to 10 cm. Spores 9 to 11(to 12) × 5 to 6 µm . . . . . (+?) **C.atrovirens**

6b Pileus at least at the marginal zone yellow or entirely tawny to bronze-foxy. Lamellae when young distinctly chrome- to lemon- or orange-yellow, tawny, to foxy

8a Pileus at least at the marginal zone yellow, the centre brown to olive-brown. Lamellae chrome- to lemon-yellow. Coniferous and deciduous forests

9a Coniferous forests. Smell unpleasant, reminiscent simultaneously of pastries and coal gas. Pileus 5 to 9 cm, sulphur- to chrome-yellow, often with olive-brown to brown spots. Alkali stains the flesh reddish brown to very dark brown. Spores 10 to 11 × 5 to 6 µm . . . . . (+?) **C.vitellinus**

9b Deciduous forests. Smell absent or faint. Pileus scarcely more than 6 cm broad, the centre with brown spots. Alkali stains the flesh light reddish ochre. Spores 9 to 12 × 5.5 to 7 µm . . . . . (++) **C.splendens**

8b With different characters . . . . . – (Various species of largely unknown edibility) –

2b Stipe not with a marginate bulb, but sometimes with a clavate base; less often bulbous without being marginate

10a Smell unpleasantly earthy . . . . . – (C.russeus, C.russeoides; useless as edible fungi; suspected of being toxic) –

10b Smell absent or spicy to marjoram-like, sometimes also with fruity components – (Various species, some of which are also indicated to be edible) –

1b Flesh of the cut fruit-body not bright yellow to lemon-yellow throughout; at least in the middle and in the pileus whitish or quite differently coloured

11a Lamellae with an olive tinge (olivaceous to fuliginous olive) and at the same time a bitter taste. Pileus with brown, grey, to almost black colours; stipe mostly concolorous; equal, clavate or sometimes also with a marginate bulb. Spores 7 to 8 µm. Coniferous and deciduous forests . . . . . (+?) **C.infractus**

11b Taste at most slightly bitterish and the pileus then with other colours . . . . – (Other species, forming the greater part of the genus) –

Cortinarius, subgenus Sericeocybe

1a Pileus and stipe violettish, bluish, or pale lilac, or the pileus of older specimens with the colours mentioned only at the marginal zone

2a Flesh becoming pink or reddish when exposed to the air . . . . . – (Species whose edibility and toxicity are unknown) –

2b Flesh not becoming pink or reddish when exposed to the air

3a Spores subglobose. Pileus when dry mostly micaceous-sericeous . . . . . – (Species whose edibility and toxicity are unknown) –

3b Spores not globose

4a Lamellae when young already saffron-ochre to umber; for the rest, all parts of the fruit-body bright lilac-violet; when old fading and then ochraceous to almost whitish. Pileus 3 to 10 cm, clavate stipe 6 to 10 × 1 to 3 cm. Tawny flesh mostly smelling unpleasantly of acetylene. Coniferous and deciduous forests. Spores (7 to)8 to 10 × (4 to)5 to 6 µm . . . . . (+) **C.traganus**

4b Fungi with different characters . . . . . – (Species whose edibility and toxicity are unknown) –

1b Pileus and stipe with other colours . . . . . – (Species whose edibility and toxicity are unknown) –

Dermocybe

Dermocybe species can bring about mild gastrointestinal poisoning.

Entoloma

The genus contains only a few species that have been found to be edible, as well as several poisonous ones and many representatives whose toxicity is unknown. As the few edible species can easily be confused with poisonous ones, the genus should in general be avoided by collectors of edible fungi.

1a Fungi relatively compact with stipes 0.3 to 1 cm broad or broader. Pileus not thin, neither conical nor campanulate nor umbilicate. Lamellae not strongly decurrent. Fungi not lilac, not violet-coloured. Not smelling sweetish of bonbons
- **2a** Pileus naked, at any rate not fibrillose or squamose
  - **3a** Growing in spring and often occurring together with Rosaceae, e.g. apple, damson, raspberry, meadow-sweet
    - **4a** Spores 9 to 10×7 to 8 µm. Pileus 2.5 to 4(to 6) cm, hemispherical to campanulate, finally expanded, papillate or umbonate, hygrophanous, when wet olivaceous to umber, when dry becoming much paler. Not associated with Rosaceae; among moss and grass at forest edges . . . . . (++) **E.vernum**
    - **4b** Smell and taste farinaceous, as well as fungi associated with Rosaceae. Fruit-body more massive and/or spores with other dimensions, e.g. E.aprile with spores 9 to 12×7.5 to 10 µm . . . . . (R) Apriles group; indicated to be edible when cooked, but great care is required because of the danger of confusion, e.g. **(R) E.clypeatum**
  - **3b** Not growing in spring and not associated with Rosaceae
    - **5a** Pileus at first convex, fairly thick-fleshed, often umbonate, when old somewhat depressed
      - **6a** Fruit-body with grey to brown colours
        - **7a** Smell of cut fungus not farinaceous, but sometimes nitrous
          - **8a** Smell strongly nitrous . . . . . (++) **E.nidorosum**
          - **8b** Smell not nitrous
            - **9a** Stipe mostly relatively thick, 5 to 12×0.3 to 1.5 cm, white or light greyish. Smell absent. Pileus 5 to 10 cm, convex to slightly umbonate, then often twisted and depressed, grey to yellowish grey. Gregarious in deciduous forests. Spores 8 to 10.5×7 to 8 µm . . . . . (++) **E.rhodopolium**
            - **9b** Fungi with different characters . . . . . – (Species whose poison content or edibility is unknown) –
        - **7b** Smell of fungus farinaceous . . . . . – (Species whose poison content or edibility is unknown) –
      - **6b** Fruit-body when dry or young more or less white or whitish
        - **10a** Fungi large, pileus 6 to 20 cm, fleshy, ivory-white to ochraceous buff, old specimens also brownish grey. Stipe 6 to 10×1 to 2.5 cm. Spores 8 to 10×7 to 8.5 µm . . . . . (++) **E.sinuatum**
        - **10b** Fungi smaller . . . . . – (Species whose poison content or edibility is unknown) –
    - **5b** Pileus campanulate-convex to obtusely conical, convex and umbonate, or papillate, mostly thin-fleshed . . . . . – (Species whose poison content or edibility is unknown) –
  - **2b** Pileus fibrillose to squamose . . . . . – (Species whose poison content or edibility is unknown) –

1b Fungi neither compact nor with the indicated shape of pileus. Also the other characters sometimes different . . . . . – (Species whose poison content or edibility is unknown) – e.g. **(?) E.sericeum**

Galerina

The genus contains deadly poisonous fungi that can be confused with Kuehneromyces; there are no edible species.

1a Hyphal septa with clamp-connexions
- **2a** Pleurocystidia present
  - **3a** Pleurocystidia thin-walled; at any rate not thick-walled like the metuloids of Inocybe, not capitate
    - **4a** Pleurocystidia with a rounded sub-capitate apex. Pileus 0.6 to 1.7 cm, conical to campanulate. Spores 10 to 12×5.5 to 7.5 µm . . – (G.pruinatipes) –
    - **4b** Pleurocystidia with an acute apex or fungi with different characters
      - **5a** Marginal zone of the pileus inflexed, at least in young, not fully expanded specimens
        - **6a** Stipe with a membranous annulus or on old specimens an annular zone as its remains. Taste mostly farinaceous. Cortical layer sometimes gelatinous
          - **7a** On wood, often among moss
            - **8a** Cortical layer not gelatinous. Mostly on conifer wood. Pileus 1.5 to 4 cm, convex to applanate, rarely slightly umbonate, ochre to ochre-brown, hygrophanous, pellucid-striate. Stipe ochre to honey-coloured, towards the base darker brown, 2 to 6×0.2 to 0.9 cm. Smell and taste farinaceous. Spores ovoid, not calyptrate, 8 to 10(to 15)×5 to 6(to 7.5) µm . . . . . (++) **G.marginata**

8b Cortical layer more or less gelatinous or spores with a separating exosporium

9a Pileus convex, then applanate, slightly umbonate, 2.5 to 6.5 cm. Cortical layer gelatinous, with 2 to 4 µm broad hyphae in a hyaline mass. On the wood of deciduous and coniferous trees. Similar in remaining characters to the previous species. Spores 8.5 to 10.5 × 5 to 6.5 µm, ovoid, with a somewhat raised exosporium . . . . . (++) **G.autumnalis**

9b Pileus conico-campanulate, mostly umbonate, less often only convex, 0.5 to 2.5(to 3) cm. Surface of the pileus when wet with a fatty gloss, but cortical layer not or only slightly gelatinous, with 4 to 8.6 µm broad, yellowish or almost hyaline hyphae. Decaying stumps, also burnt places, cut surfaces. Spores 7 to 10(to 13.5) × 5 to 7.5 µm, rounded ovoid, with a separating verrucose exosporium . . . . . (++) **G.unicolor**

7b On the ground among mosses, often in wet places. Sometimes in greenhouses on sawdust

10a Spores amygdaliform to limoniform, with a separating exosporium and hence calyptrate, (9 to)10 to 12(to 13) × 5.5 to 6(to 7) µm. Pileus 2 to 3.5 cm. In lowland moors, often among sphagnum . . . . . (++) **G.beinrothii**

10b Spores otherwise, not amygdaliform or limoniform, but with a rounded apex, not distinctly calyptrate, but exosporium nevertheless occasionally separating to some extent

11a Pileus plano-convex, scarcely umbonate, darker and more brownish red than the lamellae. Among moss in marshy places in the alpine zone. Spores 9 to 12(to 13) × 6.5 to 8 µm, almost smooth to verruculose. Exosporium not separating . . . . . – (G.moelleri) – Content of amanitins unknown

11b Pileus conico-campanulate, mostly umbonate, in colour not very different from that of the lamellae . . . . . see (++) **G.unicolor**

6b Stipe without a membranous annulus. If a veil is present, then on the stipe only as a fibrous zone

12a Spores 7.5 to 8.5 × 4.5 to 5 µm, amygdaliform, verruculose, in potassium hydroxide with a germ pore. Lamellae uncinate, distant. In greenhouses . . . . . (++) **G.sulciceps**

12b Spores 10 to 13 × 5.5 to 7(to 8) µm if from two-spored basidia, otherwise 8 to 10 × 5 to 6 µm, faintly ornamented, with an indistinctly demarcated plage. On the remains of coniferous wood, cones, etc. in forests . . . . . (++) **G.badipes** (incl. G.cedretorum)

5b Marginal zone of the pileus not inflexed . . . . . – (Various species of the genus; worthless as edible fungi) –

3b Pleurocystidia (and cheilocystidia) thick-walled . . . . . – (G.nana) –

2b Pleurocystidia absent . . . . . – (Remaining species with clamp-connexions at the hyphal septa) –

1b Hyphal septa without clamp-connexions . . . . . – (Rest of the genus) –

Gymnopilus

Owing to the bitter taste and the possible content of hallucinogenic substances, the entire genus is unsuitable for culinary purposes.

1a Stipe with a membranous annulus. Pileus 5 to 15 cm. Spores 8 to 10 × 5 to 6 µm. On stumps of deciduous trees . . . . . (+) **G.spectabilis**

1b Stipe without an annulus . . . . . – (Remaining species of the genus) –

Hebeloma

The genus does not contain any edible species.

1a Fungus large, pileus 4 to 12 cm, stipe 5 to 10 × 1.5 to 2.5 cm. Pileus ochre-brown, yellow rusty, to reddish buff or dingy brown, subconvex to applanate. Lamellae milky-coffee to cinnamon-brown, edges with the same colour or white floccose, not weeping or not spotted with dark brown. Stipe white, squamose, inside hollow with fleshy plugs reaching into the hollow from the pileus. Smell strongly radish-like. Spores 10 to 12 × 6 to 8 µm . . . . . (+) **H.sinapizans**

1b Fungus smaller or with other than the above-mentioned characters

2a Lamellae weeping at the edges and there later with dark brown spots. Young specimens with a cortina or veil. Growing on deciduous trees. Pileus 4 to 8 cm, whitish clay, pale clay, light dun. Marginal zone long involute. Lamellae pale dun. Stipe whitish grey, brownish, flocculose. Strong radish smell. Spores (9.5 to)10 to 13(to 14) × 5 to 7(to 7.5) µm . . . . . (+) **H.crustuliniforme** and related species

2b With different characters . . . . . – (Suspect or doubtful species) –

Hygrocybe

Besides a few species that are looked upon as being edible, the genus has a few that are poisonous and a majority whose effects are unknown. In the following key, the edible and poisonous species are separated from most of the remaining species of the genus.

1a Fruit-body conspicuously and persistently cherry- to blood-red, at least in the pileus, and not becoming black. Stipe dry. Pileus large, 2 to 6cm, not squamulose or fibrillose-furfuraceous. Basidia four-spored.

2a Lamellae broadly adnate to adnate with a decurrent tooth. Pileus 2 to 6cm, hemispherical, cherry-red. Surface of the stipe smooth, cherry- to blood-red, the base yellowish. Flesh cherry-red. Lamellae orange to blood-red, faces often yellowish. Spores 7 to 9×4 to 5µm . . . . – (H.coccinea; edible, but owing to its rarity should not be picked) –

2b Lamellae adnexed to narrowly adnate. Pileus more or less glutinous, stipe dry

3a Flesh in the base of the stipe white, otherwise yellow. Pileus fleshy, 5 to 12cm, campanulate, cherry- to blood-red. Stipe 6 to 10×0.8 to 2cm, yellowish orange, fibrillose-striate, base white. Lamellae yellow, orange, or red. Spores 8.5 to 11×5 to 6µm . . . . . – (H.punicea; edible, but as a rare species not to be picked) –

3b Flesh inside yellow, in the lowest part of the stipe rarely whitish. Pileus 2 to 11cm, cherry-red, carmine with a purplish tinge. Stipe orange-yellow, the base yellow, the apex reddish, the surface glabrous. Spores (7 to)7.5 to 10×(4 to)4.5 to 5.5µm . . . . . – (H.splendidissima; edible, but owing to its rarity should not be picked) –

1b Fruit-body either not conspicuously and persistently cherry-red or if red then going black, or squamose-furfuraceous on the surface of the pileus. Stipe dry or glutinous. Basidia partly two-spored

4a Fungi turning black, pileus 2 to 6cm and orange to red. Lamellae yellow to red

5a Pileus up to 7cm, typically convex and umbonate to convex. Surface of the pileus and stipe sericeous to matt, rough, coarsely fibrous, striate, streaked. Smell intensely fruity, of apples and peaches. Spores (8 to)8.5 to 14×5 to 7(to 8)µm, less often constricted than in the following species, from which it is often difficult to distinguish . . . . . (+) **H.nigrescens**

5b Pileus up to 5cm, typically more or less conico-umbonate. Surface of the pileus and stipe sericeous or matt, always finely structured. Smell fruity, of apples or peaches. Spores (7.5 to)9 to 13(to 15)×4 to 6.5(to 8)µm, occasionally constricted . . . . . (+) **H.conica** and closely related species turning black and suspected of being toxic

4b Fungi not turning black

6a Basidia two-spored, pileus more or less conico-umbonate, more or less glutinous, 2 to 6cm, lemon-yellow to orange. Stipe yellow to orange-yellow, dry, 5 to 11×0.3 to 0.6cm. Lamellae lemon- to almost chrome-yellow, adnexed to narrowly adnate, with cystidia. Spores ellipsoidal, 10 to 14(to 15)×5.5 to 7.5(to 8)µm . . . . . (+) **H.acutoconica** (= H.langei)

6b Basidia four-spored or fungi with different characters . . . . . – (Species doubtful or suspected of being poisonous) –

Hypholoma

1a Fungi fasciculate on wood

2a Taste bitter or bitterish

3a Lamellae yellow or greenish

4a Pileus greenish to sulphur-yellow, the apex more or less foxy; often umbonate, 3 to 7cm. Lamellae sulphur-yellow, then greenish to greenish brown. Stipe sulphur-yellow, base going brown. Flesh sulphur-yellow. Spores 6 to 8×3.5 to 4.5µm . (+) **H.fasciculare**

4b Pileus foxy to reddish brick, the marginal zone when young with a pale veil, 4 to 8cm. Lamellae yellowish, then yellow-brown to very dark olive. Stipe more or less rust-coloured. Spores 6 to 8×3 to 4µm . . . . . (?) **H.sublateritium**

3b Lamellae whitish . . . . . – (Species of unknown worth) –

2b Taste wholly mild. Lamellae whitish, then smoky-grey. Pileus yellow to brownish orange, 2 to 6cm. Spores 7 to 9×4 to 5µm . . . . . – (H.capnoides, edible; cf. H.elaeodes, edibility unknown, spores 6 to 7×3 to 3.5µm) –

1b Fungi at most single, not fasciculate, on wood . . . . . – (Remaining species, not edible) –

Inocybe

The entire genus is unsuitable for culinary purposes, since many species are poisonous and because harmless species can readily be confused with poisonous ones. The genus does not contain any recognized edible fungi.

Lactarius

The taste and edibility of the pungent species are much debated, or they may be palatable only after being prepared in certain ways. Only those Milk Caps yielding orange or blood-red latex — i.e. excluding those whose latex is at first white and then becomes red — are considered as acceptable edible fungi. Lactarius volemus is recognized as a good, spicy edible fungus when fried. Provided they are included in only small amounts, the other mild species can be used in mixed dishes.

1a Pileus not white, early on coloured. Amyloid ornamentation of the spores distinct
- **2a** Latex orange or blood-red right from the beginning . . . . . – (Orange-milk Lactarius and its relatives; edible) –
- **2b** Latex white and remaining so, or yellow, violet, greenish, or becoming red
  - **3a** Fungus large and at the same time with a distinct 'Maggi' smell. Pileus 4 to 16 cm, at first expanded convex, then depressed, sometimes convex and umbonate, dull reddish ochre, when old becoming paler, when moist often brownish orange, partly also with a flesh-coloured or violaceous tinge. Surface in parts tomentose-pruinose, verrucose-floccose, granulose, dry. Lamellae at first yellowish white with a delicate pink tint, later yellowish ochre. Latex watery white, mild, in the throat somewhat bitter and slightly irritating. Stipe 4 to 13 × 0.5 to 4.5 cm, somewhat lighter than the pileus, pruinose-lanose. Spores 6.5 to 9 × 5.5 to 6.5 µm . . . . (+) **L.helvus**
  - **3b** Fungi with different characters, not simultaneously large and with a chicory smell
    - **4a** Pileus 5 to 11(to 18) cm, pileus and stipe orange-foxy; lamellae yellowish white, at damaged places the surrounding area flecked with rust-brown to very dark brown from the latex, edges mostly darker than the faces. Latex white, mild, with a slightly bitterish aftertaste. Flesh with a characteristic smell, especially when old, of herring. Spores subglobose, 8 to 10 × 8 to 9.5 µm . . . . . – (L.volemus; fried, a good edible fungus) –
    - **4b** Fungi with different characters

5a Taste more or less pungent. Latex remaining white, or in some cases turning rose-red, sulphur-yellow, or violet
- **6a** Pileus 6 to 20 cm, brownish olive to blackish olive, when young with a broad olive-yellow tomentose marginal zone . . . . **(R) L.necator**
- **6b** Pileus with different characters
  - **7a** Latex at first white, then becoming rose-red . . . . . **(R) L.acris** (and the related species **L.azonites**) etc.
  - **7b** Latex not becoming rose-red
    - **8a** Latex becoming sulphur-yellow . . . . . **(R) L.chrysorrheus** (and other species such as **L.citriolens, L.resimus, L.scrobiculatus**)
    - **8b** Latex not becoming sulphur-yellow
      - **9a** Margin of the pileus with a broad villous barbate or villous fimbriate zone
        - **10a** Latex turning violet . . . . . **(R) L.repraesentaneus** (and other species)
        - **10b** Latex not turning violet
          - **11a** Pileus brownish pink, flesh-coloured, pinkish white to white. Beneath birch trees . . . . . **(R) L.torminosus** (Woolly Milk Cap) (and related species)
          - **11b** Pileus differently coloured . . . . . – (Other species of varyingly assessed worth) –
      - **9b** Margin of the pileus neither barbate nor villous fimbriate
        - **12a** Lamellae distinctly distant, bright ochre-yellow, fungi growing beneath hazel. Pileus dark to light ochre-grey. Spores 7 to 8.5 × 5.5 to 6.5 µm, with blunt, scarcely isolated, verrucosities connected by irregularly reticulate ridges of varying thickness to almost striate . . . . . **(R) L.pyrogalus**
        - **12b** Lamellae not distinctly distant, and also fungi with different characters . . . . . – (Further species of varyingly assessed worth) – cf. **(R) L.pallidus**
    - **5b** Taste more or less mild
      - **13a** Growing beneath beech trees on calcareous soils. Pileus 5 to 12 cm, dirty cream, ochraceous brown to yellowish buff, often with a light flesh-coloured tint. Lamellae essentially concolorous with the pileus. Stipe somewhat paler than the pileus. Taste mild or after long chewing ultimately pungent. Spores 8 to 9 × 5.5 to 7 µm, ridged striate . . . . . **(R) L.pallidus**
      - **13b** Fungi with different characters . . . . . – (Can be eaten when cooked in small quantities with other fungi; varyingly assessed) –

1b Pileus white; latex white, remaining so or turning violet; in contact with the flesh sometimes greenish. Amyloid ornamentation of spores sometimes faint
- **14a** Latex remaining white or becoming greenish, spore ornamentation faint . . . . . – (Peppery Milk Caps, only edible after special preparation and only in small amounts) –
- **14b** Latex turning violet . . . . . – (L.controversus, edibility unknown; to be left alone because of its rarity) –

Leccinum

A few species that when cooked are good edible fungi may possibly be toxic when raw . . . . . **(R) L.aurantiacum**; **(R?) L.duriusculum**; **(R?) L.scabrum** (Brown Birch Boletus)

Lepiota

The genus does not contain any species known or valued as being edible; a few representatives, especially the small ones, are highly toxic.

Lepista

Some species are unpalatable when raw, e.g. **(R) L.nuda** and **(R) L.nebularis**. (Discard the water in which they are cooked!)

Lyophyllum

(?) L.connatum is a white fungus growing in groups or tufts; with iron(III) chloride its lamellae turn violet. It is readily distinguished from all the other edible species of the genus.

Macrolepiota

1a Fungus reddening on bruising and in the flesh
  2a Stipe not with a brown snake-like pattern (mottled)
    3a Clamp-connexions at the hyphal septa present
      4a Spores 10 to 13 × 7.5 to 9.5 µm. Base of the stipe with an almost round, marginate bulb; annulus fairly strong, infundibuliform. Occurs in parks and gardens, on compost . . . . . (?+) **M.rhacodes** var. **hortensis**
      4b Spores (8 to)9 to 12(to 15) × 6 to 7(to 8) µm. Bulb of the stipe different. Occurs in woods . . . . . – (M.rhacodes, considered to be an edible fungus) –
    3b Clamp-connexions at the hyphal septa absent. Pileus without an umbo (with an umbo, cf. M.excoriata) . . . . . (+) **M.venenata**
  2b Stipe with a brown snake-like pattern (mottled) . . . . . – (M.permixta) –
1b Fungus not reddening on bruising or in the flesh . . . . . – (Remaining species that are edible when cooked) – The Parasol Mushroom, **(R?) M.procera**, may cause poisoning when eaten raw

Megacollybia

(+) **M.platyphylla**, the only species of the genus, is poisonous.

Mycena

1a Pileus relatively large, 2 to 6 cm; stipe 0.2 to 0.7 cm broad. Pileus and stipe pink or violaceous or if the pileus white then the fungus with the smell and taste of radish. Fungi not growing on wood, but on the ground among leaf or needle litter. Cystidia and elements of the pellicle of the pileus not with numerous appendages, thus not like broom-cells. Spores relatively small, less than 10.5 × 4 µm. Fungi never laticiferous, never with a viscid stipe
  2a Lamellae not with a dark-coloured edge. Smell of radish or sweetish with cedar-wood oil components
    3a Lamellae adnexed to sinuate, never broadly adnate. Spores amyloid
      4a Smell when fresh of cedar wood or cigar-box wood, also with sweeter components; cut fruit-body with a faint radish smell. Pileus when young strongly violet to brownish violet. Spores 6 to 9(to 10) × 3.5 to 5 µm. On litter from red beech trees (exclusively?) . . **(?) M.diosma**
      4b Smell initially radish-like and remaining so
        5a Larger species. Pileus (2 to)3 to 6(to 8) cm, pink. Lamellae whitish to pale pink. Stipe white, towards the base sometimes yellowish. Exclusively in deciduous forests . . . . . (+) **M.rosea**
        5b Mostly smaller species and/or the pileus pale lilac, violaceous, bluish, whitish, white, not pink
          6a Pileus violet, less often with bluish or yellowish tints. Stipe always some kind of violet to lilac. In deciduous and coniferous forests. Spores 5 to 8.7(to 10) × (2.5 to)3 to 4 µm . . . . . (+) **M.pura** var. **pura**
          6b At least the pileus white
            7a Pileus white, stipe becoming violaceous at the latest on drying . . . . . (+) **M.pura** var. **alba**
            7b Fruit-body white in all parts; stipe also remaining white even on drying. Spores narrower than in M.pura, 5 to 7.5 × 2.5 to 3(to 3.2) µm. Mixed forests . . . . . (?) **M.subaquosa**
    3b Lamellae broadly adnate and also adnate with a decurrent tooth. Pileus up to about 2.5 cm. Radish smell faint. Spores not amyloid, 5 to 7.5 × 2.5 to 4.5 µm . . . . . (?) **M.pearsoniana** (= M.kuehneriana)
  2b Lamellae with greyish violet faces and edged with dark purple. Pileus brownish violet, 3 to 5 cm. Smell absent or faintly radish-like. Spores amyloid, 4.5 to 6(to 7) × 2.5 to 3 µm. Deciduous forests, especially beech . . . . . (+) **M.pelianthina**
1b Pileus either smaller or fungi not smelling of radish. If fungi small and smelling of radish, cf. 1a. Fungi on wood or among litter, moss, etc.; or on bare soil. Cystidia in various species like broom-cells. Spores sometimes longer than 10 µm. Some species with latex, some others with a viscid stipe . . . . . – (Remaining species) –

Omphalotus

The species of this genus are poisonous: (++) **O.illudens** and (++) **O.olearius** in the broad sense occur only in the warmer parts of Germany and are very rare in Britain.

Panaeolina

(?+) **P.foenisecii** is toxic or at least suspected of being so.

Panaeolus

The genus does not contain any recognized edible species. Most members are poisonous or are suspected of being so.

1a Pleurocystidia present, thick-walled, pigmented, fusiform, with an acute apex. Pileus greyish white with a yellowing centre, 1.5 to 4 cm, when old fissured, sub-hemispherical. Stipe concolorous with the pileus. Flesh sometimes turning slightly blue. Spores 11 to 16 × 9 to 12 µm . . . (+) **P.cyanescens**

1b Pleurocystidia absent and fungi also differing in the other characters

2a Pileus not hygrophanous and in sufficiently young and fresh specimens the marginal zone covered by the veil

3a Pileus with a reticulate pattern of raised ribs, campanulate, 2 to 3 cm, clay-brown to reddish buff . . . . . (+) **P.retirugis**

3b Pileus not with a reticulate pattern of raised ribs . . . . . – (Further species suspected of being poisonous) –

2b Pileus hygrophanous, the marginal zone not covered by the veil

4a Pileus hemispherical to expanded convex and with a brown colour

5a Stipe 6 to 10 × 0.3 to 0.5 cm. Spores 12 to 14 × 7 to 9 µm . . . . . (+) **P.subbalteatus**

5b Stipe 4 to 7 × 0.15 to 0.3 cm. Spores 9 to 14 × 6.2 to 7.5 µm . . . . . (+) **P.ater**

4b Pileus campanulate-conical or with a grey colour . . . . . (+) **P.fimicola** and other species suspected of being toxic

Paxillus

The genus does not have any edible fungi in it and should be avoided completely. The species are to be considered at least as being unpalatable and some of them also as being poisonous.

1a Stipe more or less centric. Pileus and stipe largely concolorous; surface of the stipe occasionally somewhat tomentose, but not dark brown velutinous. Growing on the ground

2a Surface of the 5 to 15 cm broad, more or less thick-fleshed, pileus not patterned with appressed scales; strongly involute marginal zone mostly tomentose and costate. Where bruised, the lamellae becoming rapidly and strongly flecked with brown. Stipe clavate or tapering slightly towards the base. Beneath various deciduous and coniferous trees, not associated with alder . . . . . (R,+) **P.involutus**

2b Surface of the 3 to 6(to 9) cm large pileus initially covered with an appressed layer of fibres which soon resolves into likewise appressed brownish olive scales; marginal zone slightly involute, soon becoming largely straight. Lamellae after lying for some time becoming somewhat flecked with reddish brown spots. Stipe tapering or attenuate towards the base. Beneath alder . . . . . (?) **P.filamentosus** (= P.rubicundulus)

1b Stipe lateral, stumpy, or absent, or very dark brown velutinous. Growing on wood . . . . . – (Remaining unpalatable species) –

Pholiota

None of the species of this genus is suitable for culinary purposes.
The dry, straw-yellow pileus and the rust-brown stipe of (?) **P.squarrosa** have erect scales. The fungus grows in clusters on the wood of deciduous and coniferous trees.

Pholiotina

The genus has no largish species considered to be edible. As some of them contain dangerously poisonous substances or are hallucinogenic, the entire genus should be avoided by collectors of edible fungi.

Pluteus

1a Cystidia thick-walled (metuloid) and provided with one to three hooks
  2a On alder, willow, or beech wood. Pileus grey or at least with grey tinges, e.g. greyish green, and with scales on the darker ones, 3 to 6cm. Spores 7 to 8.5×5 to 6µm . . . . . (+) **P.salicinus**
  2b Pileus entirely brown or white to whitish . . . . . – (Other, edible species of the genus) –
1b Cystidia thin-walled or absent . . . . . – (Rest of the genus; small species of no culinary value) –

Psilocybe

In spite of recent contributions to its taxonomy [G33, K17], the genus is not yet sufficiently well known in Europe. The following key is therefore provisional in character. The genus has no recognized edible species; those that turn blue are hallucinogenic.

1a Stipe with a fibrillose annulus or a floccose-fibrillose annular zone. Pileus (0.5 to)1 to 2.5(to 3.6)cm, conical to convex or sub-campanulate-umbonate, occasionally slightly papillate or cuspidate, hygrophanous, pale reddish brown, honey-coloured, sepia, or ochraceous. Lamellae broadly adnate. Spores (9.5 to)11 to 14(to 16)×6.5 to 8.5(to 9.5)µm. On dung . . . . . (+) **P.fimetaria**
1b Stipe not with an annulus and not with a fibrillose annular zone
  2a Mostly species turning blue and growing on manure, with lamellae that are broadly adnate; or fungi not occurring on dung or manure
    3a Pileus conico-convex, conical, or campanulate-umbonate
      4a Spores (11 to)12 to 14(to 18)µm long. Pileus cuspidate, straw-yellow to brownish yellow, olive-yellow, sometimes also with a greenish or bluish tinge . . . . . (+) **P.semilanceata**
      4b Spores shorter than 11(to 13)µm
        5a Spores (8.2 to)9.3 to 11(to 13)µm long . . . . . (+) **P.pelliculosa**
        5b Spores (6.6 to)8.5 to 9.5(to 11)µm long. Differentiation from previous species unclear . . . . . (?) **P.silvatica**
    3b Pileus in expanded specimens ultimately convex
      6a Spores (12 to)13 to 14.5(to 16.5)µm long . . . . . (?) **P.liniformans**
      6b Spores (8.5 to)10 to 12(to 13.5)µm long
        7a Pileus convex with mostly a small umbo; pale straw-yellow. In fields. Spores 10 to 12×6.2 to 7.8µm . . . . . (?) **P.callosa**
        7b Pileus without an umbo; when soaked hazel, yellowish ochre, greyish ochre, dun
          8a Cheilocystidia scarce, 12 to 15×5 to 8µm. Spores 9 to 12.5×6.5 to 7(to 8)µm. Pileus 2 to 4cm . . . . . (+) **P.cyanescens** (= P.bohemica)
          8b Cheilocystidia numerous, 25 to 35×6.5 to 8.5(to 9)µm. Spores 9 to 11×5.5 to 6.5(to 7)µm. Pileus 1.5 to 2.5cm . . . . . (+) **P.serbica** (probably identical with the previous species)
  2b Species growing on dung or manured soil, with lamellae that are not broadly adnate . . . . . – (Species with unknown effects) –

Russula

1a Taste pungent, in some cases burningly pungent, or with a distinctly unpleasant smell . . . . . (R,+?) **R.emetica** and other poisonous or unpalatable species
1b Taste mild
  2a Spore mass not pure white, rather cream to ochre or yellow
    3a Pileus smooth, green, greyish green without any trace of violet. Flesh with iron(II) sulphate turns reddish grey. Spore mass light cream. Spores (5.7 to)6 to 10×(4.7 to)5 to 6.7µm, amyloid verrucosities occasionally connected by amyloid bars. Cortical layer with dermatocystidia turning blue-black in sulphovanillin. Preferentially beneath birch trees . . . . . (R) **R.aeruginea**
    3b Pileus not simultaneously smooth and some kind of green, or the colour of the spore mass otherwise, or deviating in the other characters
      4a Spore mass deep yellow or yellow. Flesh not turning black or grey
        5a Cortical layer without dermatocystidia that turn black in sulphovanillin and at the same time without encrusted primordial hyphae
          6a Spores with isolated spines, thin connecting lines between the spines rare; (7.7 to)8 to 10(to 12)×(6.7 to)7 to 8.5(to 10)µm. Pileus large, (6 to)9 to 16(to 20)cm, its surface mostly rimose-granulose in concentric zones, rarely smooth. Stipe entirely or in places pink . . . . . (R) **R.olivacea**
          6b Spores with reticulate ridges on the surface, or smaller . . . . . – (Various species, edible at least when cooked) –

5b Cortical layer with dermatocystidia that turn black in sulphovanillin or with encrusted primordial hyphae
7a Cortical layer with dermatocystidia that turn black in sulphovanillin
8a Pileus not with pure red or orange colours. Large species. Flesh not turning green with iron(II) sulphate and the smell not herring-like
9a Species of coniferous forests. Pileus variously coloured, but always some kind of brown, tawny, with darker marbling or also with olive-coloured spots. Hairs of cortical layer long, towards the ends gradually becoming filamentous, 2.2 to 4 µm. The dermatocystidia react with sulphovanillin and in addition have droplets or lumps as deposits on the cell walls. Spores (7.7 to)8.2 to 11 × 7 to 9.2 µm, with stout, isolated spines . . . . . **(R) R.integra**
9b Species of deciduous forests, and not showing all the other characters indicated
10a Spores with pectinate-reticulate ornamentation, 6.7 to 8.5(to 9.5) × 5.7 to 6.7(to 7.2) µm. Fungus, especially when old, fairly soft-fleshed. Pileus normally violet or green, more rarely with purple tones. Pellicle of the pileus removable. Cortical layer without encrusted primordial hyphae . . . . . **(R) R.romellii**
10b Fungi with different characters. Cortical layer with or without encrusted primordial hyphae . . . . . – (Other species) –
8b Pileus with red to orange colours or small species or flesh turning green with iron(II) sulphate . . – (Other species) –
7b Cortical layer not with dermatocystidia that turn black in sulphovanillin . . . . . – (Other species) –
4b Spore mass cream, not yellow . . . . . – (Other species) –
2b Spore mass pure white . . . . . – (Other species) –

Stropharia

1a With distinct green or blue-green (glaucous) colours
2a Pileus 3 to 8 cm
3a Cheilocystidia clavate. Spores 7 to 9 × 4 to 5 µm . . . . . – (S.aeruginosa; edible) –
3b Cheilocystidia fusiform, lageniform, not clavate. Spores 8 to 10 × 4.4 to 5.6 µm . . . . . – (S.cyanea; edible) –
2b Pileus 2 to 3 cm, only with a light bluish or greenish blue flush. Spores 8 × 4.5 to 5 µm . . . . . – (S.albocyanea; unknown worth) –
1b Without green or blue-green (glaucous) colours
4a Only the pileus viscid, or also this dry
5a Pileus slightly glutinous to more or less dry, pale dun, tawny, to deep reddish brown, or vinaceous, 5 to 12(to 17) cm. Lamellae soon greyish violet, then violet-brown. Stipe below the annulus naked, 15 to 20 × 3 to 4 cm, white, at the base with mycelial strands. Spores 11 to 13(to 18) × 7.5 to 8(to 10) µm. Outside forests on earth, remains of straw, partly in maize fields . . . . . – (S.rugosoannulata and the closely related S.eximia; although considered edible, the former can sometimes cause mild gastrointestinal symptoms) –
5b Fungus with different characters, e.g. the stipe below the annulus squamose
6a Stipe white, squamose below the annulus, naked only when old. Pileus glutinous, 4 to 10(to 15) cm, ivory to chestnut, often covered with the veil. Lamellae whitish, then smoky-grey violaceous. Spores 10.5 to 13 × 5.5 to 7 µm. Coniferous forests, on or near decayed wood . . . . . **(?) S.hornemannii**
6b Fungus with different characters. If growing on wood, pileus orange, reddish brown, to brick-red, or pileus squamose or squamulose
7a Pileus with concentric scales, ochre-yellow to wood-yellow, convex, 2 to 5 cm; fungus on wood or wood remains. Stipe above the annulus white, below it squamose. Spores 11 to 14 × 6 to 8 µm . . . . . **(?) S.squamosa**
7b Fungus with different characters. Growing on wood or not
8a Stipe white, with an evanescent annulus not striate on its upper surface. Pileus through slime first violet-grey, then yellowish or greyish yellow, campanulate to umbilicate, 3 to 5(to 7) cm. Spores 7 to 8.5 × 4.5 to 6 µm. Grassy or mossy edges along roads, edges of forests, not growing on wood . . . . . **(?) S.inuncta**
8b Stipe white, with a clearly developed annulus striate on its upper surface. Pileus ochre to yellow, rather pale, convex, 2 to 5(to 6) cm. Lamellae purple-grey with a light edge. Spores 7 to 9 × 4 to 5 µm. Meadows, pastures, and fields . . . . . **(?) S.coronilla** and other, possibly not harmless, species
4b Pileus and stipe viscid, on dung or manured soil
9a Pileus hemispherical . . . . . **(?) S.semiglobata** (Dung Roundhead)
9b Pileus conico-campanulate . . . . . – (S.luteo-nitens) –

Tricholoma

1a Clamp-connexions regularly present at the hyphal septa

2a Pileus 4 to 12cm, grey to dun, covered with broad, almost imbricate, scales. Lamellae whitish. Stipe whitish, weeping at the apex of fresh specimens, 4 to 10 × 1.5 to 2.5cm. Smell farinaceous. Spores 8 to 10 × 6 to 7µm. Coniferous and deciduous forests . (++) **T.pardinum**

2b Pileus 5 to 12cm, pileus and stipe white, copper-red, greyish green, or dun. Lamellae whitish to waxy yellowish grey, thickish, distant. Stipe 5 to 10 × 1 to 3cm, often ventricose. Fruit-body when old becoming more or less copper-red or flesh-coloured in places. Spores 5 to 6 × 3.5 to 4µm. Deciduous and coniferous forests . . . . . (+) **T.saponaceum**

1b Clamp-connexions absent or rare at the hyphal septa, or fungi with characters different from those under 2a and 2b

3a Pileus with grey to blackish colours and at the same time a burning pungent taste or first bitter then pungent

4a Stipe when young naked and lighter coloured than the pileus. Pileus often cuspidate, grey sericeous, naked, glabrous, innately fibrillose, shiny, 3 to 7cm. Lamellae white. Stipe greyish white, 6 to 9 × 0.5 to 1cm. Taste pungent immediately. Spores 6 to 7 × 5 to 6µm. Coniferous forests . . . . . (+) **T.virgatum**

4b Stipe and pileus concolorous and/or growing in deciduous forests, or lamellae grey, ash grey-pink, beige-pink . . . . . – (Species related to the previous one which must no doubt be suspected of being poisonous) –

3b Taste mild

5a With yellow colour at least in the lamellae or the flesh, or in some cases only in the pileus

6a Lamellae thickish and distant. Smell like coal-gas

7a Pileus yellow to foxy or olivaceous, 3 to 7cm. Lamellae, stipe, and flesh sulphur-yellow. Spores 9 to 12 × 5 to 6µm . . . . . (+) **T.sulphureum**

7b Pileus purple-brown . . . . . (+) **T.bufonium**

6b Lamellae not thickish and distant. Smell not like coal gas, but nevertheless sometimes unpleasant

8a Pileus white, yellow-green, yellow, and partly radially fibrillose, without grey or black colours

9a Lamellae when young white or whitish. Pileus radially fibrillose, rarely squamulose, yellow-green. Centre often browner, 3 to 8cm. Edge of the lamellae not particularly coarsely serrate. Stipe white, ventricose, 5 to 8 × 1 to 3cm. Smell and taste farinaceous. Mild to bitterish. Spores 5 to 6 × 4 to 5µm . . . . . (+) **T.sejunctum** (incl. **T.zvarae**)

9b Either the lamellae already yellow when young or their edges conspicuously serrate or the pileus not innately fibrillose-striate or the stipe not white

10a Pileus yellow, brownish yellow . . . . . – (Various edible species) –

10b Pileus white, whitish, ochre . . . . . – (Various species, partly untried or of unknown edibility) –

8b Pileus not white, yellow, yellowish green

11a Pileus reddish brown, somewhat darker squamose or fibrillose to naked, 4 to 8cm, moist glutinous-viscid. Stipe concolorous, fibrillose. Lamellae and flesh of the stipe yellowish. Lamellae when old edged with rust-coloured spots. Smell farinaceous. Taste mild. Spores 5 to 7 × 3 to 4.5µm. Especially near birch trees . . . . . (R) **T.flavobrunneum**

11b Pileus grey to black, or fungi not with the above-mentioned properties . . . . . – (Various species, some edible and some of unknown utility) –

5b No yellow colours in the flesh, pileus, or lamellae

12a Pileus with reddish or brown colours, reddish brown, tawny, dark brown, orange-brown, foxy-red

13a Stipe with a membranous, membranous-lanose, often ragged annulus

14a Pileus foxy-red, brownish orange. Marginal zone almost cinnabar, darker fibrillose-squamose, 5 to 9(to 10)cm, the margin with velar remains. Stipe tapering towards the base, foxy-brown, fibrillose-squamose as far as the lanose annulus, 6 to 8 × 1.0 to 1.5cm. Spores 3 to 4.5 × 2.3 to 3µm, shortly ellipsoidal. Coniferous forests, especially pine trees . . . . (+) **T.focale**

14b Pileus either chestnut, darker reddish brown, or tawny to chestnut or almost porphyry-brown innately squamose on a whitish ivory ground . . . . . – (Various species, some edible and some of unknown utility) –

13b Stipe at most with a fibrous veil-like annulus, with an annular zone, or entirely without such structures

15a Pileus when moist glutinous to viscid

16a Apex of the stipe essentially concolorous with the rest of the stipe, sometimes also lighter, but not sharply delimited

17a Pileus reddish brown (cocoa), more or less glutinous-viscid, convex, 5 to 12cm. Marginal zone of the pileus mostly guttate. Stipe pale, often bulbous, 4 to 10 × 1.5 to 3cm. Smell and taste farinaceous. Spores 4 to 6 × 2.5 to 3µm. Coniferous forests . . . . . (+) **T.pessundatum**

17b Fungi with different characters

18a In deciduous forests, but not near poplars. Pileus reddish brown, often with an olivaceous tinge, 4 to 8cm. Stipe brownish red with a pale centre. Lamellae at first white, then rust-spotted. Smell absent (if farinaceous, cf. T.ustaloides). Taste slightly bitter. Spores 5.5 to 7 × 4 to 5µm . . . . . (+) **T.ustale**

18b In coniferous forests or near poplars

19a Near poplars often outside the forest . . . . . – (T.populinum) –

19b In coniferous forests, mostly near pine trees

20a Spores 5 × 3 µm. Flesh not reddening. Pileus 5 to 6(to 7) cm, slightly obtusely conical or slightly umbonate, innately fibrillose, reddish brown. Stipe when young whitish, then reddish brown, somewhat squamose, towards the base darker. Taste slightly farinaceous . . . . . (+) **T.albobrunneum**

20b Spores 6 to 7 × 3.5 to 4 µm. Flesh always reddening, bitterish . . . . . – (T.stans; presumably like the previous species to be considered poisonous) –

16b Apex of the stipe clearly delimited and white . . . . . – (Various species including some of doubtful edibility. Owing to the possible confusion with poisonous species, great care is required) –

15b Pileus dry and often fibrillose-squamose-lanose . . . . . – (T.vaccinum and T.imbricatum; edible) –

12b Pileus with other colours . . – (Some edible species, but mostly not valued for culinary purposes) – here also (?) **T.josserandii**

Xerocomus

Most species are considered edible. The Bay Boletus, (?) **Xerocomus badius**, is said to be poisonous when eaten raw.

Chapter 5

Molds That Cause Human Disease

This chapter will concern itself with fungi that can infect humans and reproduce in human tissue. The following chapters will cover the molds that produce toxins that are harmful to humans.

This chapter will be divided into four parts.

1. Overview of fungal disease
2. Culture media and colony growth
3. Weapons Design Considerations
4. Charts, Tables, and Photograph Supplement

1. Overview of Fungal Disease

Fungi usually cause disease in one of three broad categories. The first is subcutaneous (skin/dermal) infection, second is superficial where the mold is able to grow opportunistically and only in a limited amount and the third is a deep-seated mycosis. This last category always includes pathogenic molds that can produce life-threatening disease.

When doctor's patients have fungal infections, their complaints range from low fever, night sweats, weight loss, lassitude, easily fatigued, cough and chest pain to the common itch of athlete's foot. The deep-seated infections can often mimic tuberculosis, brucellosis, syphilis and other infectious diseases.

The respiratory tract is the primary route of infection in which spore-saturated dust particles are inhaled and the mold encounters the ideal conditions for growth. Cough, with or without sputum, flu-like symptoms, chest pain and tachypnea are common in these infections. Cavity formation in the lung is rare but calcified nodules from chronic healed forms of this type of disease are more common. Some spores such as Asperigillus species and their products often produce allergic bronchopulmonary disease. Old tuberculosis cavities also become colonized with fungal species like Asperigillus or Zygomycetes.

Skin infection by pathogenic dimorphic (fungi that grow by budding like yeast at 37 C but produce hyphae like molds at 25 C) fungi is rare but can occur as a secondary infection after skin is inoculated with contaminated soil or vegetative matter. This usually occurs at injury sites and results in non-healing ulcers, pustules, or even draining sinuses. Scaling and itching lesions of athletes foot (caused by tinea capitis, tinea barbae, etc.) and typical ringworm infections are caused by the superficial dermatophytic fungi. Since these fungi cause itching and this disrupts the protective skin layers, it offers the potential

of a significant enhancement of various biological weapons. This will be covered later on in more detail. The dermatophytic fungi may also cause hair and nail infections.

Meningitis can be caused by Cryptococcus neoformans and brain abscesses by members of the Zygomycetes group. These infections can be insidious or abrupt at their onset and include headaches, vertigo, vomiting, memory lapses, and sometimes seizures. In advanced forms they can also cause hallucinations, drowsiness and coma.

When mycotic infections spread beyond a single organ, they can cause many symptoms relating to the organs that they have spread to and these can be quite serious such as Addisons disease (adrenal glands).

The eyes and ears can also become infected with specific organisms. Asperigillus niger is common in “swimmers ear”. Some deeply penetrate the sinuses such as Actinomycetes israeli and cause lumpy jaw and thorax and abdomen infections.

When fungi invade the tissues, a variety of inflammatory reactions are observed. This results in large-scale production in the body of leukocytes that form abscesses at the infection site. Asperigillus fumigatus and Zygomycetes (Phycomycetes) often cause necrotizing inflammation with damage to adjacent organs and tissues due to their propensity to directly invade and thrombose blood vessels which cuts off the blood supply to the tissues involved and results in cell death.

When doctors examine patients and then samples are analyzed in the lab, the fungal spores and hyphae are sometimes missed because they appear distorted in tissues. Usually two structures can always be distinguished, a mycelial form with the filamentous hyphae or pseudohyphae, and a yeast form in which only yeast type cells can be observed. The fungi that produce hyphae in tissues can be presumptively identified by observing

1. The breadth of the hyphael strands
2. The presence or absence of septa
3. The presence or absence of a brown pigmentation, which indicates it, is a member of the dematiacious (dark) group of fungi.

Charts and photos of the various groups of fungi are presented at the end of this chapter to assist in field identification.

The obvious way to obtain pathogenic fungi that are known infectious agents in humans is to go to a hospital which specializes in treating mycotic infections and take various samples from the air collectors, rooms, and trash and disposal areas (especially the lab). Many of these species are usually not regulated and can be ordered from the ATCC. Many of these also exist in nature and can easily be cultured and identified in private collections by those skilled in the art. Even the recovery of athlete’s foot is quite easy since most people have the infection in one form or another. Skin scrapings are the easiest way to recover the spores.

Dimorphic fungi can be identified positively by growing the organism in a blood based culture medium (SDA with 5-10% blood) at 37 C. This is after the filamentous form has been grown at 25 C and then transferred to the blood based media. It may take several transfers to fresh media as soon as growth is observed to eventually yield the parasitic form of the converted fungi. A small amount of the filamentous fungi can be placed in suspension and then injected intraperitoneally (in the abdomen cavity) into white mice. Usually, the parasitic forms will infect the liver, spleen and the injection site tissues. This is also a method for improving on the parasitic ability of the fungi and often converts dimorphic fungi from the saprobic to the parasitic form.

2. Culture Media and Colony Growth

As mentioned in the previous chapter, Sabarouds Dextrose Agar is the media of choice in growing almost all fungal specimens used in medical laboratories. Many modern labs prefer the formula as follows-

Dextrose	40g	
Peptone	10g	(see Volume 6-1 for preparing homemade peptone)
Agar	15g	
Water	1,000ml	

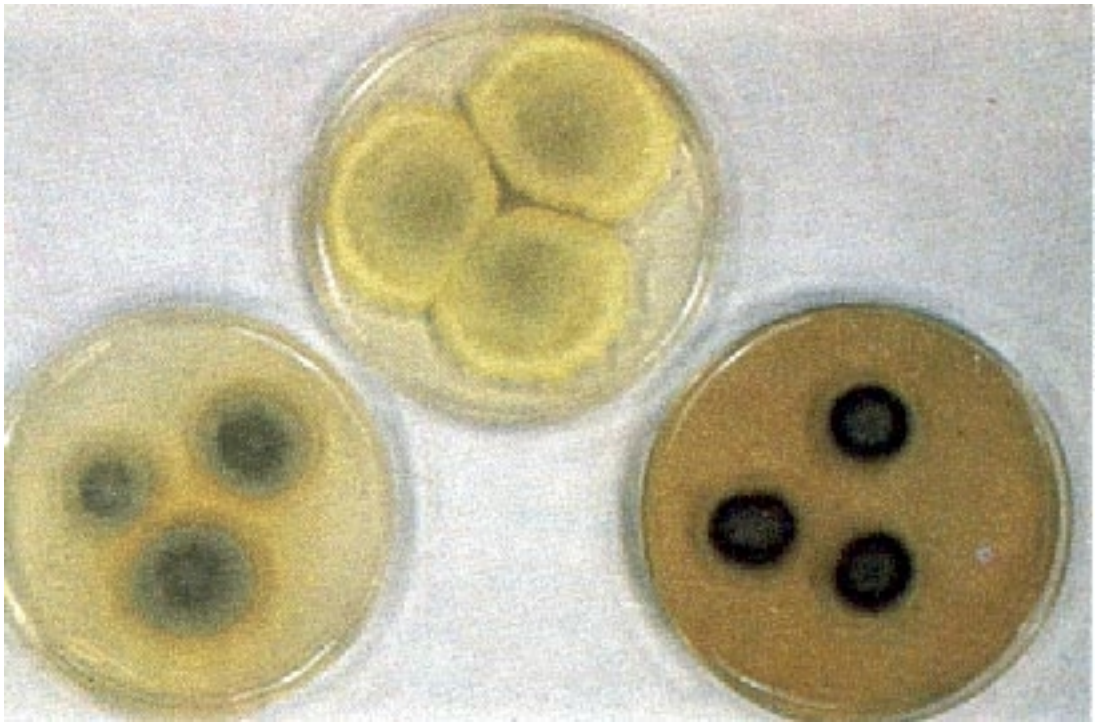
Other media that we have described can also be used. In order to prevent undesired bacteria or other organisms from growing, antibiotic such as chlortetracycline (20mcg), gentamycin (5 mcg), penicillin and streptomycin as well as others may be used. All these can be obtained at any farm supply store. The author has even used Neosporin (from Wal Mart) and smeared it on the surface of the plate to inhibit bacteria. Blood can be added to enrich these media but the blood usually inhibits sporulation. The inoculated media can be incubated at both 25 C and then later at 37 C to convert a suspected dimorphic form to the yeast phase.

Usually a mature colony develops within 5 days. The dimorphic molds can take up to 2 weeks or more to fully develop. Some rapidly growing molds produce brightly colored spores, which yields a distinct surface pigmentation. The dimorphic molds never produce pastel hues and are almost always white, gray or brown. They may produce a water-soluble pigment that diffuses into the agar and this bright color can only be easily seen by turning the plate on its side so that the bottom can be observed. Usually they produce a black to brownish discoloration while other molds may produce bright water-soluble pigments.

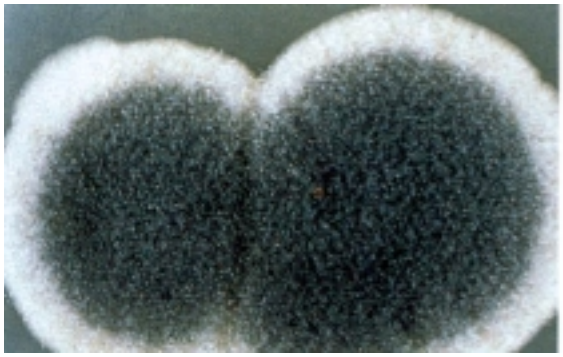
Asperigillus Molds

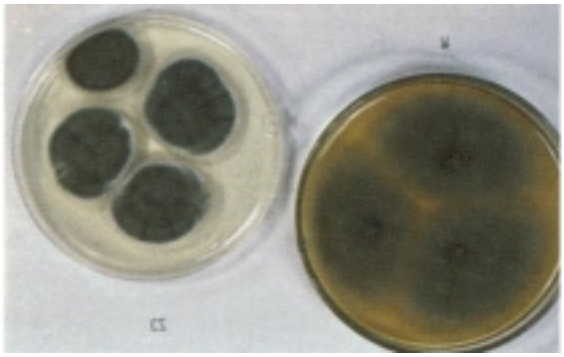
There are over 700 species of Asperigillus and three cause most of the disease encountered in human medicine. They also produce some of the deadliest cancer causing toxins known to man. The toxins will be covered in the next chapter.

The same Asperigillus species inoculated simultaneously and grown on SDA (lower left), 20% maltose agar (lower right), and Czapeks Agar (upper center). The colony appearance and growth rate can be much different on different media as this photo clearly shows.

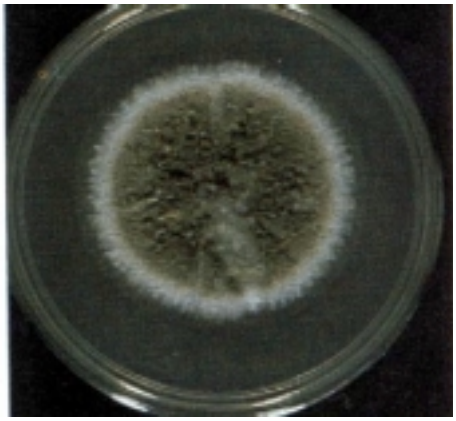


Asperigillus fumigatis produce green, green brown, or green blue colonies. Rugal folds can also be seen in some strains. They also often produce a white apron at the colony edge where growth is rapid and the black pigmented spores are produced in the mature areas behind. A comparison of growth is given on Czapeks and Maltose agar.





Different colonies of Asperigillus flavus usually appear yellow but can turn green on some media –

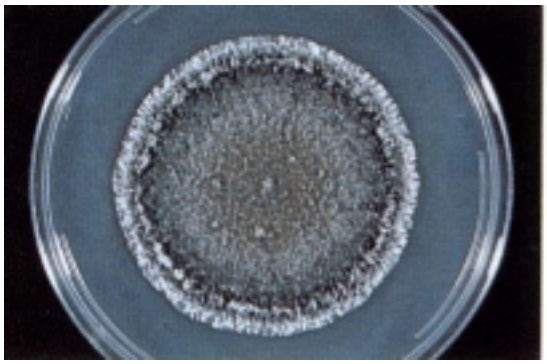


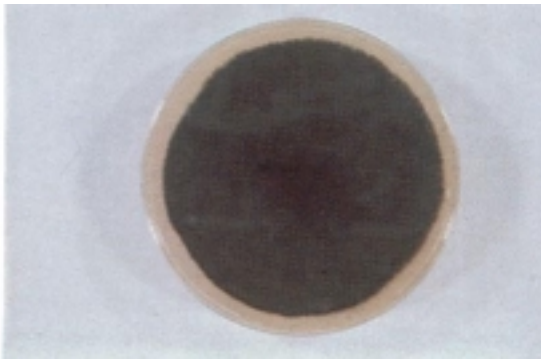
Asperigillus niger yields a dense salt and pepper effect due to the large number of black spores produced and mixed with its white hyphae. The back side of the plate is never black so this distinguishes it from a dematiacious mold.

The Dematiaceous Molds

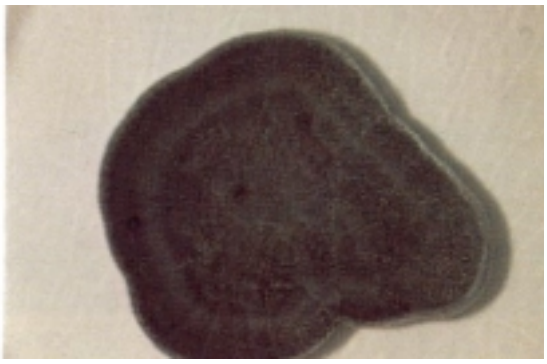
The dark (dematiaceous) molds usually develop darkmgreen, brown or black colonies with dark pigmentation on the reverse surface of the plate. Most mature within 5 days but some of the most pathogenic can take up to two weeks or more (mycetomas and chromomycosis). The following were all grown on SDA.

A colony of helminthsporium with a black surface mycelium and a deep black reverse side –

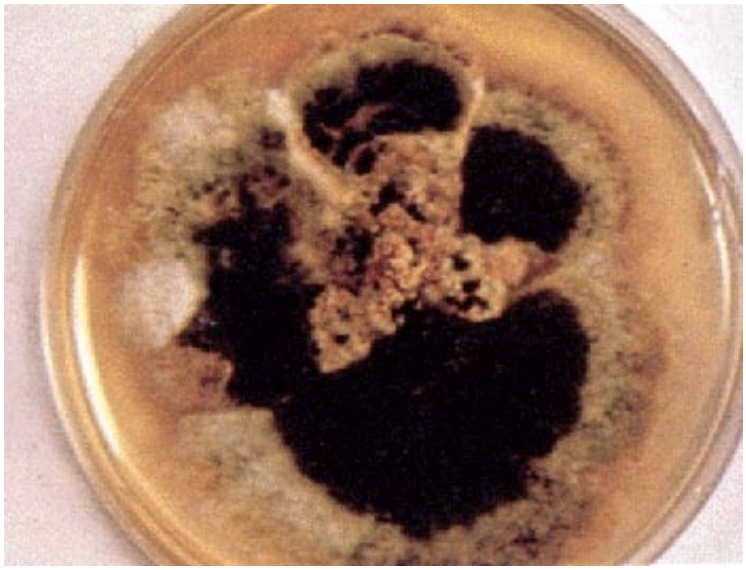




A rugose, granular olive green Cladosporium species commonly found is shown on the left. The one on the left is saprobic, the one on the right is Cladosporium carrionii, a slower growing pathogenic mold that causes chromomycosis.



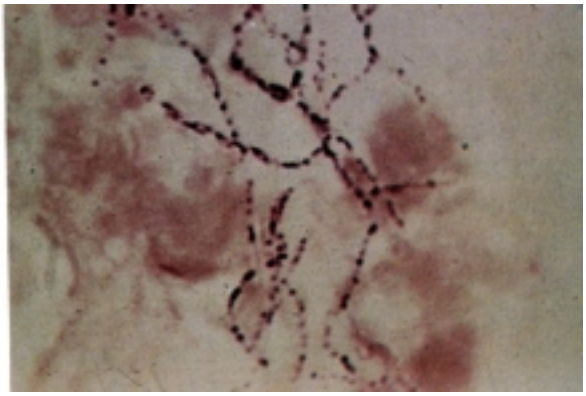
Epicoccum species with yellow, orange and black colors in different parts of the mycelium –



Flat yeast like colony with a late growth of centrally located low, white mycelium is Aureobasidium pullulans –

Actonmycetes

Nocardia asteroides on SDA agar, yields a wart-like brittle yellow colony. Most variants produce yellow or orange pigmentation but some are chalky-white. It also has a pungent earthy odor. The microscope photo on right shows its characteristic filaments –

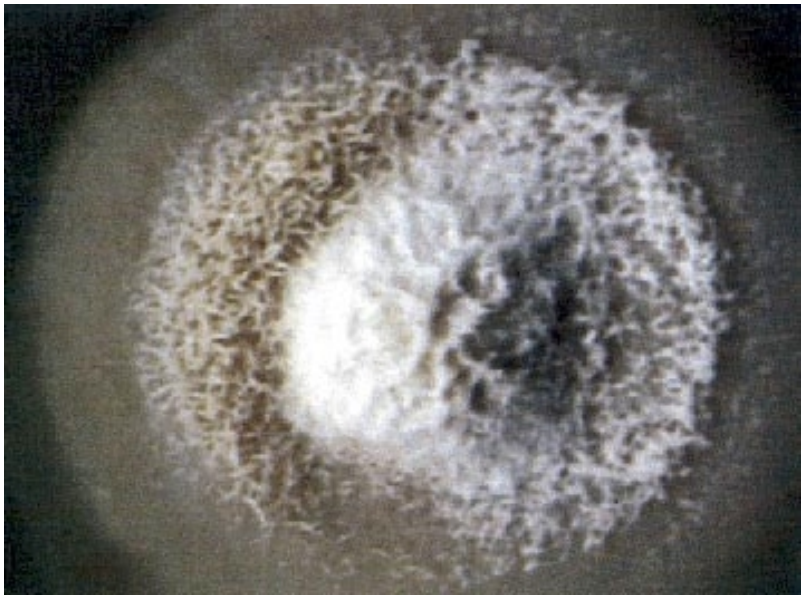


A brittle, folded, chalky-white Streptomyces. Most strains are grey or white while some are yellow. -

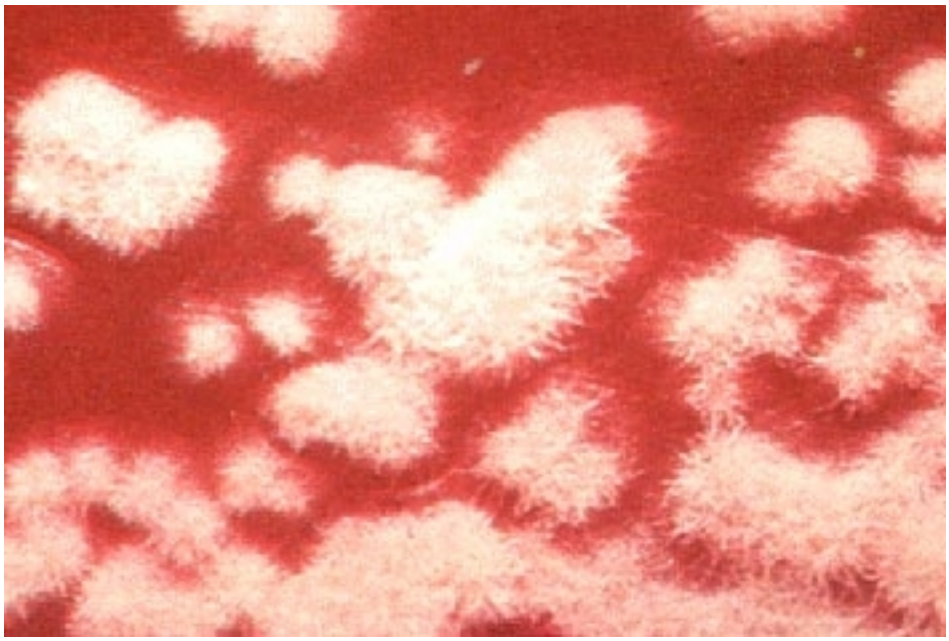
Dimorphic Fungi are called this because they grow like other molds pdoucing hyphae and mycelium at room temperature (25C) but grow like yeast at 37 C (body temperature) and are pathogenic. They are the cause of the deep seated mycoses.

SDA agar with a cottony white mold Coccidioides immitis. If growth is slow or delayed (5-10 days) care must be taken. This form is highly infectious and deadly making it an excellent weapon base.

Histoplasma capsulatum with a delicate, silky mycelium. The colony turns gray or tan at maturity and is visible in this photo in the center –

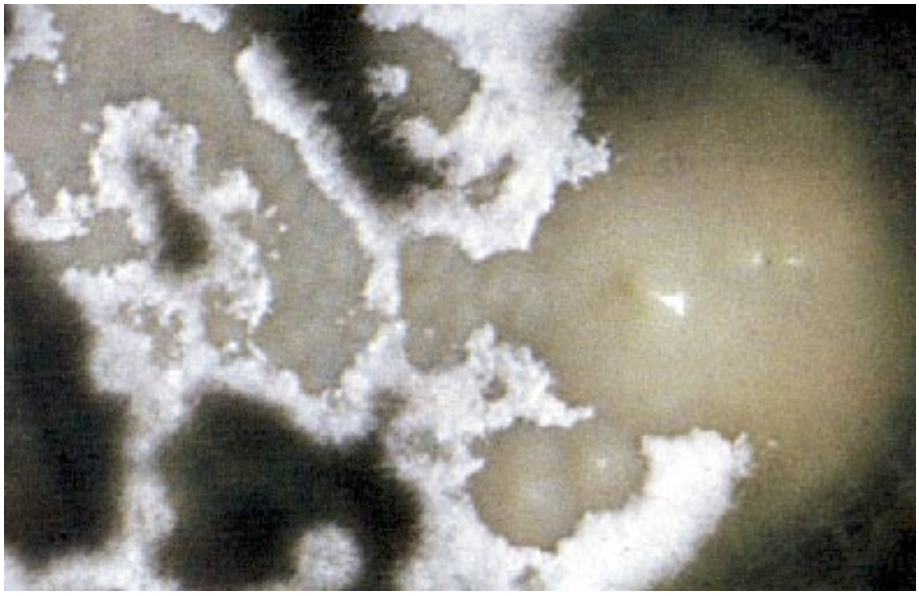


Blastomyces dermatitidis showing both yeast and fluffy white mycelium on top of the incomplete conversion to the yeast.



The same yeast on SDA agar showing both forms (yeast in the center) –

A dimorphic mold showing the “prickly” stage of yeast conversion which may be seen in both B. dermatitidis and H capsulatum –



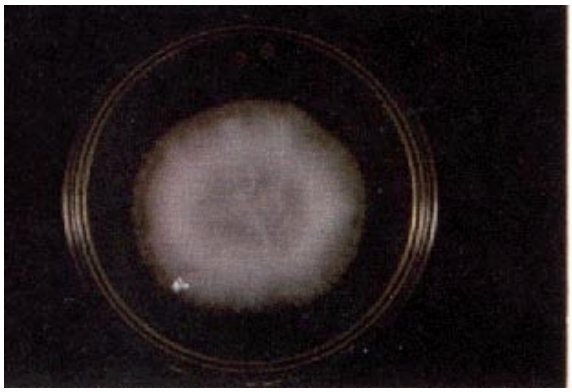
The yeast form of Sporothrix scheckii incubated at 37 C –

Dermatophytic Molds have considerable variation in strains and appearance on different media that it is difficult to identify them solely by culturing.

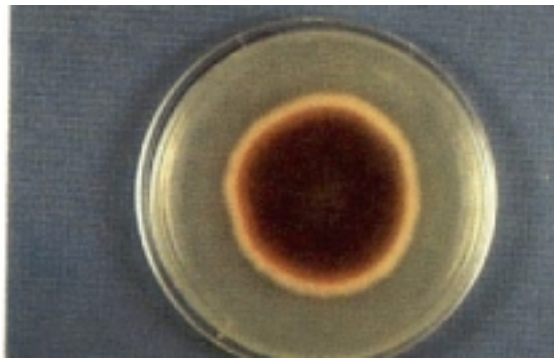
Microsporum canis with yellow-orange pigmentation. The lemon yellow apron at the margin or colony edge aids in identification –

Microsporum gypseum with a granular surface and cinnamon brown pigmentation of the dense spores produced at the center behind the fluffy white margin –

Granular and fluffy colonies of Trichophyton mentagrophytes –



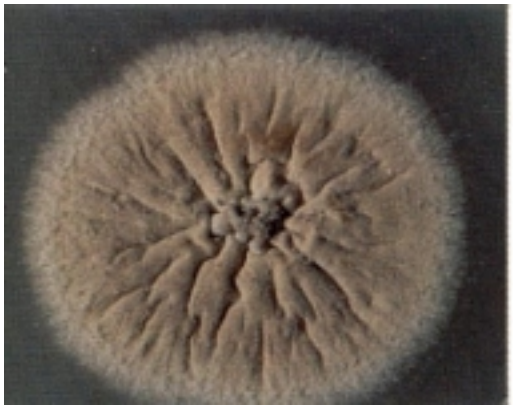
Trichophyton rubrum can also produce fluffy and granular types of growth. The plate is flipped over so the deep red pigment on the underside is visible and this is a characteristic when growing on corn meal agar –



Hyaline Molds produce mycelium with transparent (under the microscope) hyphae without dark pigmentation. They usually grow fast and mature in 3-7 days and develop a variety of colors because of the different pigmented spores that they produce. Rarely, they my cause mycotic disease in compromised humans and are most often contaminants in the laboratory.

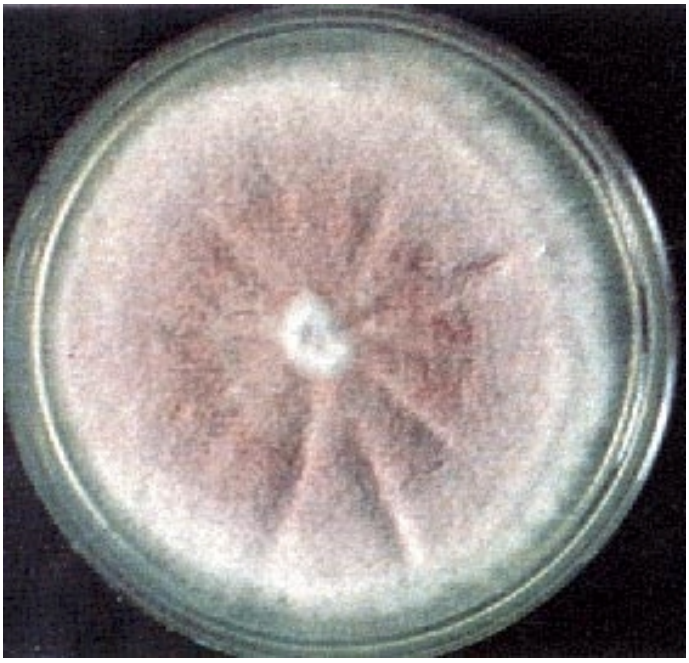
Penicillium species, usually some shade of green with a few brown or yellow variants. The surface of the colonies are granular due to the dense population of spores and radial rugal folds at the margin –

Different forms of Scopulariopsis species that always produce a shade of buff or brown. The surface is very granular from dense spore production and irregular rugal folds are often produced –



Cephalosporium species can produce light green, blue, and yellow pastel's with off white variations as seen in this photo. The aerial mycelium is delicate, low, flat and appear almost yeast-like –

Fusarium species with the classic fluffy mycelium and deep pigmentation. It can be rose red to lavender to deep purple. They cause mycotic keratitis in humans and produce some of the most potent toxins which will be covered in the next chapter –



3. Weapons Design Considerations

With the exception of only a few species, most molds are not directly infectious or contagious. The few species that are infectious are not contagious and after their presence diminishes in the target area it is safe for the user to enter and work there. In most cases, molds are opportunists and wait for a host with a compromised immune system that can't fight them off or an injury such as the tuberculosis cavities or open wounds with cut off blood supplies. These conditions create possible infectious opportunities.

In warfare, the organisms by themselves, in order to be able to infect and be effective on a battlefield, must be enhanced. Several enhancements have already been published in this series and more will appear in future volumes, however we will list and explain a few now, so the principles are well understood.

1. The addition of poison ivy and related irritants will cause scratching by the target an thereby produce a mechanical means of breaking the skin barrier and self inoculating targets with subcutaneous infective agents. Many fungi can cause subsequent infection once under the dry surface layers of the skin and these can be combined with other organisms such as various bacteria for synergistic infectious processes.

2. Mixing the fungi into a carrier such as diatomaceous earth (single cell silica organisms) or fullers earth. These single cell organisms have shapes like seashells. They are very tiny and when breathed in can reach the tiniest areas of the lungs. Their shapes make some of them hard or impossible to expel and they remain in the lungs. For most materials, the bodies defenses consume and break down materials that are not coughed up. The silica is completely immune to the defenses and provide long term safe harbor for biological weapons on the insides of their structures. When germinating proteins are added (anthrax-to improve germination) or vitamins which aid in growth of hyphae, the ability of the organism to grow where it once would not has become possible. The same holds true for other carriers such as finely ground asbestos fiber, or various types of clay.

3. Providing food for growth during dispersal also initiates growth and the organism enters it growth phase on tiny particles that are dispersed by the wind. Since all the growth requirements are met and they are growing, there is no need for germination once they eneter a part of the body they can infect (usually the lungs). If water (gelled, hydrated, etc) is part of the mix, then the organism can, for a short period, grow directly on human skin. This can increase potential infections by many orders of magnitude. It takes 5000 anthrax spores on average to initiate infection in primates. A single growing cell can produce this in a nasal cavity or on an am in a few hours and each of

these can act as seeds for new infections. It usually takes 10,000,000 salmonella cells to initiate infection when swallowed. This amount can easily be grown on a single speck of dust, inhaled, coughed up and swallowed, and then infect. Almost any organism has a limit where it can infect if the numbers are sufficiently large. US army tests in which supposedly harmless bacteria have been sprayed have resulted in hospitalizations and deaths on urban populations in the US. The principle here is not the organism. The principle is numbers. Feeding the organisms so they are already growing and increasing their numbers during dispersal is one way to improve a weapons potential.

4. The advent of a new kind of weapon has been postulated by this author. I call it the “Multiplier Effect Weapon”. It has several properties that can take advantage of the first three items listed here, and be safe for an operative to use. In some forms its production will not even require the user to have any biological education whatsoever. There are many permutations of this concept but a couple will be mentioned here.

    - Culture media can be prepared in solid form and mixed with the desired organisms and enhancements. This can then be distributed into the target area by a third party or protected operator. The mix germinates in the lungs, stomach eyes or other area of a human body that meets the moisture requirement to initiate growth.
    - A semi solid can be used and mixed into the formula with or without carrier. The water is already there so the mix begins to grow immediately. The mix can include egg white or surfactant to make it sticky so that aerosols are not produced until the media has already been distributed like toothpaste. This avoids infection of the operator. As the water is used up and the batch dries, it turns to powders and gradually releases it massive cell numbers into the surrounding area with the background clouds. This is useful in avoiding the modern ultraviolet and infrared detectors developed by the wealthy nations military forces. It also allows the covert and safe release of the weapon into or upwind of a target area.
    - A liquid can also be used. The inoculum can be soil, manure, or other prepared source. As it grows and dries, it thickens and becomes usable like the above illustration. If the user is a novice, the soil or manure can often provide at least a partial toxic growth. In these case, the medium is adjusted with baking soda or powdered limestone (to increase carbon for preferential anthrax growth or toxin production). It can have added vitamins and other specific ingredients for improved germination or toxin growth such as the aflatoxins to be discussed in the next chapter.

5. Charts Tables and photograph Supplement

Presumptive Identification of Fungi Based on Direct Microscopic Examination of Material from Clinical Specimens

Direct Microscopic Observations	*Presumptive Identification*
Hyphae relatively small (6–10 μ.) and regular in size, dichotomously branching at 45 degree angles with distinct cross septa	Aspergillus species
Hyphae irregular in size, ranging from 6 to 50 μ. ribbonlike, and devoid of septa	Zygomycetes (Phycomycetes) species Rhizopus-Mucor-Absidia
Hyphae small (2–3 μ.) and regular, some branching, with rectangular arthrospores sometimes seen; found only in skin, nail scrapings, and hair	Dermatophyte group: Microsporum species Trichophyton species Epidermophyton species
Delicate branching filaments (1 μ. or less in diameter), often contained within "sulfur granules"; gram-positive in Gram's stain. Species of Nocardia are partially acid-fast.	Actinomycetes group: Actinomyces species Nocardia species Streptomyces species
Hyphae, distinct points of constriction simulating link sausages (pseudohyphae), with budding yeast forms (blastospores) often seen	Candida species
Yeast forms, cells spherical and irregular in size (6–15 μ.), classically with a thick polysaccharide capsule (not all cells are encapsulated), with one or more buds attached by a narrow constriction	*Cryptococcus neoformans* Cryptococcus species nonencapsulated
Yeast forms, large (8–15 μ.), with cells appearing to have a thick, double-contoured wall, with a single bud attached by a broad base	*Blastomyces dermatitidis*
Large, irregularly sized (10–50 μ.), thick-walled spherules, many of which contain small (2–4 μ.), round endospores	*Coccidioides immitis*

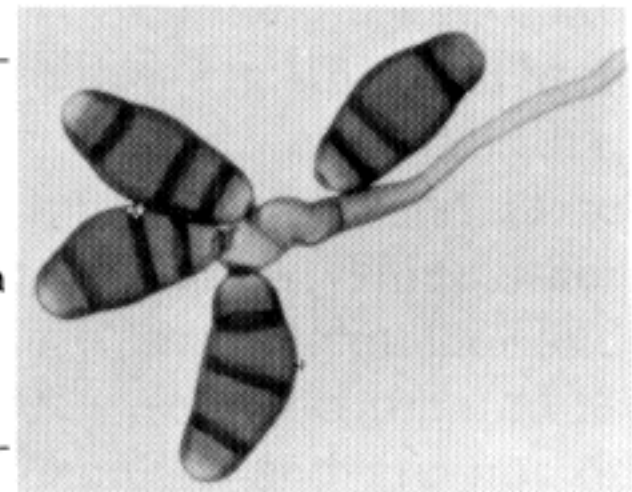
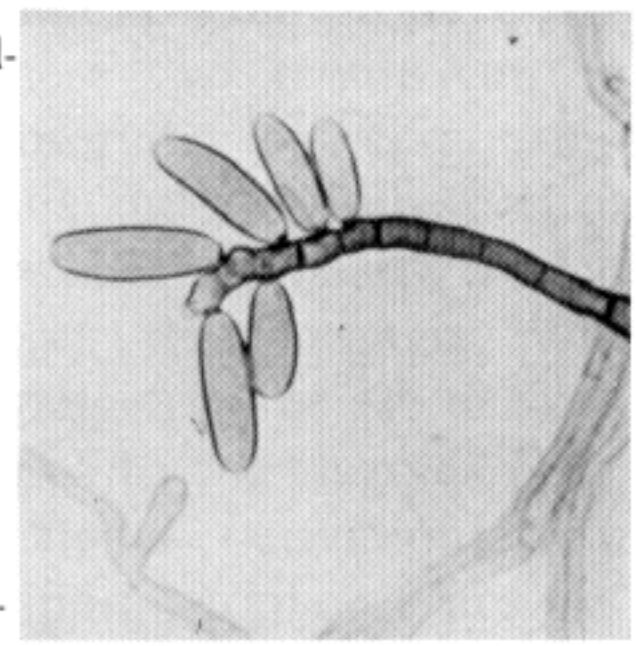
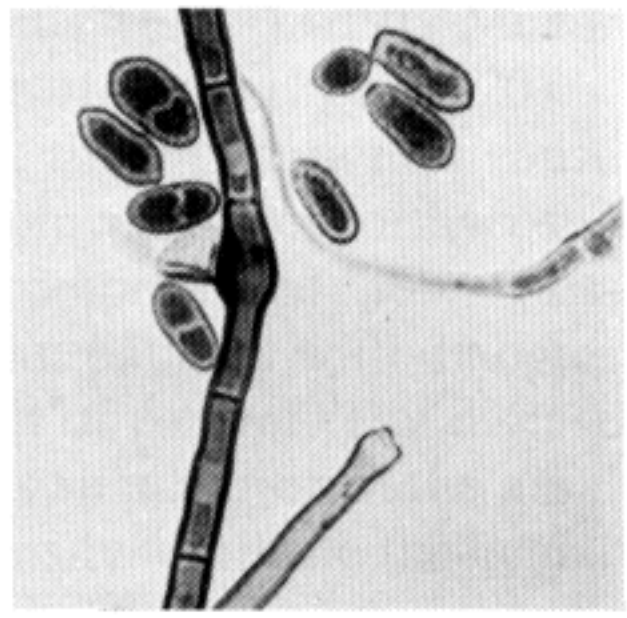
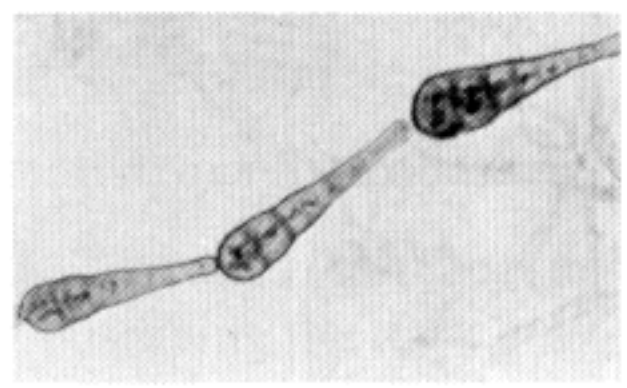
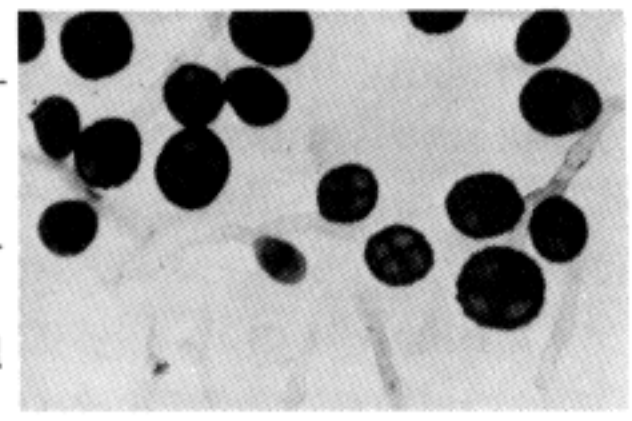
Processing and Inoculation of Fungal Specimens from Various Clinical Materials

Clinical Material	*Processing and Inoculation Techniques*	*Recommended Media*
Cerebrospinal fluid	Filter 1 to 3 ml. of freshly collected cerebrospinal fluid through a 0.45 μ. Swinnex filter (Millipore Corporation) attached to a sterile syringe. Remove the filter and place it on the agar surface so that the side containing the concentrate touches the agar surface. Examine daily and move the filter pad to another location. If less than 2 ml. of sample is received, centrifuge for 10 min. and apply 1-drop aliquots of sediment to several areas on the agar surface.	Brain-heart infusion agar Chocolate agar Sabouraud's dextrose agar *Note:* media containing cycloheximide should not be used since some important fungi such as *C. neoformans* may be inhibited.
Blood[27]	Using aseptic technique, draw 10 ml. of blood from the patient and add to the blood culture bottle. The bottle should be vented throughout the duration of incubation using a sterile cotton-plugged needle. Examine daily for growth. In small laboratories, it may be preferable to inoculate 5 to 10 ml. of blood directly to the surface of appropriate agar.	Biphasic blood culture bottle containing a brain-heart infusion agar slant bathed in brain-heart infusion broth. Flood the agar surface daily with the broth by tipping the bottle gently. For plate techniques, Sabouraud's dextrose agar or brain-heart infusion agar are satisfactory.
Urine	All urine samples should be centrifuged and the sediment inoculated onto an appropriate medium. Streak the specimen over the agar surface with a loop to ensure adequate isolation of colonies.	Sabouraud's dextrose agar Brain-heart infusion agar *Note:* the addition of antibiotics (see text) is recommended because specimens are often contaminated with gram-negative bacteria.
Respiratory secretions: Sputum Bronchial washing Transtracheal aspirations	Respiratory samples that are thick, purulent, or flecked with blood are most likely to produce positive fungal cultures. The sputum grading procedure described in Chapter 1 is not applicable to the processing of specimens for fungal culture. As much of the specimen as possible should be inoculated onto the surface of an appropriate medium. Cultures should be incubated at 30° C. and examined every other day for the visual presence of growth.	Since respiratory secretions are commonly contaminated with bacteria and rapidly growing molds which may suppress the slower-growing pathogenic fungi, media containing antibiotics should be used: 1. Sabouraud's dextrose agar with chloramphenicol and cycloheximide 2. Brain-heart infusion agar with chloramphenicol and cycloheximide or gentamicin (Cycloheximide is inhibitory to some pathogenic fungi.)
Tissue, bone marrow, and body fluids	All biopsy tissue should be minced with a sharp scalpel blade before being cultured. Grinding is discouraged since some of the hyphal forms (particularly those of the Phycomyetes) may be damaged Five to 10 ml. of tissue homogenate, bone marrow sample, or fluid specimen sediment should be placed onto the surface of appropriate media. Examine cultures daily for the presence of growth.	Sabouraud's dextrose agar Brain-heart infusion agar with antibiotics

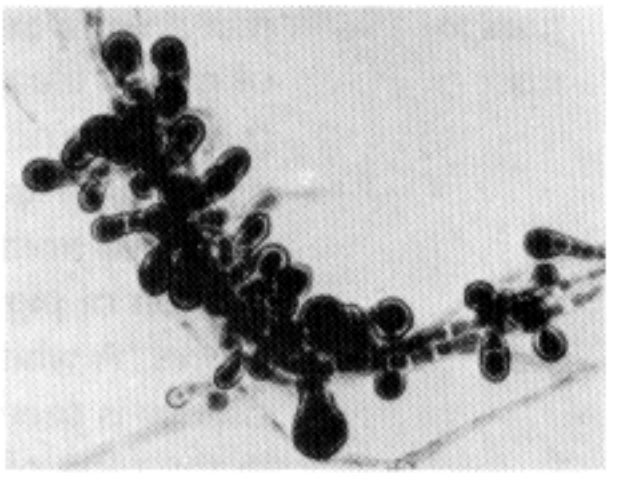
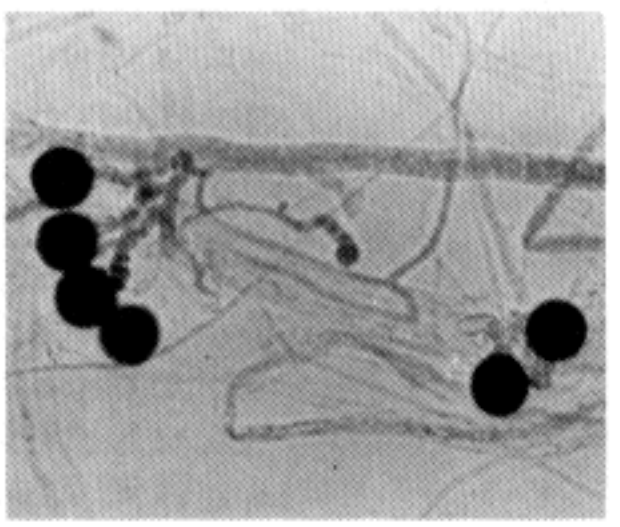
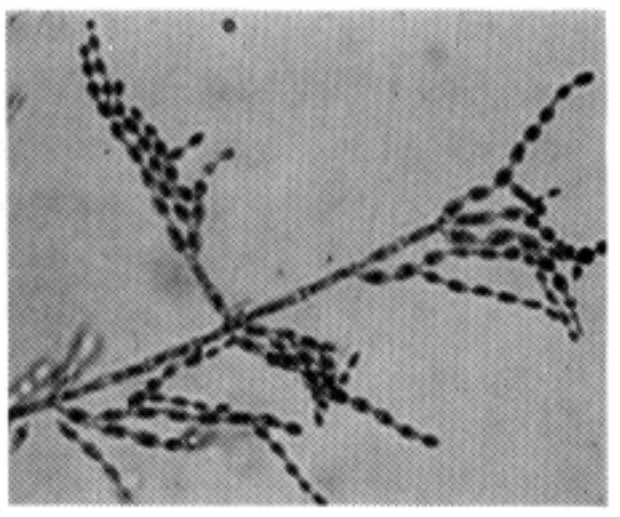
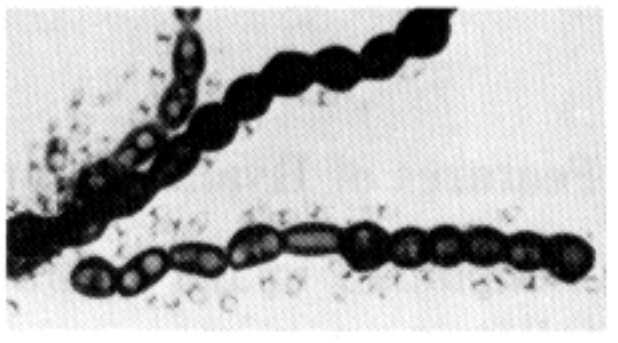
Processing and Inoculation of Fungal Specimens from Various Clinical Materials
(Continued)

Clinical Material	*Processing and Inoculation Techniques*	*Recommended Media*
Corneal scrapings and ear cultures	As much of the specimen as possible should be inoculated onto the surface of appropriate medium. Examine cultures daily for visual evidence of growth	Mycotic keratitis and external otomycosis are most often caused by the rapidly growing saprobic molds; therefore, media used should not contain antifungal antibiotics (such as cycloheximide.)
Oral mucosa	As much of the specimen as possible should be inoculated onto the surface of an appropriate medium. Cultures should be incubated for a minimum of 30 days because *H. capsulatum* is commonly recovered from lesions of the oral mucosa.	Sabouraud's dextrose agar Brain-heart infusion agar with chloramphenicol and cycloheximide
Skin scrapings, nails, and hair	Place skin scales, nail scrapings, or hairs directly on the surface of the medium. A few fragments should be submerged beneath the surface with a straight inoculating wire to produce maximal contact with the medium. Examine periodically for visual evidence of growth and hold all cultures for a minimum of 30 days.	Sabouraud's dextrose agar with chloramphenicol and cycloheximide (Mycosel or Mycobiotic agars)

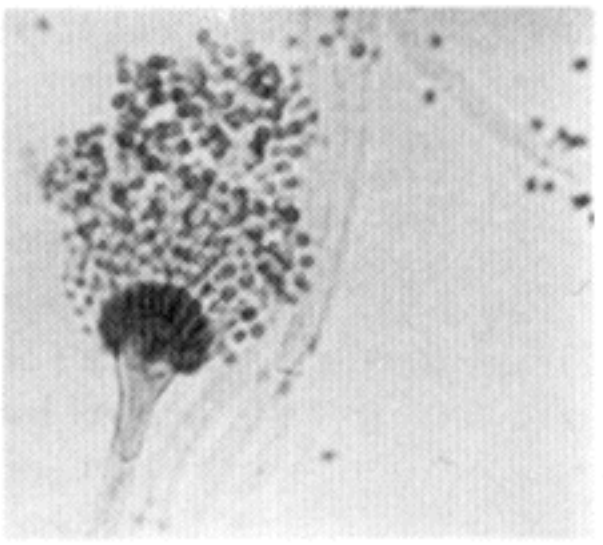
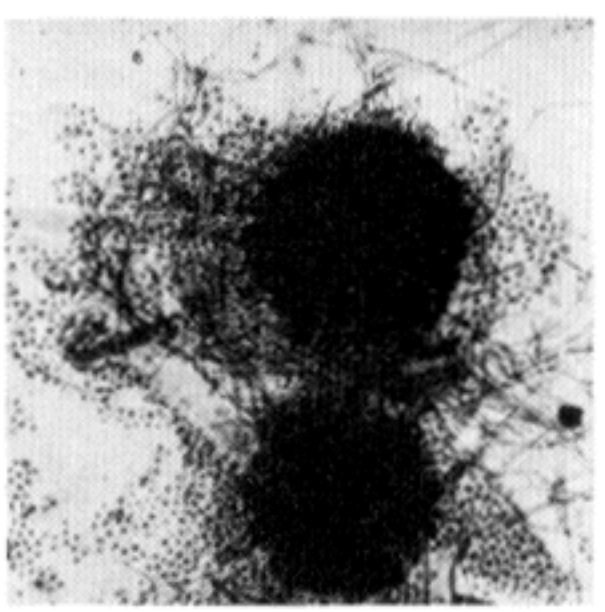
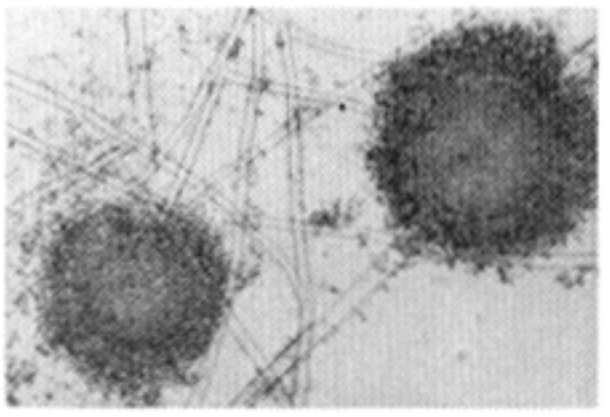
Cultural Features of the Dematiacious Molds

Genus	*Colonial Morphology*	*Microscopic Features*	*Illustration*
Curvularia	Dense, cottony, well-developed aerial mycelium. Initially gray-white, soon turning dark brown to red purple. Margins entire and sharply demarcated. Reverse is red-purple to black.	Hyphae distinctly septate and yellow-brown. Conidiophores twisted and roughened at points of conidial attachments. Dark brown macroconidia are divided into 4 to 6 cells by transverse septa having a curved or boomerang appearance.	
Helminthosporium	Colony is similar in appearance to Curvularia.	Hyphae distinctly septate and yellow-brown. Conidiophores twisted and roughened at points of conidial attachments. Elongated, cylindrical, smooth-walled, dark brown macroconidia divided into many cells by thick transverse septa. In direct mounts, macroconidia often appear vacuolated.	
Heterosporium	There are two colonial types: 1. Colony similar in appearance to Curvularia 2. Low velvety mycelium with a light gray to gray-brown coloration	Conidiophores similar to those of Helminthosporium, with roughening at points of conidial attachments. Conidia are oval to elliptical, divided into 3 to 5 cells by transverse septa, and when mature are covered by fine hairlike echinulations simulating cocoons.	
Alternaria	Colony is similar in appearance to Curvularia.	Hyphae distinctly septate and yellow-brown. Macroconidia are dark brown, multicelled, with septa both transverse and longitudinal, drumstick or beak-shaped, arranged in tandem in long chains.	
Stemphylium	Colonies spreading and covered with a low, well-developed aerial mycelium. Gray-white at onset with development of irregular, varigated dark brown to black pigmentation. Reverse of colony is dark brown to black.	Hyphae distinctly septate and yellow-brown. Conidiophores are often very short, bearing single, large, multicellular macroconidia, oval or round, divided by transverse and longitudinal septa.	

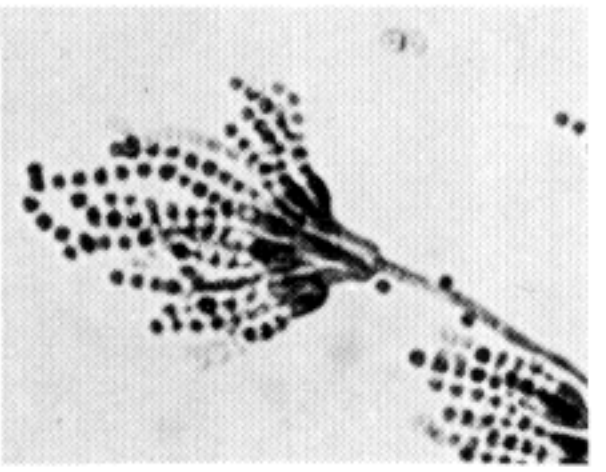
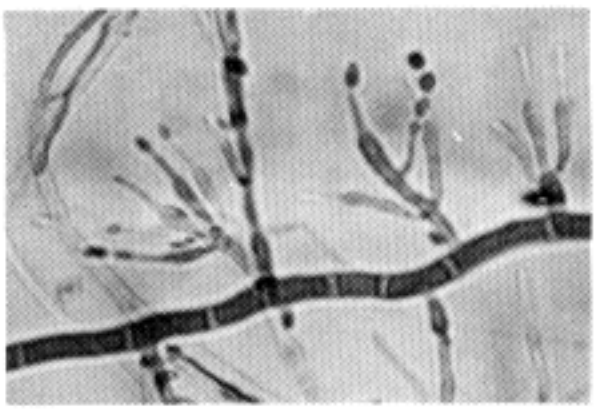
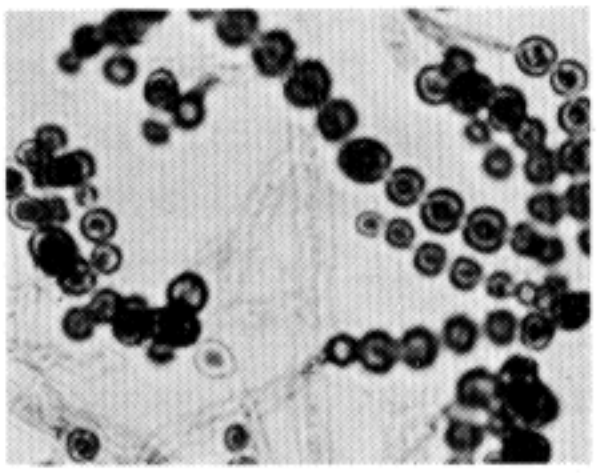
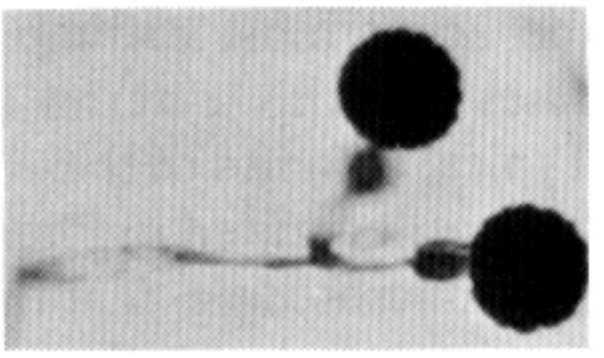
Cultural Features of the Dematiacious Molds *(Continued)*

Genus	*Colonial Morphology*	*Microscopic Features*	*Illustration*
Epicoccum	Colonies spreading but retain a distinct, serpiginous border. The aerial mycelium is well developed, presenting a cottony surface which develops a play of colors with maturity, including black, yellow, orange, red, and brown.	Hyphae distinctly septate and yellow-brown. Irregularly sized, spherical to club-shaped macrocondia are borne in clusters directly from the hyphae and are divided into multiple cells by both transverse and longitudinal septa.	
Nigrospora	Colonies spreading, gray-white, and covered by a well-developed fluffy mycelium. Darkening occurs only with maturity.	Hyphae initially hyaline and septate. Yellow-brown pigmentation occurs only with age. Conidiophores are short, somewhat helical, with a swollen urnlike tip within which are borne large, subspherical jet-black conidia, appearing as miniature cockhats.	
Cladosporium	Colonial types varying from deep brown to black, smooth, leathery, and rugose, to velvety, deep green variant covered by a low, hairlike mycelium. Early colonies may be smooth and yeastlike in nature.	Hyphae distinctly septate, yellow-brown. Conidiophores are freely branching, having the appearance of a brush from the tips of which are borne long chains of small, dark, yellow-brown oval or elliptical conidia.	
Aureobasidium (Pullularia)	Colonies grow slowly and are initially white to gray, yeastlike and glabrous, turning dark brown to jet black with age. Aerial mycelium never develops unless the colony becomes sterile.	Hyphae are broad, separated into distinct segments by thick-walled septa simulating arthrospores, giving rise to myriads of tiny elliptical nonpigmented microconidia.	

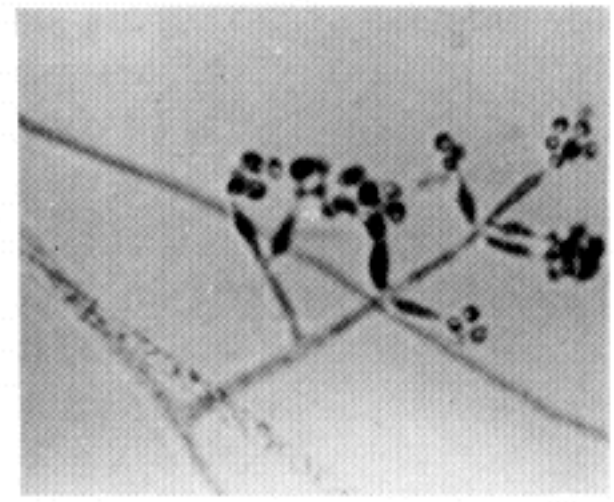
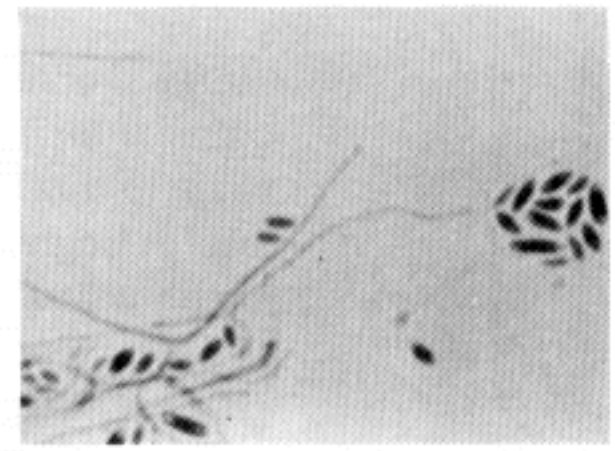
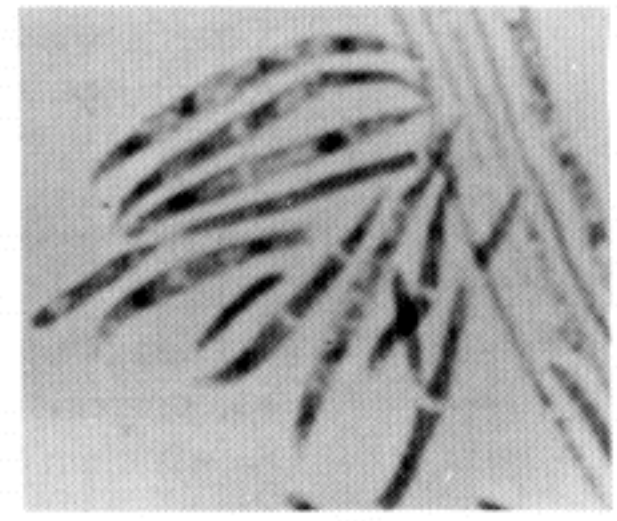
Characteristics of Three Species of Aspergillus

Species	*Colonial Morphology*	*Microscopic Features*	*Illustration*
Aspergillus fumigatus	Mature colonies have a distinct margin and are some shade of green, blue-green, or green-brown. Surface has a powdery or granular appearance from profuse production of pigmented spores. A white apron usually is seen at the edge in the zone of active growth.	Hyphae are hyaline and distinctly septate. Conidiophores are long, terminating in a large club-shaped vesicle. Chains of 2- to 3-μ. spherical conidia are borne from a single row of sterigmata that are produced only from the top half of the vesicle surface.	
Aspergillus niger	Colonies are initially covered with a white, fluffy, aerial mycelium. As colony matures, a salt-and-pepper effect is noted, with the surface ultimately covered with black spores. The reverse of the colony remains a light tan or buff color, which separates *A. niger* from the dematiacious molds.	Hyphae are hyaline and distinctly septate. Conidiophores are long and vesicle is usually not seen because it is covered with a thick ball of spores that are derived from the entire surface. Where vesicles can be seen, they have a concave undersurface simulating a mushroom. Spores are 2 to 3μ. spherical, and black.	
Aspergillus flavus	Colonies have a distinct margin, are covered by a fluffy, well-developed aerial mycelium, and when mature have a yellow or yellow-brown color.	Spherical 2- to 3-μ. spores are borne in short chains from the entire circumference of the vesicle. Vesicles are spherical and give rise to a double row of sterigmata from which the spores are borne. Hyphae are hyaline and distinctly septate.	

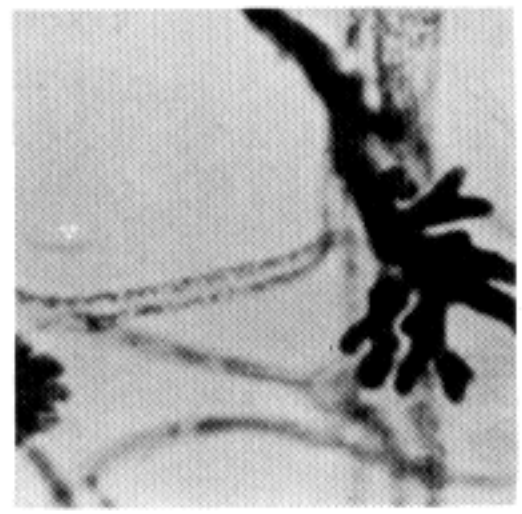
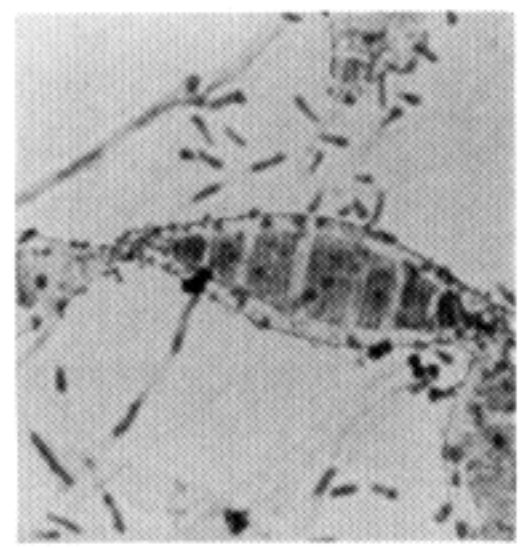
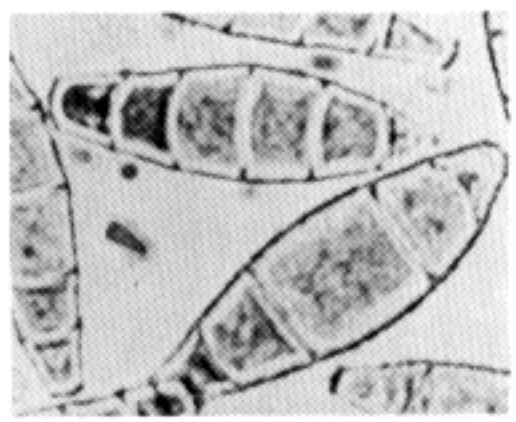
Characteristics of the Hyaline Saprobes

Genus	*Colonial Morphology*	*Microscopic Features*	*Illustration*
Penicillium	Colony is initially white and fluffy, soon turning shades of green or green-blue as pigmented spores are produced. Yellow or tan variants are occasionally seen. Radial rugae are often formed.	Hyphae are hyaline and septate. Conidiophores give rise to branching phialides forming a brush or "penicillus." Spherical or Oval 1- to 2-μ. conidia are borne in long chains from sterigmata, the tips of which are blunt and appear cut off at right angles.	
Paecilomyces	Colonies are usually powdery or granular and develop light pastel, yellow-green, green-blue, or buff as spores are produced. Margins are often not distinct.	Hyphae are hyaline and septate. Conidiophores branch freely into a brush-like structure. Oval 1- to 2-μ. conidia are borne in chains from the tips of sterigmata that are long and tapering.	
Scopulariopsis	Colonies are characteristically powdery, buff to brown in color, and develop shallow radial grooves.	Hyphae are hyaline and septate. Conidiophores branch to form penicillus; 3- to 4-μ. conidia are borne in chains. Conidia are lemon-shaped and with age develop surface echinulations.	
Gliocladium	Colonies develop diffusely over the surface as a green granular lawn. A distinct margin does not form.	Hyphae are hyaline and septate. Conidiophores branch into a brushlike structure; 2- to 3-μ. conidia are borne in clusters which obscure the tips of the sterigmata.	

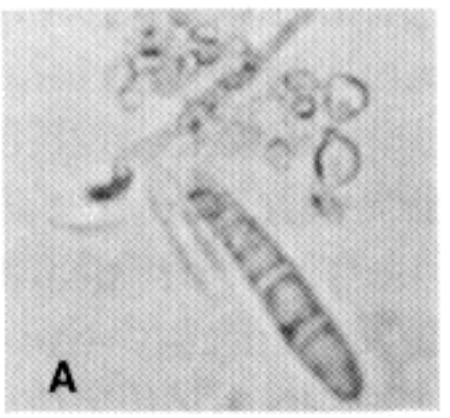
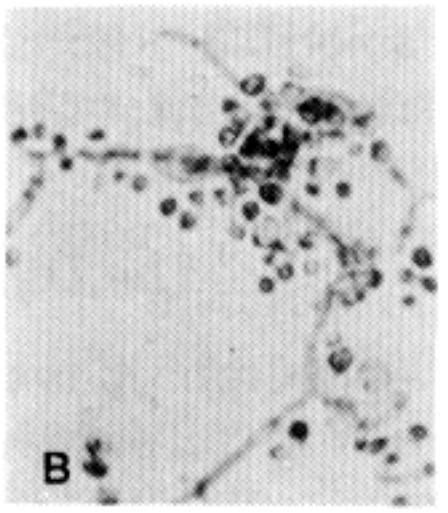
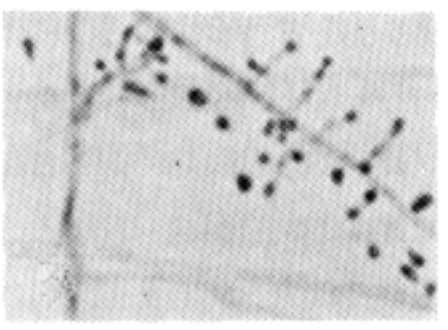
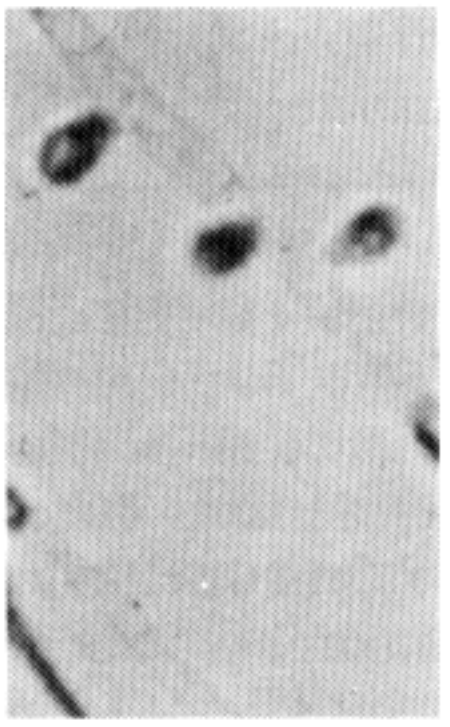
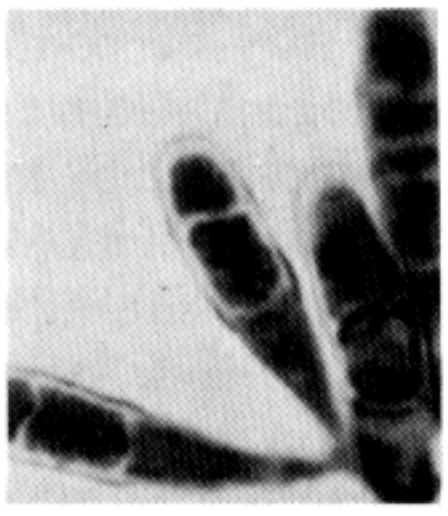
Characteristics of the Hyaline Saprobes *(Continued)*

Genus	*Colonial Morphology*	*Microscopic Features*	*Illustration*
Trichoderma	Colony is similar to that of Gliocladium forming a diffuse yellow or yellow-green lawn covering the entire surface of the agar. Colony surface is granular to fluffy.	Hyphae are hyaline and septate. Conidiophores generally are short and give rise to blunt sterigmata with tapered points. Clusters of 1 to 2 μ. in diameter, spherical to elliptical conidia form in compact clusters, held together by a thin mucinous secretion.	
Cephalosporium	Colonies are often white and covered with a fluffy, well-developed aerial mycelium. Light pastel yellow or orange colors develop with some strains.	Hyphae are quite delicate, hyaline, and septate. Conidiophores are long and slender, giving rise to elongated, elliptiform conidia clustered in a mosaic pattern simulating the cortical surface of a brain.	
Fusarium	Colonies are initially white and covered by a well-developed fluffy aerial mycelium. With maturity delicate lavender to purple-red pigment develops both over the surface and on reverse side.	Hyphae are hyaline and septate. Microconidia are 2 to 3 μ. in diameter and elliptical, form clusters simulating those of Cephalosporium. Identification is made by demonstrating pointed, banana-shaped or sickliform multicelled macroconidia.	

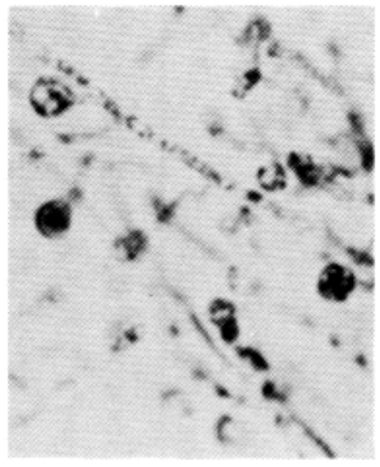
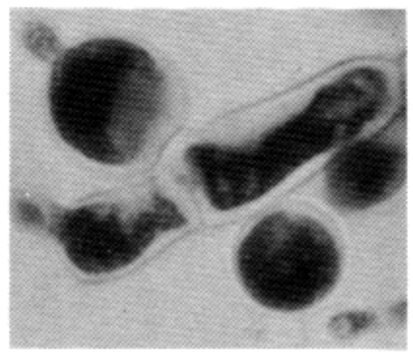
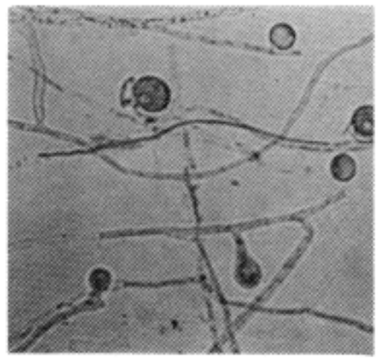
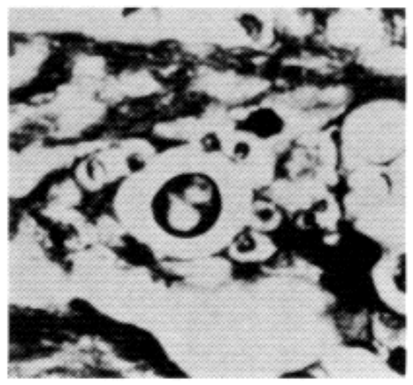
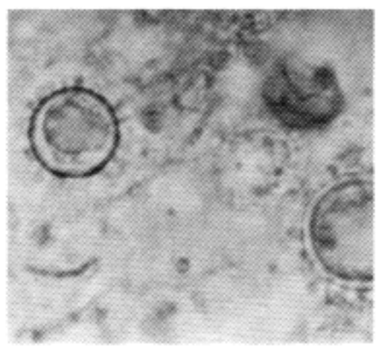
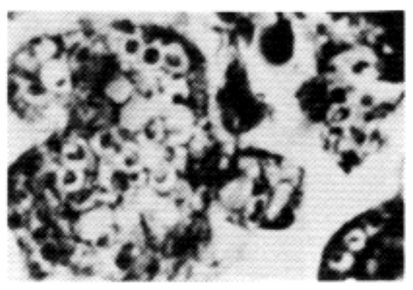
Characteristics of Three Microsporum Species

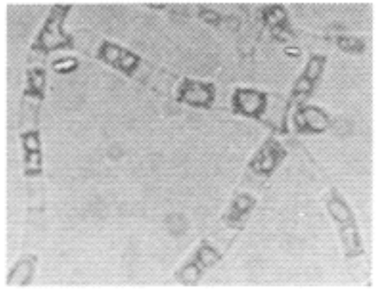
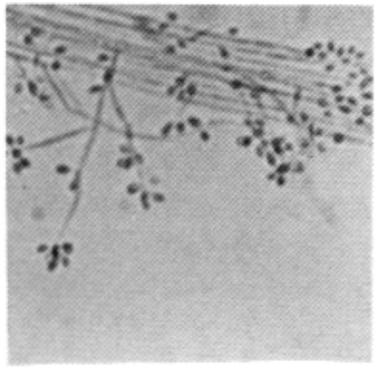
Species	*Colony Morphology*	*Microscopic Features*	*Other Features*	*Illustration*
M. audouinii	Colonies are moderately slow growing (7 to 14 days), producing a velvety aerial mycelium that is light tan or buff in color. The reverse appears salmon pink.	Macroaleuriospores are rarely produced; if present, they are bizarre-shaped. Microaleuriospores are usually rare. Terminal chlamydospores, favic chandeliers, and pectinate bodies usually abound.	No growth on rice grain medium	
M. canis	Colonies produce a granular to fluffy white to buff surface. A bright, lemon-yellow apron at the peripheral growing margin is typical. Colony reverse is usually yellow-orange.	Macroaleuriospores are thick-walled, spindle-shaped, multiseptate, and echinulate. Many have a characteristic curved tip. Microaleuriospores are generally sparse and laterally attached to the hyphae.	Grow well on rice grain medium. There are no other specific features.	
M. gypseum	Colonies are generally granular due to production of numerous aleuriospores. Surface is often cinnamon colored and the reverse is light tan.	Macroaleuriospores are thick-walled, multiseptate, and echinulate. They generally are longer and less spindle-shaped than *M. canis*, with rounded rather than pointed tips which do not tend to curve.	Grow well on rice grain medium. There are no other specific features.	

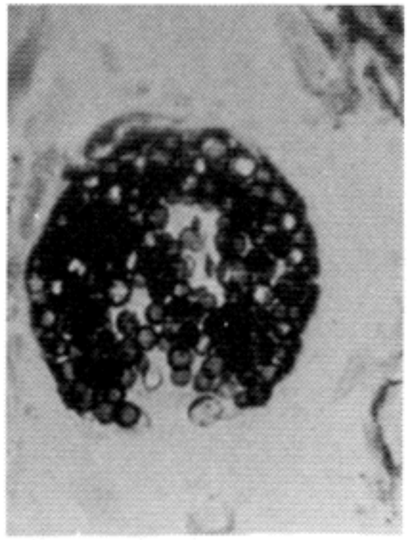
Characteristics of Three Trichophyton Species and Epidermophyton floccosum

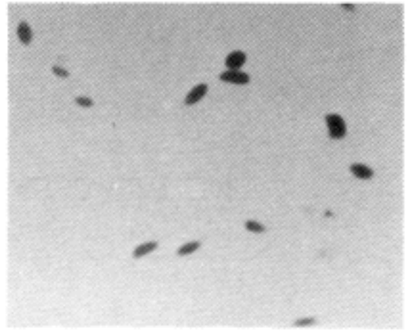
Species	Colony Morphology	Microscopic Features	Other Features	Illustration
T. mentagrophytes	There are two distinct colony types, fluffy, and granular. Color is usually white to pinkish. Reverse is buff to reddish brown. Red-brown pigment is produced by some strains, usually never as intense as with *T. rubrum*.	Microaleuriospores are usually produced in abundance, and are globose and arranged in pine-tree or grapelike clusters. Spiral hyphae are seen in 30% of isolates. Macroaleuriospores are rarely seen, are thin-walled, smooth, and pencil-shaped.	Positive urease test within two days[13] Produce conical-shaped areas of invasion of hair shafts in hair-baiting test (positive test)[2]	
T. rubrum	Colonies are generally white and downy in consistency. May be pinkish or reddish. Granular colony variants are found with strains that sporulate heavily. Reverse is often wine-red to red-yellow, particularly on corn meal agar.	Microaleuriospores are usually produced in profusion and are tear-shaped and borne laterally and singly from the hyphae. Macroaleuriospores are usually absent or are thin-walled, smooth, and pencil-shaped.	Urease not rapidly produced (Faint positive test may be seen in 7 days.) Hair baiting test negative	 
T. tonsurans	Colonies are generally tan, brown, or creamy red in color. Mycelium is usually low, giving a velvety to powdery surface. Rugal folds are common, with heaped sunken center. Reverse is yellow to tan.	Macroaleuriospores are rarely produced and are bizarre-shaped when present. Microaleuriospores are characteristically tear-shaped or club-shaped with flat bottoms and larger than other dermatophytes. Occasionally there are balloon forms.	Cannot grow on trichophyton No. 1 agar which contains only casein; good growth on trichophyton No. 4 agar which contains casein plus thiamine	
E. floccosum	Colonies are generally white and floccose; they tend to turn khaki green-brown with age. Center of colony is often folded. Reverse is yellow brown with observable folds.	Microaleuriospores are not produced. Macroaleuriospores are large, smooth-walled, clavate, and divided into 2 to 5 cells. They are borne singly or in clusters of two or three.	No special features; may be confused with *M. nanum*; however, macroaleuriospores of this species are thick-walled and echinulate.	

Characteristics of the Dimorphic Molds

	Mold Form			*Yeast Form*		
Species	*Colonial Morphology*	*Microscopic Features*	*Illustration*	*Colonial Morphology*	*Microscopic Features*	*Illustration*
Blastomyces dermatitidis	Growth in 7 days to 4 weeks. On blood agar, colonies are cream to tan, soft, wrinkled, and appear waxy. On BHI or SAB agar, colonies appear fluffy and white to-tan.	Hyphae delicate, hyaline and septate. Round to oval conidia are borne singly from the tips of conidiophores of irregular length that are borne laterally from the hyphae. They have the appearance of "lollipops."		Colonies are tan or cream in color, and very wrinkled and waxy in appearance when grown at 37° C.	Large thick-walled yeast cells having a single bud attached to the parent cell by a thick "collar" or wall.	
Paracoccidioides brasiliensis	Growth in 21 or more days. On BHI or SAB agar the aerial mycelium is white to tan-brown. Center of colony may become heaped with a crater cut into the agar surface.	Mycelium tends to be sterile and many chlamydospores may be seen. Occasional round or oval conidia similar to those of *B. dermatitidis* may be seen.		Colonies are tan to cream in color, and may become wrinkled and pasty in appearance when grown at 37° C.	Large, thick-walled yeast cells similar to those of *B. dermatitidis* except there are multiple daughter buds, forming structures simulating a mariner's wheel	
Histoplasma capsulatum	Growth in 7 to 45 days. Growth on blood agar appears moist, waxy, and cerebriform, and ranges from pink to tan in color. On BHI or SAB agar, colonies are cottony to silky and are white or turning brown with age.	Hyphae are small, hyaline, and septate. Round to tear-drop microaleuriospores are borne on short lateral branches. Macroaleuriospores spherical to pyriform and tuberculated, are the diagnostic forms.		Initial growth appears as a rough, mucoid, cream-colored colony. It turns smooth and brown with age.	Small, oval, budding cells are seen. If observed during yeast conversion phase, cells are larger and some resemble arthrospores.	

Coccidioides immitis	Growth in 5 to 21 days. Young colonies are moist and adhere to blood or SAB agar. Older colonies develop cottony aerial mycelium which becomes unevenly distributed over the agar surface in a "cobweb" appearance. It is white at first, becoming brown with age.	Early cultures have septate hyphae, and many raquet hyphae; as the culture ages, hyphae become enlarged and dissociate through points of septation into barrel-shaped arthrospores that stain alternating dark and clear, with dead cells inbetween.		No yeast form in routine culture; remains in mold form even at 37° C. incubation	10-60 μ. in diameter spherules containing 2–4 μ. in diameter endospores seen only in tissues.
Sporothrix schenckii	Growth in 3 to 5 days. Early colony is smooth and white to cream colored. With age, surface becomes wrinkled, turning brown to black. Surface remains smooth and devoid of an aerial mycelium.	Hyphae are hyaline septate, and small in diameter. Branched slender conidiophores arise at right angles from hyphae. Small pyriform conidia arranged in "flowerettes" at tips of conidiophores are diagnostic. Conidia are attached by delicate thread.		Colonies are cream to white in color and soft and creamy in consistency, resembling the typical yeast colony of many other species.	Elongated yeast cells resembling cigars with delicate buds are typically seen. Occasional yeast cells may appear more oval and bear multiple delicate buds.





TISSUE FORMS OF FUNGI OF MEDICAL IMPORTANCE TO MAN

ETIOLOGIC AGENT	DIAGNOSTIC TISSUE FORM	SIZE	COMMENTS
I. Deep-seated Mycoses			
Blastomyces dermatitidis	Thick-walled, double-contoured yeast cells, producing single bud attached by a broad base.	8–20 μ	
Coccidioides immitis	Thick-walled spherules enclosing numerous non-budding endospores.	10–60 μ	Rudimentary mycelium may rarely develop in open cavitary lesions.
Cryptococcus neoformans	Irregularly sized yeast cells, budding singly and attached by a hair-like neck, surrounded by a thick mucoid capsule.	4–15 μ	*Cryptococcus neoformans* never forms a true mycelium.
Histoplasma capsulatum	Small yeast cells located within reticuloendothelial cells. Pseudocapsules account for the species name.	2–4 μ	True capsules do not form.
Paracoccidioides brasiliensis	Large yeast cells producing multiple buds arranged in the form of a mariner's wheel.	8–20 μ	
II. Opportunistic Mycoses			
Aspergillus species	Hyaline, septate hyphae, dichotomously branching and regular in diameter with parallel opposing walls.	5–10 μ	Rarely, conidial-bearing fruiting bodies may develop in fungus ball cavities.
Candida species	Pseudohyphae composed of elongated blastospores, showing regular points of constriction simulating link sausages. Budding oval or spherical blastospores also present.	5–10 μ (Pseudohyphae) 3–4 μ (Blastospores)	
Geotrichum candidum	Hyphae producing arthrospores.		
Zygomycetes *Mucor* sp. *Rhizopus* sp. *Absidia* sp.	Broad, aseptate, irregularly branching, ribbon-like hyphae with non-parallel opposing walls.	10–30 μ	Rarely, sporangial fruiting bodies may form in fungus ball cavities.
Actinomyces israelii	Delicate, branching, minute filaments often within "sulfur granules."	Less than 1 μ	*A. israelii* is an anaerobic bacterium.
Nocardia asteroides	Delicate, branching, minute filaments often within "sulfur granules."		Branching filamentous, "partially" acid-fast bacterium.
III. Subcutaneous Mycoses			
Chromomycosis group: *Fonsecaea pedrosoi* *Fonsecaea compactum* *Phialophora verrucosa* *Cladosporium carrionii*	Dark yellow or brown, septate, hyphal segments. Also, rounded or crescent-shaped, thick-walled deep yellow or brown sclerotic bodies.	5–8 μ (Hyphae) 8–15 μ (Sclerotic bodies)	
Petriellidium (*Allescheria*) *boydii*	Production of yellow-gray granules containing wide mycelial forms often clubbed at the periphery of the granule.	6–8 μ	10–12 μ oval to round conidia may be produced in fungus ball cavities.
Actinomycetes	Delicate, branching filaments within "sulfur granules."	Less than 1 μ	*Nocardia* sp. filaments are partially acid-fast
Sporothrix schenckii	Tiny, irregular, elongated cigar-shaped yeast forms.	3–5 μ	Yeast forms are extremely difficult to demonstrate in human tissues.

TISSUE FORMS OF FUNGI OF MEDICAL IMPORTANCE TO MAN (*Continued*)

ETIOLOGIC AGENT	DIAGNOSTIC TISSUE FORM	SIZE	COMMENTS
IV. Superficial Mycoses			
Dermatophyte group: *Microsporum* sp. *Epidermophyton* sp. *Trichophyton* sp.	Slender hyphal forms, often breaking into arthrospore-like segments in the stratum corneum of the skin. Endothrix and ectothrix minute spores in hair infections.	3-5 μ (Hyphae) 1-2 μ (Spores)	Fungal forms best demonstrated in direct KOH mounts of infected skin scales, nail scrapings, or plucked hairs
Exophiala (*Cladosporium*) *werneckii*	Delicate, twisting, tortuous hyphal segments confined to the stratum lucidum.	1-2 μ	Fungal elements best demonstrated in direct KOH mounts.
Malassezia (*Pityrosporum*) *furfur*	Many short, stubby hyphal segments, admixed with budding spheroidal cells, limited to the stratum corneum.	3-5 μ (Hyphae) 4-6 μ (Cells)	Fungal elements best demonstrated in direct KOH mounts

SPECIMEN AND MEDIA REQUIREMENTS FOR THE RECOVERY OF FUNGI FROM SPECIFIC MYCOTIC INFECTIONS

INFECTION	SPECIMEN TYPE	COMMON CULTURE MEDIA
Histoplasmosis	Respiratory secretions; blood; bone marrow; urine; cerebrospinal fluid; mucocutaneous ulcers	Sabouraud's dextrose agar[a]; brain-heart infusion agar; inhibitory mold agar; Sabhi agar; brain-heart infusion blood agar with antibiotics[b]; brain-heart infusion blood agar with antibiotics and cycloheximide[c]. Biphasic brain-heart infusion agar/broth recommended for blood cultures.[d]
Blastomycosis	Respiratory secretions; skin; bone; urine; mucocutaneous ulcers	Sabouraud's dextrose agar[a]; brain-heart infusion agar; inhibitory mold agar; Sabhi agar; brain-heart infusion blood agar with antibiotics[b]; brain-heart infusion blood agar with antibiotics and cycloheximide.[c]
Coccidioidomycosis	Respiratory secretions; skin; cerebrospinal fluid; urine; mucocutaneous ulcers	Sabouraud's dextrose agar[a]; brain-heart infusion agar; inhibitory mold agar; Sabhi agar; brain-heart infusion blood agar with antibiotics[b]; brain-heart infusion blood agar with antibiotics and cycloheximide.[c]
Paracoccidioidomycosis	Respiratory secretions; mucocutaneous ulcers; skin; intestine	Sabouraud's dextrose agar[a]; brain-heart infusion agar; inhibitory mold agar; Sabhi agar; brain-heart infusion blood agar with antibiotics[b]; brain-heart infusion blood agar with antibiotics and cycloheximide.[c]
Cryptococcosis	Respiratory secretions; cerebrospinal fluid; bone; urine; skin; pleural fluid; bone marrow; blood	Sabouraud's dextrose agar[a]; inhibitory mold agar; brain-heart infusion agar; Sabhi agar; brain-heart infusion blood agar with antibiotics.[b] Media containing cycloheximide inhibit the growth of *Cryptococcus neoformans*. Biphasic brain-heart infusion agar/broth recommended for blood cultures.
Candidosis	Respiratory secretions; urine; mucocutaneous lesions; blood; stool; vagina; nails	Most common fungal and bacterial culture media are satisfactory; however, those containing cycloheximide inhibit some species. Biphasic brain-heart infusion agar/broth recommended for blood cultures.[d]
Aspergillosis	Respiratory secretions; mucous plugs; external ear	Sabouraud's dextrose agar[a]; brain-heart infusion agar; inhibitory mold agar; Sabhi agar; brain-heart infusion blood agar containing antibiotics.[b] Media containing cycloheximide are unsatisfactory and inhibit the growth of aspergilli.
Nocardiosis[e]	Respiratory secretions; blood; cutaneous abscesses	Sabouraud's dextrose agar[a]; brain-heart infusion agar; Sabhi agar; biphasic brain-heart infusion agar/broth recommended for blood cultures.[d] Media containing antibiotics inhibit the growth of nocardiae.
Zygomycosis (Phycomycosis)	Respiratory secretions; rhino-orbital lesions; skin	Sabouraud's dextrose agar[a]; brain-heart infusion agar; inhibitory mold agar; Sabhi agar; brain-heart infusion blood agar with antibiotics.[b] Media containing cycloheximide inhibit the growth of zygomycetes.
Geotrichosis	Respiratory secretions; oropharynx; stool	Sabouraud's dextrose agar[a]; brain-heart infusion agar; inhibitory mold agar; Sabhi agar; brain-heart infusion blood agar with antibiotics[b]; brain-heart infusion blood agar with antibiotics and cycloheximide.[c]
Sporotrichosis	Respiratory secretions; lymphocutaneous abscesses; synovial fluid; nasal sinuses	Sabouraud's dextrose agar[a]; brain-heart infusion agar; inhibitory mold agar; Sabhi agar; brain-heart infusion blood agar with antibiotics[b]; brain-heart infusion blood agar with antibiotics and cycloheximide.[c]

SPECIMEN AND MEDIA REQUIREMENTS FOR THE RECOVERY OF FUNGI FROM SPECIFIC MYCOTIC INFECTIONS (*Continued*)

INFECTION	SPECIMEN TYPE	COMMON CULTURE MEDIA
Mycetoma	Draining cutaneous sinuses; bone	*Eumycotic mycetoma*: Sabouraud's dextrose agar[a]; brain-heart infusion agar; inhibitory mold agar; Sabhi agar; all media should contain antibiotics and cycloheximide.[c] *Actinomycotic mycetoma:* Sabouraud's dextrose agar[a]; brain-heart infusion agar; Sabhi agar. Media containing antibiotics inhibit the growth of aerobic actinomycetes.
Chromomycosis	Skin; brain	Sabouraud's dextrose agar[a]; brain-heart infusion agar; inhibitory mold agar; Sabhi agar; All media should contain antibiotics and cycloheximide.[c]
Dermatomycosis	Hair; skin; nails	Mycosel agar or mycobiotic agar.[f]
Mycotic keratitis	Corneal scraping	Sabouraud's dextrose agar[a]; brain-heart infusion agar; Sabhi agar. Media containing antibiotics or cycloheximide are unsatisfactory.
Otomycosis	External ear	Sabouraud's dextrose agar[a]; brain-heart infusion agar; inhibitory mold agar; Sabhi agar; brain-heart infusion blood agar containing antibiotics.[b] Media containing cycloheximide are unsatisfactory and inhibit the growth of several etiologic agents.

[a]Contains 2% dextrose, pH 7.0.

[b]Contains gentamicin, 5 μg/ml, and chloramphenicol, 16 μg/ml, or penicillin, 20 units/ml, and streptomycin, 40 units/ml.

[c]Contains gentamicin, 5 μg/ml, and chloramphenicol, 16 μg/ml, or penicillin, 20 units/ml, and streptomycin, 40 units/ml, and cycloheximide, 0.5 mg/ml.

[d]See Roberts, 1975.

[e]Not a mycotic infection; however, organisms are often recovered on fungal culture media.

[f]Contains chloramphenicol, 50 μg/ml, and cycloheximide, 0.5 mg/ml.

COMMON FILAMENTOUS FUNGI IMPLICATED IN HUMAN MYCOTIC INFECTIONS

ETIOLOGIC AGENT	TIME REQUIRED FOR IDENTIFICATION	PROBABLE RECOVERY SITES	CLINICAL IMPLICATION(S)
Alternaria species	2–6 days	Skin, nails, conjunctiva, and respiratory secretions	Skin and nail infections, conjunctivitis, hypersensitivity pneumonitis
Aspergillus flavus	1–4 days	Skin, respiratory secretions, gastric washings, nasal sinuses	Skin infections, allergic bronchopulmonary infection, sinusitis, myocarditis, disseminated infection, renal infection, subcutaneous mycetoma
Aspergillus fumigatus	2–6 days	Respiratory secretions, skin, ear, cornea, gastric washings, stool, nasal sinuses	Allergic bronchopulmonary infection, fungus ball, invasive pulmonary infection, skin and nail infections, external otomycosis, mycotic keratitis, sinusitis, myocarditis, renal infection
Aspergillus niger	1–4 days	Respiratory secretions, gastric washings, ear, skin	Fungus ball, pulmonary infection, external otomycosis, mycotic keratitis
Blastomyces dermatitidis	6–21 days (recovery time) [additional 3–14 days required for confirmatory identification]	Respiratory secretions, skin, oropharyngeal ulcers, bone, prostate	Pulmonary infection, skin infection, oropharyngeal ulceration, osteomyelitis, prostatitis, arthritis, CNS infection
Cephalosporium (*Acremonium*) species	2–6 days	Skin, nails, respiratory secretions, cornea, vagina, gastric washings	Skin and nail infections, mycotic keratitis
Cladosporium species	6–10 days	Respiratory secretions, skin, nails, nose, cornea	Skin and nail infections, mycotic keratitis. Chromoblastomycosis, brain abscess and tinea nigra palmaris caused by *Cladosporium carrionii*, *C. trichoides*, and *E. werneckii*, respectively.
Coccidioides immitis	3–21 days	Respiratory secretions, skin, bone, cerebrospinal fluid, synovial fluid, urine, gastric washings	Pulmonary infection, skin infection, osteomyelitis, meningitis, arthritis, disseminated infection
Epidermophyton floccosum	7–10 days	Skin, nails	Tinea cruris, tinea pedis, tinea corporis, onychomycosis
Fusarium species	2–6 days	Skin, respiratory secretions, cornea	Mycotic keratitis, skin infection (in burn patients)
Geotrichum species	2–6 days	Respiratory secretions, urine, skin, stool, vagina, conjunctiva, gastric washings, throat	Bronchitis, skin infection, colitis, conjunctivitis, thrush
Helminthosporium species	2–6 days	Respiratory secretions, skin	Pulmonary infection (rare)
Histoplasma capsulatum	10–45 days (recovery time) [additional 7–21 days required for confirmatory identification]	Respiratory secretions, bone marrow, blood, urine, adrenals, skin, cerebrospinal fluid, eye, pleural fluid, liver, spleen, oropharyngeal lesions, vagina, gastric washings, larynx	Pulmonary infection, oropharyngeal lesions, CNS infection, skin infection (rare), uveitis, peritonitis
Microsporum audouinii	10–14 days (recovery time) [additional 14–21 days required for confirmatory identification]	Hair	Tinea capitis
Microsporum canis	5–7 days	Hair, skin	Tinea corporis, tinea capitis, tinea barbae, tinea manuum
Microsporum gypseum	3–6 days	Hair, skin	Tinea capitis, tinea corporis

COMMON FILAMENTOUS FUNGI IMPLICATED IN HUMAN MYCOTIC INFECTIONS (*Continued*)

ETIOLOGIC AGENT	TIME REQUIRED FOR IDENTIFICATION	PROBABLE RECOVERY SITES	CLINICAL IMPLICATION(S)
Mucor species	1–5 days	Respiratory secretions, skin, nose, brain, stool, orbit, cornea, vitreous humor, gastric washings, wounds, ear	Rhinocerebral infection, pulmonary infection, gastrointestinal infection, mycotic keratitis, intraocular infection, external otomycosis, orbital cellulitis
*Nocardia asteroides**	4–25 days	Respiratory secretions, skin, urine, blood, brain, conjunctiva, bone, cornea, gastric washings	Pulmonary infection, mycetoma, brain abscess, conjunctivitis, osteomyelitis, mycotic keratitis
Penicillium species	2–6 days	Respiratory secretions, gastric washings, skin, urine, ear, cornea	Pulmonary infection, skin infection, external otomycosis, mycotic keratitis, endocarditis
Petriellidium (Allescheria) boydii	2–6 days	Respiratory secretions, gastric washings, skin, cornea	Pulmonary fungus ball, mycetoma, mycotic keratitis
Phialophora species	6–21 days	Respiratory secretions, gastric washings, skin, cornea, conjunctiva	Some species produce chromoblastomycosis or mycetoma; mycotic keratitis, conjunctivitis, intraocular infection
Rhizopus species	1–5 days	Respiratory secretions, skin, nose, brain, stool, orbit, cornea, vitreous humor, gastric washings, wounds, ear	Rhinocerebral infection, pulmonary infection, mycotic keratitis, intraocular infection, orbital cellulitis, external otomycosis
Scopulariopsis species	2–6 days	Respiratory secretions, gastric washings, nails, skin, vitreous humor, ear	Pulmonary infection, nail infection, skin infection, intraocular infection, external otomycosis
Sporothrix schenckii	3–12 days (recovery time) [additional 2–10 days required for confirmatory identification]	Respiratory secretions, skin, subcutaneous tissue, maxillary sinuses, synovial fluid, bone marrow, bone, cerebrospinal fluid, ear, conjunctiva	Pulmonary infection, lymphocutaneous infection, sinusitis, arthritis, osteomyelitis, meningitis, external otomycosis, conjunctivitis, disseminated infection
Trichophyton mentagrophytes	7–10 days	Hair, skin, nails	Tinea barbae, tinea capitis, tinea corporis, tinea cruris, tinea pedis, onychomycosis
Trichophyton rubrum	10–14 days	Hair, skin, nails	Tinea pedis, onychomycosis, tinea corporis, tinea cruris
Trichophyton tonsurans	10–14 days	Hair, skin, nails	Tinea capitis, tinea corporis, onychomycosis, tinea pedis
Trichophyton verrucosum	10–18 days	Hair, skin, nails	Tinea capitis, tinea corporis, tinea barbae
Trichophyton violaceum	14–18 days	Hair, skin, nails	Tinea capitis, tinea corporis, onychomycosis

*Although *N. asteroides* is a bacterium, it is commonly recovered on fungal culture media due to its slow growth rate.

Chapter 6

An Introduction to Mycotoxins

Molds produce a wide range of secondary metabolites as they grow. Some of these substances are pigments, some are antibiotics and some are toxic to plant, animals and humans. Those substances produced by molds that are toxic are called “mycotoxins”. Some of these toxins are produced in the fruiting bodies of mushrooms and are among the most poisonous materials known. Some are only produced by the fungi when growing on certain grains and others cause formation of toxins when combined with a host plant.

As mentioned in the first chapter, history vividly recounts the stories of outbreaks of gangrenous ergotism during the 9th and 10th centuries where limbs literally rotted and fell off of infected humans. In the 11th century, the order of St. Anthony’s was founded to provide hospitals for those afflicted with “St. Anthony’s Fire”. The disease was caused by the consumption of rye grains and seedheads which were contaminated with the sclerotia or resting structures of the fungus *Claviceps purpurea*. The ergot “alkaloids” also affected the nervous system causing convulsions and spasms of the limbs. Those poisoned by the fungus described the sensation as “ants running underneath their skin”. The ergot alkaloids killed hundreds of thousands throughout history but because of modern science is known mainly for their contributions to human medicine and the narcotics trades (LSD).

In 1959, the most significant event in the history of mycotoxins took place. A turkey farm in East Anglia in Britain lost thousands of turkey poults over the course of a few days. It was quickly learned that they had died of a poison present in the pelleted feed they consumed. Examination of the groundnut meal used in the pellets revealed the mold mycelium and new laboratory methods using thin layer chromatography revealed several new and previously unknown compounds. These new substances would fluoresce intensely under ultraviolet light. The mold was called *Asperigillus flavus* and the metabolites it produced became known as “aflatoxins”.

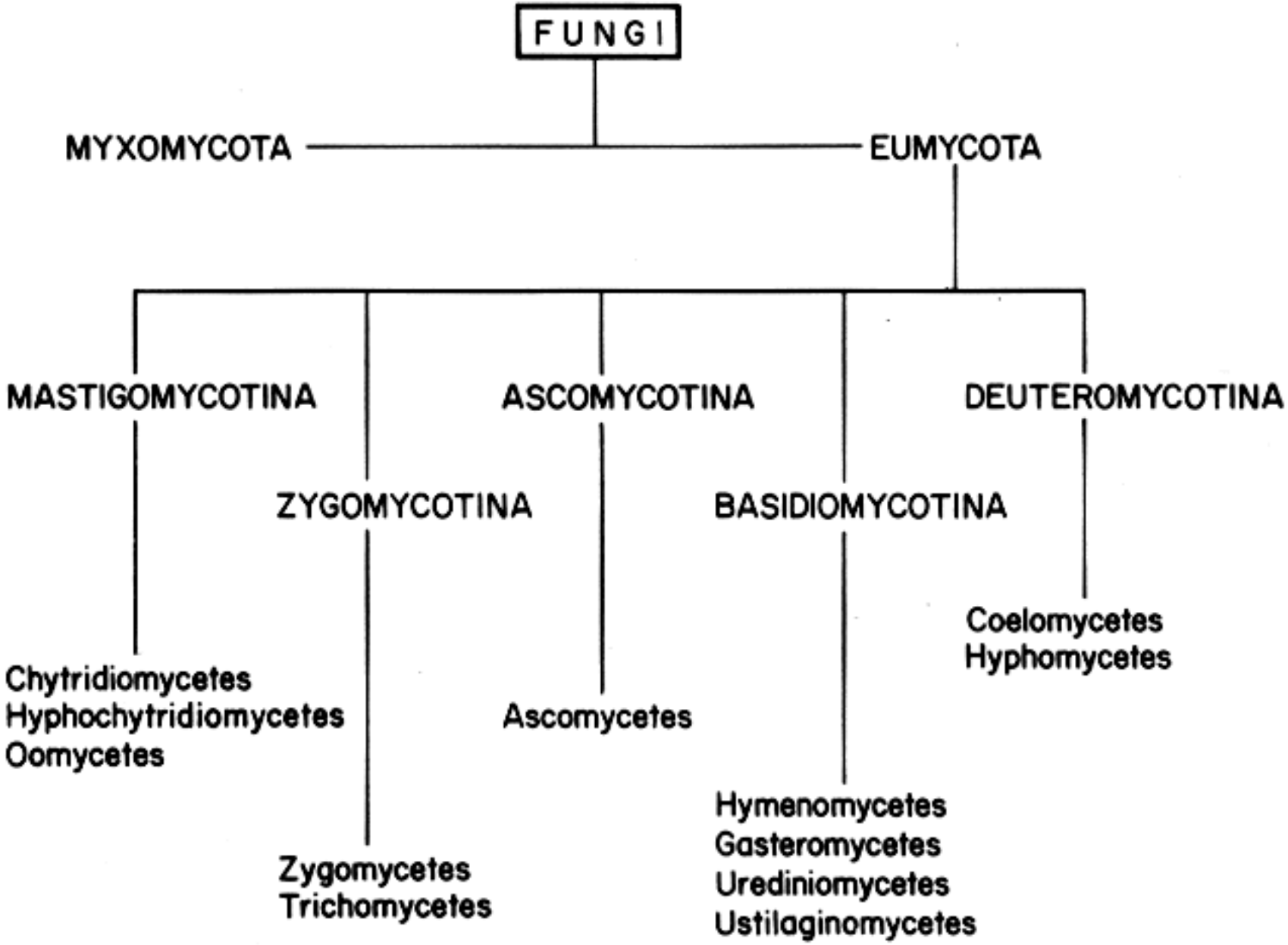
Aflatoxins soon became well known as some of the most deadly toxins known to man. The LD50 for a single oral dose in mg/kg of body weight for Aflatoxin B1 was soon established –

Rabbit	.3	Guinea Pig	1.4-2.0
Duckling	.34	Baboon	2.0
Cat	.55	Chicken	6.3
Pig	.6	Rat (male)	5.5-7.2
Rainbow Trout	.8	Rat (female)	17.9
Dog	.5-1.0	Macaque	7.8
Sheep	1.0-2.0	Mouse	9.0

Many previously unknown causes of animal deaths were now understood. It was also soon discovered that when cottonseed meal was used in fish farm pellets as a replacement protein, the rainbow trout began to show almost universal liver carcinomas. The cause was soon traced to the aflatoxins and it was soon learned that even the tinniest presence of aflatoxin, as little as .4 mcg per kg(-1) causes significant incidence of hepatoma. This made the Aflatoxin one of the most carcinogens known to man.

In third world countries, aflatoxin has been responsible for thousands of deaths when moldy grain was used to make bread. Starving, poor and desperate people eat whatever is available.

It is helpful to provide a few charts of the fungi and the classes, orders and genera that contain the toxin producing species –


FUNGI
MYXOMYCOTA
EUMYCOTA
MASTIGOMYCOTINA
ZYGOMYCOTINA
ASCOMYCOTINA
BASIDIOMYCOTINA
DEUTEROMYCOTINA
Chytridiomycetes
Hyphochytridiomycetes
Oomycetes
Zygomycetes
Trichomycetes
Ascomycetes
Hymenomycetes
Gasteromycetes
Urediniomycetes
Ustilaginomycetes
Coelomycetes
Hyphomycetes

Major groups of terrestrial filamentous fungi

Class	Order	Examples of genera	Comments
Oomycetes	Peronosporales	*Phytophthora*	Plant pathogens including potato blight. Not known to produce mycotoxins
Zygomycetes	Mucorales	*Mucor* *Rhizopus*	Important as agents of food spoilage. Occasional reports of mycotoxins
Ascomycetes	Clavicipitales	*Claviceps* *Eurotium*	Plant pathogens, ergotism Saprophytes able to grow at low a_w. Food spoilage some toxigenic
	Hypocreales	*Nectria* *Gibberella*	Plant pathogens, some toxigenic.
	Sphaeriales	*Chaetomium*	Saprophytes, toxigenic
	Pezizales	*Helvella* *Gyromitra*	Poisonous 'toadstools'
Ustilaginomycetes	Ustilaginales	*Ustilago*	Plant pathogens, smuts
Urediniomycetes	Uredinales	*Puccinia*	Plant pathogens, rusts
Hymenomycetes	Agaricales	*Amanita* *Agaricus*	Poisonous and edible 'toadstools', mushrooms
Coelomycetes	–	*Phomopsis*	Pycnidial 'fungi imperfecti'
Hyphomycetes	–	*Aspergillus* *Penicillium* *Fusarium* *Pithomyces* *Alternaria* *Stachybotrys*	The 'fungi imperfecti' including many toxigenic species (Table 2.3)
Mycelia Sterilia	–	*Rhizoctonia*	Plant pathogens including at least one toxigenic species

Ascomycetes and their anamorphs associated with mycotoxin formation

Teleomorph	Anamorph	Mycotoxins
Claviceps purpurea	*Sphacelia segetum*	Ergot alkaloids
Eurotium chevalieri	*Aspergillus chevalieri*	Xanthocillin
Eupenicillium ochrosalmoneum	*Penicillium ochrosalmoneum*	Citreoviridin
Monographella nivalis	*Fusarium nivale*[a]	Trichothecenes
Gibberella zeae	*Fusarium graminearum*	Zearalenone Trichothecenes
Nectria haematococca	*Fusarium solani*	Trichothecenes
Hypocrea spp.	*Trichoderma viride*	Trichodermin

[a]Also referred to as *Gerlachia nivalis.*

Toxigenic species of Deuteromycetes other than aspergilli, penicillia and fusaria

Species	Toxins
Alternaria alternata	Tenuazonic acid
Pithomyces chartarum	Sporidesmins [a]
Trichothecium roseum	Trichothecin
Rhizoctonia leguminicola [b]	Slaframine
Stachybotrys atra	Satratoxins
Myrothecium roridum	Roridins
Phomopsis leptostromiformis	Phomopsin

[a] The name of these toxins reflects an earlier name given to the mould: *Sporidesmium bakeri.*

[b] Strictly a member of the Mycelia Sterilia.

The more important toxigenic species of *Penicillium* on cereals and other foods

Species	Toxins	Comments
P. citrinum	Citrinin	Common biodeteriogen, worldwide on foods, decaying plant materials, textiles
P. cyclopium	Penitrem A Cyclopiazonic acid Penicillic acid Ochratoxin A	(= *P. aurantiogriseum*). Common on cereals and other foods
P. expansum	Patulin Citrinin	Predominantly from rotting apples and pears, but also other fruits
P. islandicum	Luteoskyrin Islanditoxin Cyclochlorotine	Cereals, particularly in the tropics
P. purpurogenum	Rubratoxins	(= *P. rubrum*). Primarily a soil fungus associated with the decay of many substrates
P. roquefortii	P.R. toxin Roquefortine	Blue cheeses, also cool stored products
P. viridicatum	Ochratoxins Citrinin Viridicatin Xanthomegnin Viomellein	Worldwide, cereals and cereal products

The more important toxigenic species of *Aspergillus*

Species	Toxins	Comments
A. chevalieri	Xanthocillin	Low a_w, stored cereals and cereal products
A. clavatus	Patulin Cytochalasin E Tryptoquivaline	Alkali-tolerant, animal dung, soil and decomposing organic material
A. flavus	Aflatoxins Aflatrem	Tropical and subtropical soils, plant products such as groundnuts and maize
A. fumigatus	Viriditoxin Gliotoxin Fumagillin Verruculogen	Thermophilic, decomposing organic material, pathogenic to birds and mammals
A. niger	Malformins Oxalic acid	Cosmopolitan but particularly in the tropics
A. ochraceus	Ochratoxins Penicillic acid Destruxin B	Soils, decaying vegetation, grain, adventitious pathogen
A. parasiticus	Aflatoxins	Insect pathogen, saprophyte on plant products
A. ustus	Austocystins Austamide Austdiol Brevianamide	Widespread in soil
A. versicolor	Sterigmatocystin Cyclopiazonic acid	Soil, mature cheeses, cured meats, decaying vegetation

Toxin formation and teleomorphs of species of *Fusarium*

Species	Toxins					Teleomorph
	Trichothecenes	Zearalenone	Moniliformin	Fusarin	Butenolide	
F. moniliforme	–	+	+	+	–	*Gibberella fujikuroi*
F. oxysporum	+?	+	+	–	–	–
F. culmorum	+	+	–	–	–	–
F. avenaceum	+	+	–	–	–	*Gibberella avenacea*
F. equiseti	+	+	+	–	–	*Gibberella intricans*
F. graminearum	+	+	–	–	–	*Gibberella zeae*
F. lateritium	+	+	–	–	–	*Gibberella baccata*
F. solani	+	–	–	–	–	*Nectria haematococca*
F. nivale[a]	+	–	–	–	+	*Monographella nivalis*

[a]Also referred to as *Gerlachia nivalis*.

Trichothecene-producing genera other than *Fusaria*

Anamorph genus	Toxins (some spp.)	Teleomorph (some spp.)
Myrothecium	Verrucarins, Roridin	*Nectria*
Dendrodochium	Verrucarins, Roridin	*Nectria*
Cylindrocarpon	Roridins	*Nectria*
Stachybotrys	Satratoxin, Roridin	–
Trichoderma	Trichodermin	*Hypocrea*
Trichothecium	Trichothecin	*Hypomyces*
Cephalosporium[a]	Crotocin	–
Verticimonosporium	Vertisporin	–

[a] *Cephalosporium* = *Acremonium*

Commonly used mycological media

Medium	Composition (per litre)	Uses
Rose-bengal agar	Glucose (10 g) Peptone (5 g) KH_2PO_4 (1 g) $MgSO_4.7H_2O$ (0.5 g) Rose-bengal (35 mg) Agar (15 g) Tetracycline (35 mg)	Initial isolation from soils, plant materials and foods
Czapek dox agar	Sucrose (30 g) $NaNO_3$ (2 g) K_2HPO_4 (1 g) $MgSO_4.7H_2O$ (0.5 g) KC1 (0.5 g) $FeSO_4$ (10 mg) $CuSO_4$ (5 mg) $ZnSO_4$ (10 mg) Agar (15 g)	Identification and maintenance of *Aspergillus* and *Penicillium* (*P. digitatum* will not grow on this medium)
Malt extract agar	Malt extract (20 g) Agar (15 g)	Good general medium for mucorales and the majority of moulds
Potato sucrose agar	Potato extract (500 ml) Sucrose (20 g) Agar (15 g)	Growth and identification of *Fusarium*

The mycotoxins usually cause marked signs of disease or death in animals which consume feed infected with the mold and toxin. A single high dose or a series of small doses is all that is required to produce fatalities. The measurement of the ability of a single substance to produce fatalities is usually given as LD50 or LD100 which represents the dose necessary to kill 50 or 100% of the test animals. The ability of a mycotoxin to kill is strongly affected by the animals sex, age and strain as well as by the route of administration (IV, Oral, Injection itraperitoneally).

Generally, most pathological studies show that at the heavier doses, ones that approach LD50's, the mycotoxins affect nearly every system of an animals body. It has also been shown that many of the mycotoxins, when used in combination are synergystic. This means that the damage they do when combined is much greater than either alone at the total doses used.

It has also been discovered that exposure to low levels of some mycotoxins results in impaired immune systems making the animals much more susceptible to disease and these have caused known failure of livestock vaccines. This property is very useful in combination bio-weapon design. An example is the effect of aflatoxin in poultry feeds where the level to infect chickens with salmonella drops in dose size from 10,000,000 CFU to as little as 100,000. The amounts fed to produce this level of immunosuppression varied from 250 mcg/kg (-1) in poults to 625 mcg in broilers. The tricothecenes also makes animals much more susceptible to inhalation and ingestion disease.

Mycotoxins also cause a range of mutagenic (mutations), teratogenic (malformed, dead or reabsorbed fetuses), and oestrogenic (atrophied ovaries, reduced testes) effects. The carcinogenic effects have been measured with many mycotoxins. In rats, aflatoxin B1 produces tumors in 10% of the populations when fed at 1 mcg per kg(-1) of the diet for one year. At 50 mcg, liver cancer was produced in 75% of the animals and at 100 mcg, cancer reached 100% in surviving rats. Most aflatoxins cause cancer primarily in the liver, colon and kidney tissues, and most mycotoxins are tissue specific when carcinogenicity and other effects are measured. Aflatoxin B1 is considered the most mutagenic of the mycotoxins causing chromosal abberations and DNA breakage in plant and animal cells.

In late 1974 in northwest India, a large number of villages suffered outbreaks of epidemic jaundice which involved liver disease. Over 100 people died. Studies showed that they had been exposed to levels of aflatoxin at .5-2 mg per Kg of food that they had recently eaten. It was found in a grain portion of their diet.

When aflatoxin is fed to dairy cows it becomes slightly altered and is produced in the cows milk. The aflatoxin M1 (M for milk) is about 40% as potent as B1 in producing liver tumors in rainbow trout.

An epidemiological study of workers in the animal feed and grain industries showed that workers have increased incidence of cancer when exposed to as little as .87-72 nanograms of aflatoxin per cubic meter of air in the workplace. This translated to a weekly exposure rate of 39 nanograms to 3.2 micrograms. These amounts are invisible to the naked eye and since their effects are measurable and significant, this commends their consideration of these as part of a biological weapons program.

In 1942-1947, severe famine occurred in parts of the Soviet Union. It was a repeat of several similar historical episodes in which the famine was accompanied by desparate consumption of moldy grains and bread. The symptoms included discomfort of the mouth, throat and stomach, followed by inflammation of the intestinal mucosa.Vomiting and diarrhea soon occurred with damage to the bone marrow and haematopoietic system (making blood cells) as more mold toxin is consumed. This is followed by anemia, a drop in erythrocyte and platelet counts, capillary walls hemorrhage, and the necrotic and dead tissues that form become infected with bacteria. The mold was *Fusarium* and the toxins as a group are known as Trichothecenes. They would kill thousands of Russians. The world would hear more about them in the early 1980's when the Soviets were accused of using the Trichothecenes as a weapon in Afghanistan where it was nicknamed "Yellow Rain".

In Japan, there have been many records of nausea, vomiting and diarrhea associated with eating wheat and rice contaminated with Fusarium. There it became known as the red mold disease which produced different but related toxic Trichothecenes.

Tests of corn in the USA have shown that 46% of all samples contained tiny trace amounts of Trichothecenes and more than 50% harbored spores of aflatoxin producing Asperigillus (1977). This makes the recovery of the Fusarium and Asperigillus toxin producing species simple for anyone with the skills desire and knowledge.

In 1938, scientists found that rice contaminated with penicillium species caused illness and disease in humans (yellow rice). The molds produced several toxins including "cardiac beri-beri" (Citreoviridin). The blue green penicillium molds prefer water reduced substrates like bread and fruit preserves. In this case several species were documented growing, often invisibly on the rice and sometimes tainted its color yellow.

Nature, as we have seen regularly conducts biological warfare on the human inhabitants of this planet. In parts of Africa, liver cancer occurs in very high rates. It is known now that aflatoxin and Fusarium toxins are a low level and continuous part of the grain diet of the populations of those areas.

Virtually all agricultural crops in the world have potentially toxin producing spores on them. In modern practice, the spores rarely germinate or do so and then die off (sporulate) as the grains are harvested and dried or the temperature becomes too cold to grow.

Any grain seed that has not been pressure cooked can harbor the spores of toxin producing fungi and provide the raw materials for a mold based biological weapons program. These will be described by toxin and species in the chapters ahead.

Mycotoxin Extraction

All grains contain tiny levels of various mycotoxins. These levels are at or below parts per billion and are sometimes undetectable but present. When the mold is being cultured deliberately and all the grain is being used as food substrate, the toxins can be produced in quantity. Extracting the toxins then becomes important. The process for each toxin type may be slightly different but a few general rules apply to almost all of them.

Usually, grain or substrates that are being used to grow the mold are finely ground and in a very thin layer so all the grain is used as mold food. In some cases, solid grains have been used. Usually, these need to be finely ground to allow liquids to soak into and reach all the potential fungi parts and grain cells to extract toxin.

Most mycotoxins are soluble in polar solvents like chloroform, acetonitrile, methanol, acetone, ethylacetate, and dichloromthane. These are usually used to mix into the media and then to solubulize and extract the toxins. Small amounts of water or acids are added because they more easily penetrate hydrophilic tissues and increase extraction levels.

If fats, lipids or pigments are present, they reduce the extraction levels. In this case, lab workers use a fat solvent such as hexane. The hexane takes up the fats and lipids and keeps them dissolved in its partition. When hexane is used with another solvent that it is immissible (does not mix with and separates into two or more layers) with, it can carry away the fats and be discarded with the hexane. Partitioning is usually done using a funnel with a valve. The bottom layer is drained through the funnel until it is gone. The valve is shut off and the two layers are now separated.

The solvent with the toxin is usually evaporated away in a vacuum or in a steam bath or on an enclosed hot plate.

Summary of extraction solvents used in Official AOAC Methods of Analysis for several mycotoxins

Toxin	Commodity	Extraction solvent
Aflatoxins	Corn, cottonseed	Acetone : water (85 : 15)
	Green coffee beans, soybeans, coconut, copra, copra meal	Chloroform : water (91 : 9)
	Cocoa beans	Defat with hexane then chloroform
	Peanut products, pistachio nuts	Chloroform : water (91 : 9) or methanol : water (55 : 45) plus hexane (39 : 32 + 29)
	Powdered milk	Acetone : water (70 : 30)
Ochratoxins	Barley	Chloroform + 0.1 M phosphoric acid
Patulin	Apple juice	Ethyl acetate
Sterigmatocystin	Barley, wheat	Acetonitrile : 4% potassium chloride (9 : 1)
Trichothecenes	Cereals	Methanol : water (9 : 1)

A reliable way to detect and measure many mycotoxins is the use of UV light. Many of the mycotoxins such as aflatoxin absorb UV light but they also re-emit part of the energy of the absorbed UV light as visible light (they fluoresce). Mycotoxins are often easily detected by using fluorescence. This is also a good measure of the concentration of the toxin. In the case of aflatoxin, the B and M toxins fluoresce blue while the G toxins fluoresce green. Ochratoxin A fluoresces greenish-blue, sterigmatocystin fluoresces dull brick red when exposed to long wave UV light and Zearelenone fluoresces a bluish green in short wave UV light.

Patulin and penicillic acid can be made to fluoresce by spraying with 3% aqueous ammonia. Patulin fluoresces pale blue and penicillic acid bright intense blue.

The following visualization procedures have been developed for these toxins –

TLC visualization procedures for trichothecenes

Procedure	Trichothecene	Limit of detection[a] (μg per spot)	Colour
p-Anisaldehyde (MeOH, acetic acid, H_2SO_4 soln.)	Deoxynivalenol	0.05	Yellow
	Diacetoxyscirpenol	0.10	Purple
	T-2 toxin	0.10	Brown
	HT-2 toxin	0.20	Brown
20% H_2SO_4 soln.	Deoxynivalenol	0.05	Yellow
	Diacetoxyscirpenol	0.20	Purple
	T-2 toxin	0.20	Grey
	HT-2 toxin	0.50	Grey
10% Aluminium chloride	Deoxynivalenol	0.10	Blue (fluor)
	Nivalenol	0.10	
	Fusarenon-X	0.10	
4-(*p*-Nitrobenzyl) pyridine	All trichothecenes	0.02–0.2	Blue spots
Nicotinamide/2-acetylpyridine	All trichothecenes	0.02–00.05	Light blue (fluor)

[a] Determined as pure reference standards.

Visualization techniques used for detection of mycotoxins in feedstuff extracts

Mycotoxin	Visualization of spots	Interpretation at	Colour of spot
Aflatoxin B_1		360 nm	Blue fluor.
Ochratoxin A	Treatment with NH_3 vapour (10 min)	360 nm	Blue fluor.
Patulin	Spraying with MBTH solution[a] followed by heating for 15 min at 110°C	360 nm	Yellow fluor.
Sterigmatocystin	Spraying with $AlCl_3$ solution followed by heating for 10 min at 110°C in oven (20 g $AlCl_3$ in 100 ml ethanol)	360 nm	Yellow-green fluor.
Zearalenone	Before spraying with $AlCl_3$ solution for sterigmatocystin	254 nm	Blue-green fluor.
Penicillic acid	Treatment with NH_3 vapour (10 min), followed by heating plate for 5 min at 110°C in oven	360 nm	Blue fluor.
Citrinin		360 nm	Yellow fluor.
Cyclopiazonic acid	Spraying with Ehrlich reagent[b]	Daylight	Violet

[a] MBTH-solution: 0.5 g 3-methyl-2-benzothiazoline hydrazone hydrochloride in 100 ml H_2O.

[b] Ehrlich reagent: 2 g *p*-dimethylaminobenzaldehyde in 100 ml 10% HCl.

Aflatoxins, once formed are somewhat resistant to sunlight, and resist heat up to their melting point of 250 C. Moisture and heat together at boiling or above will degrade aflatoxins as will strong acids, formaldehyde and strong bases. In the general environment, aflatoxin is very stable until other microorganisms gradually degrade it. This makes it a very effective short to mid term potential weapon.

Chapter 7

Mushroom Toxins

There are seven predominant types of mushroom poisonings and related toxins that have been classified and described. This author will confine himself to these seven groups although there are many other toxic mushrooms that could be harvested, or home cultivated and extracted. These seven are –

	Poisonings by fungi reported to CCTU-Munich 1975-1987			
	Total #	Patients	Inquiries	Fatalities
Phalloides syndrome	419	105	315	14
Orellanus syndrome	10	1	1	-
Gyromitra syndrome	-	-	-	-
Muscarine syndrome	23	7	16	-
Pantherina syndrome	90	29	61	-
Psilocybin syndrome	37	1	36	-
Coprinus syndrome	32	3	29	-
Paxillus syndrome	14	2	12	-

Phalloides Syndrome (Cyclopeptide Poisoning)

Phalloides syndrome accounts for about 90% of all fatal cases of mushroom poisoning. After consumption, a latent period of about 6-24 hours passes after which progressive liver dysfunction occurs and requires immediate hospitalization. The later the occurrence of the symptoms, the smaller amount of toxin that has been absorbed and the milder the poisoning and liver damage. The symptoms include sudden onset of nausea, abdominal pain, colic, with vomiting and cholera like watery diarrhea, followed by bloody diarrhea. The symptoms caused by electrolyte loss include lowered blood pressure, rapid pulse, shock, dehydration and leg cramps. This phase can last from 12 hours to 4 days.

There is no fever and a deceptive period of improvement which lasts 12-24 hours. Then the first signs of liver damage become apparent. These worsen according to the amount of toxin absorbed and if serious enough leads to death during hepatic coma in 4-7 days. Otherwise, regeneration of the liver leads to a slow and often complete recovery.

The primary fungi responsible is the *Amanita* species. The most famous is the death cap (A. phalloides) from which the syndrome derives its name. The toxins present form two groups called Amatoxins and Phallotoxins. There are 16 different types of toxins whose chemical structures have been identified in these two groups. Nine are amatoxins of which eight are found in the death cap and all are found in other Amanita species. Purified amatoxins are colorless, usually crystalline solids that are soluble in polar solvents such as water, ethanol, liquid ammonia and methanol. They are insoluble

in weakly polar organic solvents. They are stable at boiling temperatures which is why they continue to remain deadly after cooking. They are resistant to acids and enzymes in the gastrointestinal tract as well. Most of the amatoxins produce LD50's in mice at .3 mg/kg of body weight making them among the most deadly substances known.

Seven phallotoxins have been discovered and are also colorless solids with properties similar to the amatoxins. Their toxicity is somewhat less with LD50 for mice ranging from 1.5-4.5 mg/kg body weight. These are not absorbed from the GI tract and are toxic only by direct administration into the bloodstream, subcutaneously or by conversion to a different toxic form.

A simple test for the amatoxins has been developed called the newspaper test-

[A small piece of the fungus is squeezed out onto the unprinted edge of a sheet of newspaper (which contains wood fibers). After drying the spot, it is moistened with 1-2 drops of hydrochloric acid (25%). If the juice contains more than .02 mg of amatoxins per ml, the spot will turn blue-green to blue after 5-10 minutes].

This tests utilizes the known ability of indole compounds (to which amatoxins belong) to form colored substances with aromatic aldehydes which are liberated from the newspaper by the acid acting on the lignin content.

This test allows a simple method for confirming the mushroom ID and positively identifying the presence or absence of the deadly amatoxins.

Amanita Phalloides (Death Cap) are large, white-spored fungi with hanging ring on the stem and with a large sac-like membranous sheath at the bottom of the stem. The gills are free (not attached to the stem), and white with a slight flesh colored tinge coming from the depths. They are never purplish. The cap is convex to flattened convex, is greenish-olive to grayish-olive or yellowish-green with fine radially arranged fibrils embedded in the surface, but has no scales. The species forms fruiting bodies in deciduous forests.

Amanita phalloides contains amatoxins at up to 3 mg/g of dry weight or about .3%. The LD50 for humans is less than .1 mg/kg body weight so a single mushroom can be fatal.


Amanita phalloides (Death Cap)


Amanita inaurata

Amanita phalloides are found mainly in Europe but many deadly Amanita species are found in North America including A bisporigera also known as A verna (or Destroying Angel), and A muscaria (Fly Agaric), and these are also rich in toxins. Many amanita species contain some amatoxin. Destroying angel can contain up to 5mg/g dry weight of amatoxin.

Other species that produce amatoxins include –

Galerina marginata
Galerina sulciceps
Lepiota helveola
Lepiota brunneoincarnata
Lepiota citrophylla
Pholiotina (all members)

Orellanus Syndrome

The toxins associated with this syndrome attack the kidneys. Early gastrointestinal symptoms are rare and after a latent period of 2-17 days, symptoms of the damaged kidneys appears. This syndrome was only discovered after a mass poisoning in Poland in 1952 because the symptoms took place so long after the mushrooms were eaten.

The symptoms include exhaustion, lack of appetite, intense thirst, headache, dry mouth, burning lips and tongue, polyuria, vomiting, diarrhea, shivering and chills. Later there are pains in the lumbar region and anuria with constipation. There can also be hepatic and neurological symptoms.

The main toxin is called *orellanine*. It is a colorless, polar, blue fluorescing compound that is somewhat unstable and yields the non-toxic orelline slowly at 150C and explosively at 267C. The LD50 for this toxin is 4.9-8.3 mg/kg in the cat and mouse.

The fresh or dry fungus containing orellanine shows a distinct fluorescence under UV light. The toxin is water soluble and is extracted with 50% ethanol and 50% water solvent with stirring for 15 minutes.

Cortinarius is a large genus with several hundred species, some of which are deadly poisonous although a few are edible. In North America, these species include C. rainierensis, C. atrovirens, C vitellinus and other yellow to yellow green Cortinarius.

Cortinarius species are usually quite fleshy, with convex caps. They have a cobwebby partial veilof silky hyphae when young, somewhat fleshy stalks and rusty brown to cinnamon brown spores. Many are mycorrhizal and are found under trees.

Examples of Cortinarius species.

Gyromitra syndrome

Also called helvella syndrome, mycetismus sanguinareus, gyromitrin poisoning and monomethylhydrazine [NMH] poisoning. The toxin is similar to the amatoxins with symptoms delayed and subsequent liver damage. There are also haemolytic and neurological damage. The affected individual may have a slight feeling of unwellness in minor poisoning to death in the higher doses of ingested toxin. Recovery occurs in 2-6 days in non-fatal cases. The fatal cases experience liver damage with the symtoms described for amatoxin, as well as restlessness, crying, delirium, pupil dilation, muscle twitching, convulsions, and in 2-3 days circulatory collapse and respiratory arrest while in coma.

About a century ago, scientists isolated an oily substance from *Gyromitra (Helvella) esculenta*, also known as the false morel. They called it helvellic acid. It was not until the 1960' that scientists discovered this was a harmless substance and isolated the actual toxin. The toxin called "Gyromitrin" is a colorless, volatile, oily liquid at room temperature and forms tetragonal crystals at lower temperatures. It is unstable in water and acid environments which made it hard to extract and work with. The laboratory method for extraction involves heating the fungi with water in a sealed tube for several hours at 120C so that the chemically bound poisons are liberated. It is then extracted with chloroform under a nitrogen atmosphere. The recovery of gyromitrin is in micrograms for each mushroom. The LD50 in humans is estimated at 10-30 mg in children and 20-50mg in adults although symtoms are observed at 35 mcg.

Species containing gyromitra include –

Gyromitra esculenta
Sarcosphaera crassa
Cudonia circinans

Muscarine Syndrome

Symtoms of this syndrome include bouts of sweating (perspiration) with salivation and lachrymation (PSL syndrome). Muscarine poisoning appears within a few minutes to two hours of eating the mushrooms. They can include vomiting, diarrhea, colic, pupil constriction, slowed pulse and asthma.

The toxin is found in many Inocybe and Clitocybe species. It was first isolated in the 1930's from 1250 Kg of fungus that was extracted with methanol (reflux-circulated). After filtration through glass wool, the extract is dried in a rotary evaporator at 40C. This yields a muscarine concentrate. The pure material is achieved through several fractionation steps. Some Inocybe species contain up to .8% dry weight muscarine. The LD50 in mice is estimated at .23 mg/kg. An adult human dose is estimated at 180 mg. The antidote is atropine.

The number of species containing the muscarine is extensive and the reader should consult field guides on mushrooms for identification and recovery of the particular species in their areas.

Pantherina Syndrome

This syndrome is caused by toxin in the “Fly Agaric” and “Panther”. They begin very much like alcohol intoxication and this has caused much self experimentation among mushroom hunters. The latent period is 30 minutes to three hours after which the intoxication begins. Confusion, slurred speech, impaired vision, ataxia, exhaustion, and sometimes anxiety and depression or possibly euphoria will occur. The affected individual may cry, shout, laugh, sing, dance, or rave (as in raving lunatic). It may cause a sensation of floating, superhuman strength, illusions of color and hallucinations have been reported. Tremors, cramps and muscle tremors have also been observed. The syndrome ends in 10-15 hours by deep sleep. The affected individuals rarely remember the experience.

The chemical compound causing the symptoms is íbotenic acid” with secondary metabolites formed from it and contributing. In the monohydrate it forms colorless crystals that melt at 145C that are difficult to dissolve in cold water. On drying, the acid yields muscimol. Muscimol forms colorless crystals which melt at 155-156C (hydrate) or 174-175C (anhydrous) and dissolve readily in water but with difficulty in ethanol and is insoluble in less polar solvents.

Extraction is carried out using 10g fresh fungus soaked for two hours in 10ml of water. It is placed in 100ml of methanol and 1ml of formic acid (which reacts with ibotenic acid to form muscimol) and refluxed for one hour. After filtration, the residue can be further extracted with 80% methanol. The combined filtrates are dried under vacuum at 40C. This yields a concentrate of muscimol and muscazone which are more active than the ibotenic acid.

The Amanita muscaria (Fly Agaric)

The Amanita muscaria on the previous page is well known with its bright red cap and white scales. The swollen bulbuous base of the stem has several warty rings round it. The flesh and spore mass are white. 19th century travellers in Siberia reported that the Fly Agaric was (and still is) ingested to produce intoxication and that the urine is passed around to maintain and recycle the "high".

It has been used as a recreational narcotic in the US and around the world. Usually, the skin of the caps are removed, dried and smoked for a mild psychoactive effect.

Other species containing ibotenic acid and muscimol include –

Amanita regalis
Amanita pantherina (The Panther)
Amanita gemmata
Tricholoma muscarium

Psilocybin Syndrome

The toxins in this group are usually ingested deliberately and are hallucinogens producing symptoms similar to those of LSD. The symptoms occur from 15 minutes to 2 hours after ingestion and include headache, vertigo, confusion, slowed pulse, lowered blood pressure, and numbness. Psychotropic effects accompany these and can result in positive or negative experiences. They range from happiness, liberation of inhibitions, laughing, erotic feelings, hallucinations, altered perceptions of space and time, to anxiety, depression, attacks of rage, violence, and delirium ending in unconsciousness. The action subsides at 6-10 hours, usually without after-effects.

The main compound, psilocybin forms colorless crystals and dissolves in water with an acid reaction. It chemically resembles some of the ergot alkaloids (LSD). One of the accompanying compounds oxidizes and forms blue colored products in the fruit bodies which is an indicator of the presence of the hallucinogens.

Extraction is accomplished by taking 100-200g of dried and ground fungus, shaken with 10 liters of methanol overnight at room temperature. The product is filtered and the liquid dried. This yields a concentrate that can be purified with fractionation. The pure psilocybin produces mild intoxication at 4mg and marked effects t 6-20mg.

Approximately 81 of the recorded 144 Psilocybe species occurring throughout the world are hallucinogenic.

Other species containing Psilocybin include –

Panaeolus cyanescens
Panaeolus subbalteatus and several other Panaeoulus species
Pholiotina cyanopus
Panaeolina foenisecii
Gymnopilus spectabilis
Pluteus salicinus
Pluteus nigroviridus

Coprinus Syndrome

Also known as Antabuse syndrome, acetaldehyde syndrome and Coprine poisoning, these toxins act in combination only with alcohol. After the mushrooms are eaten a single glass of beer can, within a few minutes to 72 hours later, produce an intense reddening of the face, neck, and chest. A feeling of being hot, a metallic taste in the mouth, and tingling in the arms and legs often follows. Palpitations, raised pulse rate, tightness, headache, shortness of breath, disturbance of cardiac rhythm, sweating, a fall in blood pressure and collapse also are observed. The patient usually recovers in 2-4 hours and fatalities are extremely rare.

The principal toxin is called coprine. It is a colorless, crystalline solid that dissolves readily in water and is insoluble in less polar organic solvents. It is stable at room temperatures to weak acids and alkalis but is broken down by them in a few hours at 60C. Coprine is a precursor of the toxin that is formed with the consumption of alcohol. This is what induces the sensitization to alcohol.

Coprine is found in the Common Ink Cap (Coprinus atramentarius). Other Coprinus species include C. picaceus, C. alopecia, and C. insignis.

Paxillus Syndrome

This syndrome is associated with the eating of raw or uncooked Brown Roll Rim (Paxillus involutus). After 1-2 hours, the individual suffers abdominal colic, vomiting, diarrhea and collapse. Haemolytic anemia symptoms appear including subicterus, oliguria, anuria, and renal pain. Usually, these are regarded as a food allergy reaction to individuals whoo have eaten this mushroom for years.

An antigen of unknown structure is present in the fungus and stimulates the formation of antibodies in the blood serum. In subsequent meals, complexes may form that attach to erythrocytes.

Chapter 8

Aflatoxins and other Asperigillus Toxins

Aflatoxin is one of the most potent carcinogens known to man. It is one of the more deadly direct toxins and at sub-toxic levels can damage the immune system increasing mans susceptibility to bacterial and viral disease. It can be mass produced in the field by untrained or novice troops. It can be made undetectable in certain forms and the effects of its covert mass use could rival that of Anthrax, VX, Ebola, and Nuclear Weapons. The ability to create these weapons anywhere, even from bread and water, to mass produce them and to arm large populations with instructions on how to do so commends it as one of the most potent potential armaments in human history.

Because of this, extra care will be given in presenting the technical information in this chapter. Your author offers no pretense that he is an expert in this field. Almost all the information that follows has been derived from university and military sources. By providing the knowledge that they have accumulated, I am able to stand on the shoulders of giants. These experts and the knowledge that they have provided will enable all people to arm themselves in times of upheaval and great social change.

The aflatoxins will be covered as follows –

1. Introduction and history
2. Aflatoxin producing species
3. Aflatoxin production
4. Separation and purification
5. Toxicology and animal testing for measuring toxicity
6. Other Asperigillus Toxins

1. Introduction and history

Although the introduction to mycotoxins (chapter 6) gave a good basic account of the history and effects of aflatoxin, we will cover some of the same ground here in more detail.

Most of the early discoveries of toxins from molds came about from drug companies screening of mold samples for antibiotics following the discovery and worldwide use of penicillin. Many molds produce toxins that have never been published because the information from screening all the known strains of molds remains in the private hands of drug companies until it can become useful to them. It is known that almost all fungi produce some level or type of substance that is toxic in some amount to humans and other animals.

A significant range of data began to accumulate, starting in the 1960's on the aflatoxins and other toxins produced by Asperigillus species. Huge livestock losses and related threats to human health caused much basic research to take place in the incidence, formation, biology and chemistry of this group of toxins.

The primary producer of aflatoxins is Asperigillus flavus. A mold that is widely distributed in the soil and almost every grain seed on the planet. Members of this species are broadly identified by the production of greenish-yellow spores and the absence of ascospores.

It has been learned that aflatoxins cause consistent liver injuries in all mammal species tested although some are more resistant than others. They cause mutations, immunosuppression, and can mutate themselves into different toxin producing strains. The earliest aflatoxins discovered were B1 and G1 and were so named because the B group would fluoresce blue under long wave ultraviolet light. The G group would fluoresce green. Other toxins designated B2 and G2 were soon discovered. Both of these, chemically were dihydro derivatives of the B1 and G1 toxins. When these are dehydrated under certain conditions, or exposed to certain other microorganisms, they have converted from B2 to B1 and G2 to G1. The first form is the most toxic by far. When the toxin is ingested by cows it is excreted in their milk in a modified form that is designated M1 and M2. When ingested by humans, we excrete the M1 and M2 forms in our urine which allows medical laboratories to make quick identification of exposure to aflatoxins.

Chemicals that are used to destroy aflatoxins include bleach, alkali's, strong acids, and oxidizers. These are used to maintain sterile and sanitary (safe) conditions in labs that work with these materials.

2. Aflatoxin Producing Species

Almost all strains of A. parasiticus produce aflatoxins. Most produce all four toxins. Some are prodigious producers and some produce small amounts. A. parasiticus is primarily a tropical mold but is found in the southern US on various cereal crops, peanuts and cottonseed. The primary producer of aflatoxin is A. flavus. This species varies greatly on the type and amount of aflatoxins produced. Almost all produce B1 and the few that do not usually produce B2 and G1. Most strains also produce B2 but only about 9% of all strains tested produce all four toxins. A. globosus also has variants that produce all four toxin types. A study in Israel in 1969 analyzed 1,626 isolates of A. flavus from peanuts and soil and found that 90% produced aflatoxin B1.

Other studies have shown that almost all strains that produce G1 will produce B1 but not the reverse. Many other strains have been reported to produce aflatoxins including –

A. Niger	Penicillium citrinum
A. Ruber	P. frequentans
A. wentii	P. variable
A glaucus	P. puberulum
A. oryzae	P. expansum

The easiest way to initially identify and quantify the presence and amount of aflatoxin is to observe the mold growth under black light. The toxin coverage and presence can be roughly judged by observation alone. This method is about 95% accurate with a few strains producing other toxins or substances that fluoresce also.

The easiest way of obtaining aflatoxin producing strains is by purchasing cottonseed, peanuts, corn, wheat, and/or rice or their respective meal, flour, or cake forms and moisten them to grow the species on them directly. Almost all of these grains contain A. flavus spores and some, especially those originating near the tropics may contain A. parasiticus. A simple test with a black light (long wave ultraviolet lamp) can confirm the presence of the toxin visually and there is a good correlation between the amount seen and the actual amount of toxin present. The mold growth and spore production can also be observed directly. American grown corn almost universally contains A. flavus spores (app. 80% of samples tested). Dried sweet potato, sorghum, oats, dried spaghetti and almost all other cereal crops and their consumer products have been found to carry A. flavus spores at some level. Almost all peanuts and peanut products will contain aflatoxin producers although the spore counts may be low. This includes peanut brittle, peanut butter (over 50%), and other peanut products.

In soil and forest tests in which samples were taken at random in Georgia, A. flavus was recovered and grown on 10-27.5% of the isolation plates. In samples of air taken from chick hatcheries, A. flavus accounted for 64.3% of the 10,440 fungus spores isolated. A 1956 test showed that 27% of all unblemished peanut kernels were invaded by, and contained A. flavus.

In 1979 in Georgia, tests were conducted in which whole, untreated, surface disinfected grains of corn were examined. The kernels were taken from the tip, middle and bottom of the ear at first silk and weekly to 60 days after silk. The kernels were incubated to support growth of Asperigillus. At full silk, none to very few of the kernels were colonized. At 60 days after full silk (maturity), 50-75% of the kernels were colonized. A, flavus was the colonizer in 92% of the samples and A. parasiticus in 8%. A handful of contaminating bacteria and other fungi were discarded and not included in the test. Kernels with insect or other physical damage had much higher rates of colonization. It has also been found that the mold tends to invade and concentrate around the germ end of the kernel.

In an interesting test in the early 1980’s it was found that the Aspergillus species growing on corn from the southeastern US generally had much higher levels of aflatoxin production in the grain they colonized than those found in the midwest. This may have been due to factors relating to moisture, humidity and growing season.

The different species can be isolated and grown in culture media until the desired best producers can be selected. A. flavus and A. parasiticus are found in nearly every

field of cereal grains on the planet, either on the grain itself or in the soil. A. flavus has even been cultured from 40 year old samples of peanuts. Soil samples from cereal crop fields can also be used to inoculate a moistened grain for testing and isolation.

A flavus and A. parasiticus are differentiated by the sterigmata which is bisereate in A flavus and unisereate in A. parasiticus.

3. Production

Some strains of A. flavus do not produce aflatoxin, although most will to some extent. The strains are genetically different and under the same conditions some will produce much more aflatoxin than others. Other factors under the control of the lab technician (and nature) which influence aflatoxin production levels are moisture, temperature, substrates (food), aeration and growth factors.

Moisture affects the sporulation and growth of A. flavus more than most molds.It is the dominant species of mold found in corn stored at 30C with a relative humidity of 80%. It was also the most common in stored grains at 16.2-24.4% moisture. It would not invade corn samples at 17.5% moisture but extensively grew at 18.5% in a separate research test. In wheat there was little growth at 14% moisture and moderate growth at 16%. Other Asperigillus species would grow at 13-14% moisture but the A. flavus was the only one that invades and deteriorates most grains, using them as substrate. This is why most grains, especially corn are dried to 13% moisture or less at harvest.

Tests of aflatoxin on corn show that moisture content of 18-19% at a temperature of 20-25C support abundant production of the toxins. Research from the 1960's has shown that aflatoxin production occurred at –

Hum.	Kernel Moisture	Temperature	Aflatoxin mg/kg B1	B2	G1	G2
83	12.2%	30C	Trace	Trace	Trace	Trace
85	9.3	30	0	0	0	0
87	10.9	30	7	6	18	2
87	13.6	30	125	67	320	250
89	14.8	30	15,700	4,700	39,700	8,000
92	15.7	30	2,140	1,330	2,140	833
99	14.2	30	26,660	20,000	25,700	10,000
99	32	30	10,130	13,330	8,530	6,670
97+	16.9	12	0	0	0	0
97+	19.1	20	84,200	19,900	213,300	46,600
97+	14.7	30	94,900	33,300	106,600	20,800
97+	13.1	40	2,500	1,000	1,000	166
97+	12.3	46	0	0	0	0

Other tests show that toxin yield is abundant at kernel moisture content of 23-34% with dramatic reductions above or below these levels. High humidity almost always fosters toxin production and mold growth at some level. A test on stored rice showed that A. flavus, when incubated at 80F with 26.2% moisture, required 6 days to reach aflatoxin production of 30 ppb (parts per billion). When the moisture was reduced to 22.6%, it required 9 days to reach 30 ppb. At 19.8% moisture, only traces of toxin were produced. Maximum production on rice usually develops at 20-22% moisture after 15-21 days of incubation.

In laboratory tests, the moisture content is usually achieved by soaking the grain for one hour and then using towels or straining to remove free water. Different grains will take up different quantities of water and in some cases, two hours of soaking may be necessary.

Temperature affects A. flavus. It is classified as a mesophilic fungus which grows at 6-8C minimum, 36-38C optimum, and 44-46C maximum. A. flavus produces aflatoxin between 11C and 37C with the optimum range for maximum production for rice of aflatoxin B at 28-32C and 28C for G toxin. Groundnut production peaked at 30C for both A. flavus and A. parasiticus. In various testing conducted throughout the last 40 years, there is wide variation in the results. Generally, A. parasiticus strains produce more aflatoxin than A. flavus species under the same conditions. Aflatoxin B levels peaked at temperatures of 24C while maximum mold growth took place at 29-35C. The levels of B vs G production also varied with temperature with B produced in greater ratio at higher temperatures ideal for the mold growth. This was believed to be due to accelerated catabolism of G toxin at the higher temperatures.

Short exposures to high temperatures (40-50C) during incubation at an average of 25C retarded toxin production. Short exposures to cold temperatures (10C) had no effect. In a 1969 test at refrigerator temperatures of 7.5-10C, strains of A. flavus produced significant toxin quantities after 3 weeks. [70F=21.1C, 90F=32.2C]

Aeration is important since all fungi and mycotoxin producers are aerobic and require oxygen to grow. A. flavus does not grow under 100% nitrogen or carbon dioxide atmospheres. It does not die either. It returns to growth as soon as a suitable atmosphere returns. Increased levels of CO2 gas up to 20% does not reduce mold growth but above 20%, sporulation and growth are inhibited. Aflatoxin production declines as CO2 levels increase from 0-100%. Both mold growth and toxin production declines dramatically as oxygen content declines from 5% to 1%.

Studies indicate that toxin yields increase when the samples are shaken during incubation (which increases aeration). Individual kernels will yield higher levels of production than those bound up in a mycelial mass together. It was also found that toxin production increased by up to 10 times when medium is shaken as compared to static. Aeration is crucial in liquid mediums and the normal production range of toxin is 20-30mg per 100 ml of growth medium when aerated and agitated. This means that aflatoxin can be mass produced in fermentors when aerated properly.

Substrate that is used to feed the Asperigillus influences the toxin production. In 1968, it was discovered that unidentified growth factors exist in peanuts that improve toxin production. When 6 mg of Vitamin E was added per 100 grams of peanuts, the toxin accumulation more than doubled from 310 ppm to 720 ppm. Vitamin E appears to serve as a structural precursor to part of the toxin molecule (coumarin nucleus). Adding yeast extract (.7%) or dried yeast products also provides unidentified growth and toxin factors.

The following feed sources have been tested for aflatoxin production –

Substrate	Incubation(days)	Temperature	Toxin B1(mg/kg)B2		G1	G2	Total
Peanut	7	30					133-650
Yeast (Liquid)	10-14	25	11.5		100		
Peanut	10-14	25	17		61		
Wheat	10-14	25	55		96		
Sorghum	6	28	84	67	80	25	256
Wheat	6	28	336	89	916	143	1,484
Peanuts	6	28	152	40	256	40	488
Soybeans	6	28	8	4	96	1	109
Rice	6	28	253	100	213	25	591
Corn	6	28	164	33	321	33	551
Wheat	7	30	720	60	200	20	1,000
Peanuts	7	30	250	30	160	20	460
Cottonseed	7	30	690	160	490	110	1,450
Coconut	9	24					8,788
Rice	9	24					1,563
Sweet Clover	6	28	18.4	2.3	25.4	2.0	48.1
Oat Straw	6	28	34.1	3.4	81.5	4.0	123.0
Cheddar Cheese	10	Room Temp	50		50		100

Mold growth and toxin production occurs in both cottonseed meal and hulls although the meal supports higher production of both. The fresh grated coconut in the above tests showed it to be by far the most efficient medium for producing aflatoxin of all substrates tested. This is believed be a result of the coconut fatty acids, carbohydrates, and/or a toxin stabilization factor. Shredded wheat biscuits (bite size) have also been used as good growth substrate in laboratories.

A. flavus has also been grown and produced aflatoxin on -

Peanuts	Grapes	Bread	Cocoa Beans
Potatoes	Cantaloupe	Peaches	Cheese
Beef Infusion	Grape Juice	Grapefruit Juice	
Orange Juice	Pineapple Juice		Apple Juice
Apple Juice	Vegetable Juice (V8)		Tomato Juice
Apricot Nectar	Peach Nectar Butter		Margarine
Red Pepper	Cassava	Stored Meats	Egg Solids

Skim Milk Powder	Wheat Meal	Corn Meal	Soy Meal
Peanut Meal	Sesame Seed	Hazelnuts	Almonds

The addition of corn steep liquor to liquid mediums (8%) maximized yields for A. parasiticus to 100-200mg toxin/ml. An addition of 1% peptone, .4% citric acid and 5.8-8.6% glucose maximized production in liquid artificial medium for A. flavus. Other mediums were enhanced to maximum production with 2% yeast extract added. Oilseeds tend to allow lower production of aflatoxins because the oil content is not immediately available for synthesis by the organisms.

Ammonium Sulfate and Potassium Nitrate are the best inorganic sources of nitrogen for toxin production. Natural occurring amino acids support less than optimum production, with the exceptions of yeast extract, peptone, and other casamino acid bearing substrates which always support good toxin yields.

Best toxin production in liquid or artificial media occurs with sucrose, fructose and glucose as carbon sources. The ideal nitrogen sources were organic sources peptone and yeast extract. The addition of Zinc at .4% was required in artificial media when ammonia was used as the nitrogen source for maximum yields. In natural media, added zinc at .4ppm was beneficial. Thiamine and Biotin were the only B vitamins capable of stimulating toxin production while the addition of ethyl alcohol (ethanol) at 1-4% dramatically increased aflatoxin yields. Toxin producing strains of A. flavus form ethanol and subsequently uses it in its metabolism.

Another important factor in toxin production is the time of incubation. Maximum toxin yield is usually achieved after 5-12 days of mold development followed by a distinct decline in aflatoxin level. On most solid substrates, optimum toxin levels are followed by a reduction in toxin to a uniform level in a few days. At 10-15C, A. flavus takes up to 3 weeks for mold and toxin development.

Exposure to sunlight also reduces aflatoxin production during incubation. This suggests that opaque media be used to screen the mycelium from light sources (grown on the bottom). Extreme acid conditions (<pH of 1.0) also reduce toxin viability. The toxin is produced in high levels at pH of 5.0-5.5 and continues to be produced below 4.0 Aflatoxin synthesis is also lowered when the inoculum had been subcultured for less than 7-11 days or more than 25 days. This means that the age of the inoculum exerts an effect on toxin yield (for the same strain).

4.) Separation and Purification

Aflatoxins are usually produced on and agricultural commodity like corn, wheat or rice. The toxin is usually extracted with a solvent and then precipitated with hexane, however, the extraction procedures vary from substrate to substrate because of the other materials present in the sample. The amount of toxin is usually small, in the microgram to milligram per kilogram of sample. Distribution may be uneven in the sample due to differences in the mixture, aeration, humidity, and moisture. The fungus should be killed before the aflatoxins are extracted because the spores may contain aflatoxin and could be inhaled and germinate in the lungs.

Peanuts and Coconut are extracted by reducing the product to a paste and then using chloroform to soak into the media and solubulize the toxin. The chloroform is separated and evaporated. The solids are then washed with hexane and then ether to remove lipids. The aflatoxin can be dissolved into a 3% methanol and 97% chloroform mix.

Corn is extracted with chloroform in the presence of water. It is cleaned up with hexane extraction, and lead acetate precipitation.

Cottonseed is extracted with aqueous acetone, and lead acetate treatment to remove gossypol pigments. (Cottonseed fluoresces greenish-yellow and foreign matter may fluoresce blue.)

Coffee, Tea and Cocoa are extracted with 25% Silver Nitrate solution. It is defatted with n-hexane and chloroform may be used and reshaken.

Aflatoxins can also be dissolved into methanol 30% chloroform 70%, water chloroform mixtures, and water-acetone-chloroform mixtures. The addition of water or benzene to the solvents often improves the extraction results. Extraction typically requires 1-6 hours to liberate the toxin from mycelium and spores.

Other extraction mixtures include –

Acetone 54%, hexane 44%, water 2%
Acetone 70-90%, water 10-30%
Hexane 79%-ethanol 21% (peanut meal)
Hexane 73%-methanol 27%
Hexane 41%, acetone 59%
Hexane 85%, ethanol 12%, water 3%
Hexane 44%, Acetone 55%, water 1%
Isopropanol 80%, water 20% (oilseed meals)
1% sodium bicarbonate or 1% calcium chloride (peanut meal)

The 1% sodium bicarbonate dissolves 33% of the protein while the calcium chloride dissolves only 6% making it the preferred choice in most extractions.

Aflatoxins are bound to constituents in various meals that are water soluble which requires the extraction of these materials as well, often using a small amount of water with the solvents.

5) Toxicology and Animal Testing for Toxicity

The amount and potency of any given sample containing aflatoxin can be measured by exposing it to animals in a variety of tests.

Ducklings are the most susceptible of the laboratory animals to aflatoxins. The toxin bearing sample is measured and dissolved in water or propylene glycol. This is given by capsule or stomach tube to one day old ducklings. The oral 7-day LD50 of aflatoxin B1 is 18.2 mcg, B2 is 84.8 mcg, G1 is 39.2 mcg, and G2 is 172.5 mcg. The dose is diluted or increased (usually in tenfold increments) until the survival rate is app. 50% and then the level in the sample can be calculated. Aflatoxin M1 has an LD50 of 16 mcg and M2 is 61.4 mcg.

Embryonated Eggs can be used to measure aflatoxin. The aflatoxin bearing sample is injected into the yolk of 5 day old chicken embryos. The toxin causes death of the embryo at levels of only $1/200^{th}$ of that of the ducklings making this test much more sensitive. Very small amounts of aflatoxin can be measured in this manner. The best results come from toxin injection into the yolk or air cell before incubation. Toxicity is greater with injection via the air cell route than the yolk. The toxicity is measured by mortality at time of hatching. The LD50 for toxin B1 at 21 day incubation is .048 mcg for the yolk and .025 mcg from the air cell route. G1 toxicity is 60% in 21 days at 1 mcg. Nonsurviving embryos show severe growth retardation, edema and hemorrhage in most cases. Mottled and granular liver surface, short legs and slight clubbing of the down is also observed.

Nine day old chick embryos incubated in egg cartons were much more sensitive to toxins incubated on cotton padding or in a commercial incubator. The LD100 for B1 in carton incubated embryos was .01 mcg while it was greater than 5 mcg for the commercial incubator. The reasons are unknown.

Trout are very sensitive to aflatoxin. It requires only 1 mcg per ml of B1 to kill all embryos in 72 hours. They show abnormal movement within a few minutes in exposure to 1 mcg toxin per ml and are all moribund in 5-6 hours. 30 Hours are normally used for an LD 50 test.

Dried aflatoxin is elctrostatic and readily attaches to dust particles. This produces great risk to handling in concentrated and pure samples. This property also strongly enhances dust based weapons. The use of “glove box, hood, or double ziploc bags is recommended for laboratory workers. You should also use a face mask for safety purposes. The inhalation or ingestion of milligram quantities can be fatal by direct effect

(liver damage) or induced cancer. If some is accidentally inhaled or ingested, the mouth should be treated with 1% sodium perborate and sodium bicarbonate solution. The stomach should be pumped. Exposed skin tissues should be washed thoroughly and immediately with undiluted bleach followed by soap.

The fungal spores, if inhaled are also a danger. In farming and grain elevator conditions where repeated and long exposure to asperigillus occurs, asperigillosis can become a problem. This can also be useful as a long term invisible weapon.

In 1964, rhesus monkeys were tested. Two male monkeys were fed .5mg of aflatoxin daily for 18 days and then 1 mg daily until they died. Four monkeys were dosed with 1 mg/day via stomach tube and two monkeys were used as controls. The two male monkeys on the low dose died at 32 and 34 days. The higher dose monkeys died between the 19^{th} and 27^{th} days. All of them showed anorexia and drowsiness leading to coma during the last few days. Liver damage was much more severe in the high dose animals which also had measurable kidney damage. Until these tests were completed in the mid 1960's, no substance tested had ever caused production of hepatic fibrosis and cirrhosis in primates by either dietary or other toxic means.

Monkeys receiving as little as 100 mcg of aflatoxin per day developed fatty livers and biliary fibrosis within 16-30 days on low protein diets. High protein appears to provide primates with some protection from small levels of aflatoxin. In Africa, south of the Sahara, there are areas where aflatoxin is found sporadically in mold contaminated porridges and brews. There is a high incidence of liver cancer and cellular disturbances in these areas. More than 50% of all cancers are liver tumors which occurs at rates of 5-50 times higher than that found in the United States.

The only direct human fatality results of aflatoxin exposure are from accidental ingestion. In Taiwan in 1968, three children died after consuming moldy rice containing 18-22 mcg of aflatoxin B1. Autopsies were not performed. Studies of workers in oilseed plants who inhaled spores and aflatoxin contaminated dust particles had higher mortality and respiratory cancer rates than did the general population. There is also evidence that individuals exposed to aflatoxin B are more susceptible to hepatitis B virus.

The cancer causing ability of aflatoxin B1 has been measured in rats and other mammals. The following are results for liver tumor induction in rats. Other carcinogens were also included in the test and all others required doses of 10 times or moree to produce similar effects.

	Mcg/day dose	Days fed	Total dose	Tumor Frequency
Aflatoxin B1	12	245	2.9mg	80%
	4	245	1mg	14%
	.4	364	.15	54%
	.2	364	.07	0%
	.2	476	.095	100%

The results of the last two pairs clearly indicates that prolonged exposure of incredibly small amounts will induce tumors where the same or larger doses shorter term did not. Applied to weapons and humans, large scale warfare could take place for over a year with no one aware a war had taken place. The result could still be 100% casualties with low level exposures that would be nearly immeasurable in the general environment.

Tumor incidence in rainbow trout as measured in parts per billion are –

	Levels	Days Fed	Tumor Frequency
Aflatoxin B1	7.9 ppb	365	42%
	4 ppb	365	15
	.8 ppb	365	0
	.8	605	10
	42	14	60
	42	28	75

It has also been found that aflatoxin inhibits germination in seedlings as well as causing chlorophyll deficiency and albinism.

Aflatoxins also have immunosuppressive activity. It binds to DNA, suppresses DNA dependent RNA production and in this manner interferes with transcription. Basically, they inhibit protein synthesis. The net specific effects are that aflatoxins suppress phagocytosis by macrophages,, cause thymic aplasia, suppress cell mediated immunity and formation of humoral substances related to resistance and immunity and impairs immunogenesis.

The effect seen in test animals is that they become more susceptible to a range of other diseases. The potential use of aflatoxins in combination with other types of microorganisms (bacteria and fungi) and chemicals to induce disease is obvious. Aflatoxins may act as an enhancement at the site of any disease initiating system by suppressing the immune reaction.

6 Other Asperigillus Toxins

Other strains of Asperigillus produce various toxins. The most important of these include –

Ochratoxin and related dihydoisocoumarins
Aspergillic Acid
Kojic Acid

Out of concern for the aflatoxin problem, other species were examined. It was discovered in 1961 that three out of five strains of Asperigillus ochraceus produced a different type of toxicity. A ochraceus occurs widely in nature and is found worldwide on decaying vegetation and in soil samples. A highly toxic sample was recovered from sorghum in these tests and was maintained on sterile soil. Sterilized corn was used for large scale cultivation of the strain and its metabolites.

The main toxic component was “Ochratoxin A”. Related derivatives were also discovered but were minor in comparison. Ochratoxin B was also isolated but was less toxic than its “A” counterpart. Ochratoxin A was found as a natural contaminant of poor grade corn. Soon two new species, A. sulphureous and A. melleus were found to yield “A”. In 1968, a penicillium species was isolated from a surface growth on packaged ham and found to produce “A” as well.

Five strains of A. ochraceus from peanuts were tested in 1969. They were grown on sterile, moist corn and fed to day old Babcock cockerels. Two were highly toxic, two were moderately toxic and one strain had no effect. The A. ochraceus would invade grain with a moisture content of more than 16% at 20-25 C.

Ochratoxin A was produced in bulk on moistened sterilized cornmeal. The dried moldy meal was extracted with 50% chloroform and 50% methanol over 72 hours. The toxic extract (about 10% of the moldy cornmeal) was taken up in the chloroform and washed with water. The chloroform layer was extracted with aqueous sodium bicarbonate. The aqueous phase was acidified and then reextraced. This yielded a neutral and acidic fraction. The lipid material was removed from the neutral fraction with benzene and glacial acetic acid (25:1) as mobile phase. The fractionation of the crude extract was tested on day old ducklings. The LD50 for “A:” was 25 mcg/duckling. Ochratoxin B has an LD 50 of 135-170 mcg/duckling. The LD50 in rats is about 20 mg/kg.

The pure toxin is a colorless, crystalline compound that will crystallize with benzene and contain one mole of benzene. They melt at 94-96 C with loss of the benzene.

A production study of Ochratoxin A was undertaken in 1970. A high toxin producing strain of A. ochraceus was cultivated on shredded wheat (100 gm) in 2.8 liter flasks at 72 F. At water levels of 40-70 ml/100gm shredded wheat the toxin production averaged 239 mg/100gm shredded wheat. Production rates were higher on solid media and it was found that wheat, rye, rice, buckwheat, soybeans and peanuts can all be rendered toxic to experimental animal by inoculation with a toxic strain of A. ochraceus.

Asperigillus flavus was discovered long ago to produce a substance with antibiotic properties. It was the first one noted and published after the discovery of penicillin and the authors named it “aspergillic acid”. Different strains of A. flavus serve as sources for this material.

The substrate used to produce it is different than that used for maximum mold growth. The best nitrogen sources are corn steep liquor, peptone and tryptone. Casein hydrolyzate also is used. Glucose, brown sugar or lactose also stimulated growth and added to the antibiotic titer. Yields vary from 5-300mg of crude crystalline material per liter of culture filtrate. A simple medium of 2% difco yeast extract and 1% glycerol also produced good yields. The initial pH of the medium was 6.3-6.6. A heavy inoculum of spores initiated growth and in 48 hours at 25 C, a heavy, white, wrinkled pellicle had formed. The pH and antibiotic titers continued to rise until day 6-7 where a pH of 7.8 was observed.

In stationary cultures, after removal of the initial culture broth, the intact, unfolded mycelium mat may be used for additional production by re-flooding the medium.

Acidified culture filtrate can be extracted with chloroform followed by concentration of the solvent and extraction of the antibiotic with sodium bicarbonate solution. The crude aspergillic acid is precipitated by acidifying the bicarbonate solution. This concentrate is then dissolved in boiling hexane and filtered. The solution is then concentrated to allow separation of nearly pure aspergillic acid crystals that melt at 90-95C. Further crystallizations from acetone or methanol can be carried out if required.

The crystals occur as yellow elongated rods and have a sharp characteristic odor similar to black walnuts. They are soluble in many organic solvents including ether and ethylene dichloride and are slightly soluble in water which increases with heating. Because it is acidic, it is soluble in dilute sodium bicarbonate and sodium hydroxide. Aspergillic acid reacts to form salts with silver and copper. There are many derivatives and metabolites related to aspergillic acid which have been published and studied. The substances have strong antibiotic properties towards many disease causing bacteria and is also toxic. The LD50 in mice is 25 mg/kg.

The fungus that is used in oriental food preparations for centuries as a starter inoculum is called “koji”. In 1907, in one of the earliest toxic extracts for a fungus ever recorded, a toxic substance was removed as a filtrate and given the name “Kojic Acid”. More than 20 asperigillus species as well as penicillium and other molds produce kojic acid.

Substrates used for production of kojic acid have included ethanol, glycine, acetate, rice (from which it was isolated) and corn. The optimal carbon sources for fungus and acid production are glucose and xylose. Nitrogen is limited and carbon sources should not exceed 10% of the formula. The acid is usually detected in a few days after fungal growth commences and peak production is reached by 10-20 days. Most of the aspergilli grow luxuriantly at 25-30C which is suitable for acid synthesis. The optimal pH is 2-3 and small upward changes tend to reduce yields sharply.

The acid is recovered through acidification of the culture broth and extraction into ether or other solvents. Neutralized solutions permit precipitation of the acid with dilute copper sulfate to form an insoluble complex with characteristic rhombic, light green crystals. The kojic acid will form many other salts and metal chelates.

Kojic acid is soluble in water and lower alcohols at 5-7% at 60C. It readily dissolves in acetone and ethyl acetate but is less soluble in ethyl ether, pyridine and chloroform.

Kojic acid has strong antibiotic properties including effectiveness against tuberculosis. It is lethal at 30mg given intraperitoneally in aqueous solution to 17 g mice. It is 100% lethal to 12 day old chick embryos at 12 mg/100gm egg weight. In tests on mammals including dogs, kojic acid acts as a convulsant and produces seizures similar to epilepsy in man. Those animals that did not go into coma usually survived. In other tests, kojic acid killed human leukocytes in 3 hours in 1% solutions. It also produces cardiotoxic effects on frog hearts which discouraged human testing trials.

Chapter 9

Trichothecenes (Yellow Rain) and Fusarium Toxins

Trichothecenes are a group of chemically related toxins produced by various fungi, most notably by *Fusarium* species. These toxins and others produced by Fysarium will be covered in the following sections –

A) Introduction & History of Trichothecenes
B) Organisms That Produce Trichothecenes
C) Toxin Production
D) Toxin Separation & Purification
E) Toxicology
F) Other Fusarium Toxins
    Zearalenone
    Moniliformin
    Butenolide
    Fusarins
    Stachybotryotoxicosis

A) Introduction & History of Trichothecenes

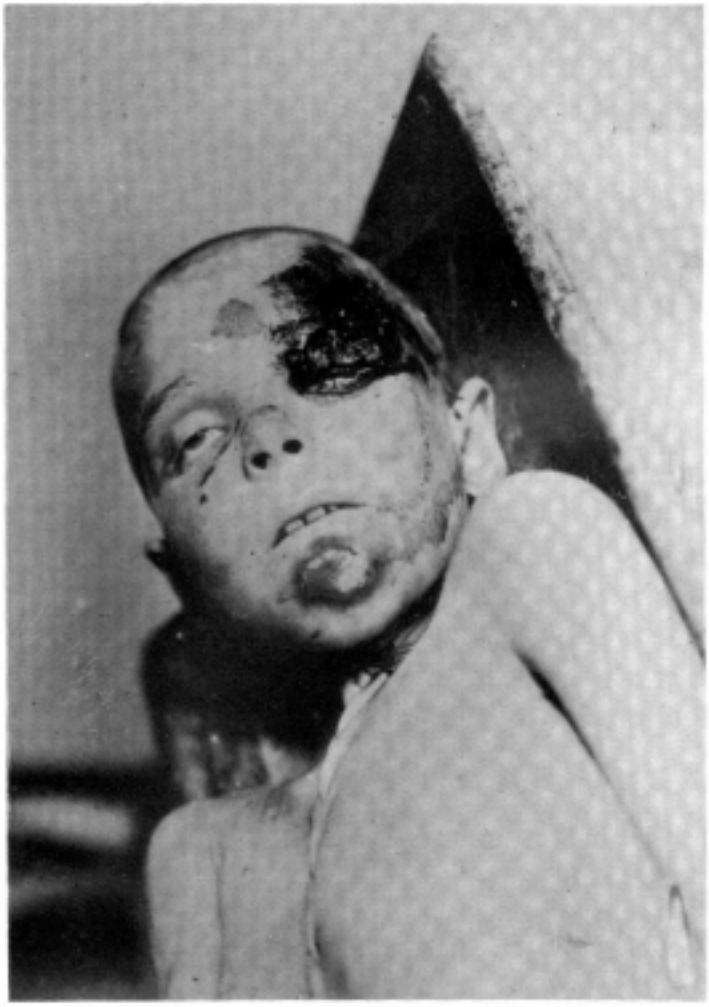
Various species of Fusarium, Myrothecium, Trichoderma, Cephalosporium, Verticimonosporium and Stachybotrys are considered plant pathogens, whereby they invade various agricultural products and plants. These species also produce a group of chemically related toxins when growing on these plants and tissues that are collectively known as **Trichothecenes**. They cause various intoxications when they are consumed, some of these being quite famous.

The earliest well documented report of this came from the Ussuri district in Siberia in 1891 in which “staggering grains” occurred. People who consumed grains and their products exhibited headache, vertigo, chills, nausea, vomiting, and visual disturbances. Farm animals refused to eat the grain and even dogs were affected. It was also described in the literature as “drunken bread” for those who ate bread made from the suspect grain.

In 1931, massive intoxication of horses, swine, poultry and cattle developed in the Ukraine with similar fatal cases recorded in Eastern Europe. The symptoms included shock, somatitis, dermal necrosis, hemorrhage, nervous disorders and death from respiratory failure. It was associated with moldy fodder. In the spring of 1932, it became endemic with human illness in several western districts of Siberia. Human symptoms included bleeding from the nose throat and gums, necrotic angina, fever, sepsis, and exhaustion of the bone marrow. At the time, the symptoms could not be reproduced in laboratory animals making the actual cause a mystery.

In 1942-1947, and mainly in 1944, over 10% of the population of Orenburg near Siberia died from the consumption of overwintered wheat, millet and barley. These grains had been left under the snow during the winter and were harvested or grazed following snow melts as had been the practice for decades. Near famine conditions existed in many parts of the Soviet Union in 1944 due to the scorched earth practices of nations at war and this contributed to the use of the remaining feedstuffs.

A variety of fungi that could live at these temperatures infected the grains and produced unknown toxins that caused vomiting, skin inflammation, diarrhea, leukopenia (loss of white blood cells), multiple hemorrhage, necrotic angina, sepsis, and exhaustion of bone marrow. This condition was given the name “Alimentary Toxic Aleukia” (ATA) although its cause was unknown at the time. ATA affected entire families and communities with mortality rates reaching as high as 60%. which made it appear as if an infectious disease was at work. The toxic fungi developed in over-wintered grains, which remained under the snow all winter. They are harvested only after the snow melts in the spring. It is during this period that that the fungi produce the toxin.



. Necrotic lesions around the eye and on the face in a child who died from ATA.

The occurrence of ATA usually occurred after the consumption of about 2 Kg og grain with near 100% fatalities associated with 6Kg consumption. The first symptoms would appear at 2-3 weeks with death occurring at 6-8 weeks. Millet and wheat were the most toxic grains and those grains harvested after the spring thaw were the most deadly. It was also noted that the higher the elevation (above sea level), the less the toxicity. The incidence of the disease was reduced in areas where humans washed the grains in boiling water before grinding. Some of the toxins were extracted and washed away with the water.

In 1958 and 1959 in the Ukraine, moldy grain related illness affected a horse population and quickly spread to thousands of cattle. From the 1940's to the 1980's, a similar disease afflicted wintered horses in Hokkaido, Japan. They were fed bean hulls and exhibited convulsions, disturbed respiration, decreased heart rate, and retarded reflexes. Ten to fifteen percent of the afflicted horses died in 2-3 days. Studies began on the fungi populations that could grow at near freezing temperatures and this would soon reveal the true causes of the epidemics. Test plots were deliberately infected with fungi, mostly strains of Fusarium poae and F. sporotrichioides. These trials were finally able to reproduce the ATA symptoms in animals.

Samples of grain were taken at harvest and the stored grains were used as controls. Some of the grain was stored deliberately in the soil and under the snow. Grain was also stored in warm, near freezing and subfreezing conditions in the laboratory. Soil samples were also taken periodically to investigate which fungi could grow best in the grains at low temperatures and produce toxins. These were also grown on solid agar and identified. They also yielded toxins on the agar. Members of the Fusarium species were the predominant toxin producing organisms.

These various diseases became known by different names. Moldy corn toxicosis in the United States, Red Mold and bean hull poisoning in Japan, Stachybotryotoxicosis and other labels in the Soviet Union and Eastern Europe. All of these would soon be attributed to a common cause.

By 1970, the cause of the illnesses were finally identified. Various species of fungi that had grown on the respective grains had been cultured and extracted. These extractions were then tested, chemically purified and found to all be members of a family of closely related "sesquiterpinoids". All of these contain a ring system named tricothecane and hence the name "trichothecenes" was born. There is a chemical group at a location called C12 and C13 so in the chemical and biology books, this class of toxins is called "12,13 trichothecenes". There are 37 known toxic trichothecenes that have been isolated and tested from the various fungi.

The extracts of these compounds are stable solids. They can react in solution at extreme pH ranges. Their esters (alcohol forms) are saponified by treatment with alkali without affecting their toxicity.

In 1972, 20% of a Wisconsin dairy herd died from eating moldy corn. They found the toxin T-2 at 2 ppm in the corn and recovered the fungi Fusarium tricinctum in the corn. In 1974, tests conducted on field corn at elevators in the mid-western US found that 54% of 173 samples contained .5-1 mcg of trichothecenes that irritated the skin of test animals directly. It has since been found that the toxins are present in parts per billion in almost all types of feeds and many feed extracts will cause dermal irritation when applied to the skin of rats or guinea pigs. Acute tests on the dermal injuries caused by the trichothecenes in the 1970's show that they resemble radiation, alkylating agents or nitrogen mustard (poison gas).

In the areas of Japan facing the Pacific Ocean, the "red mold disease" has been prevalent for over a century. It usually coincides with the rainy season resulting in outbreaks of Fusarium and resulting intoxication's of humans and animals who ingest the grains an downstream feed and food products. Major outbreaks have been recorded in 1890, 1901, 1914, 1932, 1946, 1958, 1963, and 1970.

The first appearance of the fungi is in the form of a scab appearing on the budding head of wheat when there is rain during the heading period. Part or all of the spikelet turns brown during the early phase of the infection followed by a salmon colored growth between the layers of the glumes. With additional rain, the entire head rots and minute black bodies appear on its surface which consist of the perithecia with ascospores. This is why the infection is also called black spot disease. Human and animal consumption of the infected grain and products usually resulted in anorexia, vomiting, diarrhea and death in sustained or larger doses.

The Fusarium species are also widely distributed in the rice fields on both plants and in the soil.

In 1984, the Soviet Union reportedly used Fusarium produced Tricothecenes in a weapon nicknamed "Yellow Rain" because of the color of the aerosol that rained on the victims. Those affected experienced symptoms of skin injury and illness which appeared to be consistent with T-2 and other toxins. More detail will be explained in the toxicology part of this chapter.

B Organisms That Produce Trichothecenes

The trichothecenes are produced by a wide range of fungi and around 20% of the isolates of all these species produce more than one of these compounds. Studies of Canadian grain show that there is significant presence of Fusarium in all grains and fields there.

	Wheat-Kernels/Sample		Barley Kernels/Sample		Oats	
Eastern Canada	1.45%	41.79%	3.88%	76.22%	5.67%	79.53%
Western Canada	.23%	13.76%	.7%	36.29%	1.11%	38.95%

Fusarium is also very common in the rice fields (soil and plants) of California. They have also been found in field and sweet corn, sorghum, tall fescue, turf grass, carnation, cranberry, pea, and cooked rice that has been left uncovered for several days. Myrothecium species that produce tricothecenes are found on the leaves of the *Gardenia*, tomatoes, violets, kidney beans, snapdragons and other common plants. Trichoderma species are among the most numerous soil species on earth and they too produce these toxins. Trichothecium roseum is commonly found in the soil as well as Cephalothesium which can also be cultured from fruit, paper and wood. The Cephalosporium genus may also cause mycetomas in man (a foot fungus) and many are pathogenic to plants.

Trichothecene-producing fungi

Type	(A) T-2 toxin type	(B) Nivalenol-type	(A) and (B)	(C) Macrocylic
	T-2 toxin	nivalenol	diacetoxyscirpenol (DS)	roridins
Trichothecenes	HT-2 toxin diacetoxyscirpenol neosolaniol	monoacetylnivalenol diacetylnivalenol deoxynivalenol	diacetylnivalenol 7-hydroxy-DS 7, 8-dihydroxy-DS	verrucarins satratoxins vertisporin
Fungus	*F. tricinctum* *F. roseum* "culmorum" "avenaceum" "scirpi" *F. sporotrichioides* *F. poae* *F. solani* *F. rigidiusculum* *F. lateritium* *F. semitectum* *F. equiseti*	*F. nivale* *F. episphaeria* *F. roseum*	*F. equiseti* *F. scirpi* *F. oxysporum* *F.* sp. K5010	*Myrothecium verrucaria* *Myrothecium roridum* *Stachybotrys atra* *Verticimonosporium diffractum*

The toxicity of the grains affected by these fungi vary considerably depending on humidity, temperature, culture nutrients, and the local area and strains. F. tricinctum produces two toxins at 8 C and a third toxin at 25 C which accounts for the severe toxicosis seen in overwintered grains that are used in foods in hungry areas and in grazing livestock.

During 1943-1949, grain samples were taken in the Soviet Union to identify the species of fungi responsible for ATA. These included soil and grain samples and these were identified and tested for toxicity. The following chart shows which species were recovered and the percentage of highly toxic and mildly toxic isolates in each genus.

GENERA OF FUNGI ASSOCIATED WITH TOXIN PRODUCTION IN OVERWINTERED GRAIN

Genus	Isolates[a]			Species		
	Total number	Highly toxic (%)	Mildly toxic (%)	Number isolated	Highly toxic (%)	Mildly toxic (%)
Fusarium	501	22.4	13.3	25	60	28
Cladosporium	480	5.4	8.5	15	60	20
Alternaria	506	2.8	5.3	6	30	0
Penicillium	830	1.6	3.8	36	22	33
Mucor	335	3.0	7.2	18	33	22

[a]Highly toxic isolates were also found in *Piptocephalis freseniana* (with *Mucor albo-ater*), *Trichoderma lignorum*, *Rhizopus nigricans*, *Trichothecium roseum*, *Thamnidium elegans*, *Verticillium lateritium*, and *Actinomyces griseus*.

TABLE II

TOXICITY OF *Fusarium* FUNGI ISOLATED FROM OVERWINTERED CEREALS, SUMMER-HARVESTED CEREALS, AND THEIR SOILS

Fungus	Number of isolates											
	Overwintered cereals: grains and vegetative parts				Soils				Summer-harvested cereal: grains and vegetative parts			
	Toxic	Mildly toxic	Non-toxic	Total	Toxic	Mildly toxic	Non-toxic	Total	Toxic	Mildly toxic	Non-toxic	Total
Fusarium arthrosporioides Sherb	–	1	7	8	–	–	2	2	–	–	5	5
F. avenaceum (Fr.) Sacc.	3	3	26	32	–	–	10	10	–	–	3	3
F. culmorum (W.G.Sm.) Sacc.	2	1	13	16	–	–	–	–	–	–	–	–
F. equiseti (Cda.) Sacc.	7	3	41	51	–	1	9	10	–	–	27	27
F. graminearum Schw.	–	1	2	3	–	–	–	–	–	–	–	–
F. javanicum Koord.	–	–	8	8	–	–	–	–	–	–	5	5
F. kühni (Fuck.) Sacc.	–	1	9	10	–	–	–	–	–	–	–	–
F. lateritium Nees	2	2	24	28	–	1	3	4	–	–	–	–
F. moniliforme Sheld.	1	3	22	26	–	1	10	11	–	–	–	–
F. nivale (Fr.) Ces.	–	2	11	13	–	–	–	–	–	–	–	–
F. oxysporum Schl.	1	2	16	19	–	1	13	14	–	–	2	2
F. poae (Pk.) Wr.	44	17	2	63	2	3	–	5	–	–	–	–
F. redolens Wr. Wr.	1	–	5	6	–	–	2	2	–	–	–	–
F. sambucinum Fuck.	1	1	14	16	–	–	–	–	–	–	–	–
F. semitectum Berk. et Rav.	2	2	23	27	–	–	–	–	–	–	–	–
F. solani (Mart.) App. et Wr.	–	3	16	19	–	–	5	5	–	–	–	–
F. sporotrichioides Sherb.	42	15	4	61	2	2	–	4	–	–	2	2
F. tricinctum (Cda.) Sacc.	2	1	19	22	–	–	5	5	–	–	–	–

These organisms occur widely in nature and are found in soils and on plants such as cereal grains, vegetables and feeds throughout the world. After fall harvest, the vegetative parts of the cereal grains fall to the ground and provide a good medium for the development of the fungi in the soil. Rain provides the moisture to support the mold growth. The temperature, humidity, and other conditions effect the resulting growth.

Fusarium species not only produce toxins, they also are among the most deadly plant pathogens with species causing root rots, seedling deaths, and canker of mature plant tissues. The Fusarium genus is characterized by the production of multiseptate, hyaline microconidia which are curved in the long axis. The spores are produced from phialides and the basal cell has a distinctive heel which is diagnostic for the genus. The fusaria usually become established on a crop before harvest.

Samples of grain tested in different parts of the world illustrate the widespread nature of the Fusarium toxins –

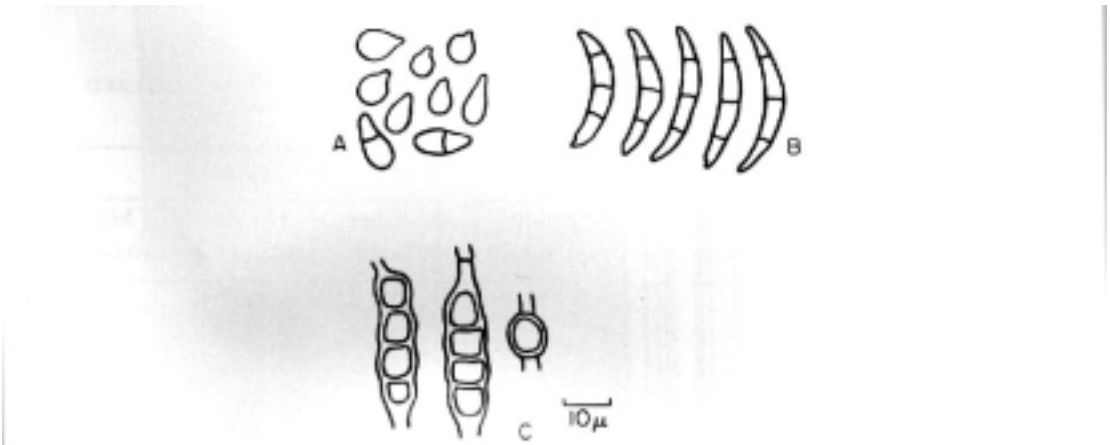
Country	Date	# samples	Commodity	% Contaminated	Toxin Conc.
USA	1977	52	Corn	46	DON(5.0ppm)
Japan	1970-80	130	Barley/Wheat	81	DON(2.5ppm)
Canada	1980	45	Wheat	98	DON(4.3ppm)
Finland	1972	160	Cereals	2	T-2 (.03ppm)
Hungary	1979	464	Animal Feeds	6	T-2 (5 ppm)
Denmark	1980	36	Cereals	2.8	DON(1.0ppm)

The four principal toxins produced in cereals in the US are T-2, diacetoxyscirpenol, nivalenol, and deoxynivalenol (vomitoxin).

The toxic fungi are believed to develop first in the embryo of the grain. The mycelium spreads from their to infect the whole grain. In the case of prosomillet in the USSR, it was found that the light and heavy grains could be separated by floating the light ones in 10-25% sodium chloride solution. The heavy grains would sink. The floaters would almost always be highly toxic while the heavy grains were mostly non-toxic or mildly toxic. The light grains could be ground to a powder and the greatest amount of toxin and Fusaria recovered. The percentage of germination of overwintered grains infected with the fungi is much lower than uninfected grains.

The main toxic fungi are cultured as follows –

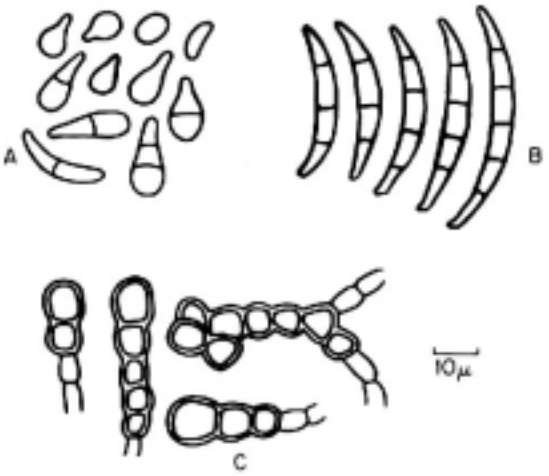
Fusarium poae is grown on potato agar or potato acid agar. A white, yellow, brown or sometimes pink mycelium develops. Pseudopionnotes form on the 30th day. Chlamydospores are intercalary and in pale brown chains.



Fusarium poae: (A) microconidia; (B) macroconidia; (C) intercalary chlamydospores.

The primary mycelium is yellow, pink, and yellow-brown when grown on rice. The rice grains turn to a yellow-carmine color. Secondary mycelium are weakly developed and white with a delicate tint. On potato slices, many turn dirty yellow to pinkish brown mycelia. The slice margin turns carmine or dark brown. The microconidia are mostly unicellular, roundish to lemon or pear shaped. Two celled conidia are usually ellipsoidal, spindle and sickle shaped. The macroconidia are sickle shaped with three septa.

Fusarium sporotrichiodes on potato agar or potato acid agar show a well developed aeril mycelium which is white with a pink or carmine hue. Pseudopionnotes form on the 15th day to 30th day. Chlamydospores are mainly intercalary, unicellular, arranged in chains and colorless, light brown or sometimes pink with a rough or smooth surface.



Fusarium sporotrichioides: (A) microconidia; (B) macroconidia; (C) intercalary chlamydospores.

The primary aerial mycelium is yellow or brown on rice. The rice grains turn shades of olive-brown, pink or carmine. The grain margin turns dark brown or yellow brown. The secondary mycelium weakly develops, and is white with a yellow brown tinge. The sclerotia are round or oval and brown in color.

Potato slices turn brown at the margins. Aerial mycelium is light yellow to brown with luxuriant growth and spreads all over the plate or tube.

Microconidia are borne on conidiophores or scattered in the mycelium. They are pear shaped or sherical, or ellipsoidal, unicellular and non-septate.

Fusarium graminearum, or *F, nivale* the causes of red mold disease in Japan produces a floccose, pink colored colony with a purple pigment on the reverse side (bottom). They have crescent or spindle shaped conidia (with 1-3 septa) attached at the top of 1-3 sterigmatos extending from twig-like branches of conidiophores.

The greatest frequency of toxin producing strains occurs in the soil. These apparently spread to plant parts affecting the vegetation first and then the grain last. Toxin accumulation is most favorable in the grain which is why the toxicity is observed most often there. Field tests indicated that the infection of grains is preceded by infection of the vegetative parts during the previous seasons. Infection and formation of toxin in the grain are evidently secondary processes which are greatly influenced by environmental conditions.

Studies of meteorological data from the ATA outbreaks suggest that variations and fluctuations of temperature intensifies toxin accumulation and Fusaria infection. The Fusaria also grow well at –7 to –10 C but not at lower temperatures. Toxin formation was most active at just below 0 C in the field trials, which is slightly above the growth minimum. Dense snow cover also prevented the soil from freezing to its normal depths.

In laboratories, the Fusaria are best cultured at refrigerator or cellar temperatures close to 0 C. They will also grow at 20-25 C. Non toxic species would also grow at 20-25 C but would not grow at all at 0-2 C. This allows for differentiating the toxic from non toxic strains. The toxic strains grow at both temperatures, although more slowly at the colder ones. The non-toxic strains would rarely, if ever grow at near freezing. This means that the toxic strains are *cryophilic*. The optimum growth temperature for most Fusaria is estimated to be 10-12 C.

Tests on F. poae at that time indicated that the Fusaria was always toxic when inoculated on sterilized grain if it was toxic when first isolated.

There is also great variability in the Fusarium genus and this variability increases because they mutate readily and cultures have been known to weaken in their toxin producing properties. The pigmentation, conidia, sclerotia, type of sporification all can

vary with environmental conditions and age of the culture. Loss of toxic properties is usually associated with culturing on liquid and carbohydrate-peptone agars, or on sterile millet at room temperatures or even at 0-5 C. The changes included the appearance of a slimy layer in the culture, the appearance of fat globules in the mycelia and the gradual disappearance of aerial mycelium. These changes paralleled the loss of toxicity.

Various vegetable substrates have been used for the Fusaria including millet, wheat, barley, oats, rice and potato. The best nutrient sources in artificial mediums were carbohydrates (starch, glucose), while peptone and asparagine were the best nitrogen sources. Ammonium Sulfate also was a good source of nitrogen. Ideal pH for toxin production is 4.8-5.4.

Fusarium grown under conditions of alternating freezing and thawing were characterized by abundant spore production and high toxicity. The presence of non sporifying mycelium coincides with low toxicity. Pigmentation was unrelated to toxicity.

Another fungi, *Cladosporium epiphyllum*, also produces trichothecenes. It grows at temperatures as low as –2 to –10 C and has also been identified as a major toxin contributor to ATA. Its growth at 25 C is weak compared to Fusaria. In the autumn to winter periods, C. epiphyllum produces abundant growth on the ears of cereals with little Fusarium in evidence while the Fusaria predominate in the spring.

A final comment on Fusarium and genetics should be made here. Fusarium species reproduce sexually. A fertile cross between strains and species of Fusarium is possible using one as a female and another as a male. A successful cross yields new strains in which the ascospores can then be grown and tested for improved toxin and infectious properties. Conventional genetic techniques have already been described in the textbooks regarding this with successful results. DNA mediated genetic transformation technologies have been developed as well as other types of genetic engineering that can significantly change the potential for weapons in these organisms. [Imagine C.immitis, highly infectious, successfully crossed with aflatoxin and/or tricothecene producing species. Now combine this on a single grain of dust or diatoms with anthrax, and/or Clostridium species].

In actual practice with F. sambucinum, crosses are made by pairing two strains of opposing mating type and appropriate sex. Individual strains can be mating type 1 or 2, either male or female or hermaphroditic or neuter. No female strains have been found in F sambucinum but they do exist in other fertile bisexual Fusarium species. The female (recipient) strain is grown alone first on mulberry twigs/water agar at 20-25 C to allow formation of the female reproductive structures called protoperithecia. The male (donor) strain is than added by covering the surface of the protoperithecia with a freshly prepared suspension of conidia. The cross is then incubated two or more weeks at 15 C after which mature perithecia appear may begin to appear.

At maturity in 2-4 weeks, the perithecia are filled with clusters or rosettes of asci. Each of the ascus contain 8 haploid spores called ascospores. These spores represent the four products of a single meiosis, each one having undergone an immediate mitotic cell division whereby every ascus has four sets of spores that are twins.

It then takes 2-8 weeks for the culture to produce mature perithecia which can then be studied. Genes for mating type, femaleness, pigmentation, auxotrophy, tricothecene production and other processes are inherited in the normal 1:1 mendellian fashion.

F. sambucinum is also amenable to genetic manipulation, mutagenesis and transformation. Mutants are obtained by UV irradiation treatment of the target strain.

C) Toxin Production

Almost all trichothecenes exhibit blue fluorescence under UV light which means they can be detected under growth conditions in moldy grains and cultures.

During the red mold disease outbreak in Japan in 1970, Fusarium and other species were cultured to produce the toxins responsible. Detection rates of Fusarium correlated with visual damage of the grains and several species were isolated. Twenty one of these species were then tested for their toxicity's to mice. Seven produced trichothecenes in detectable amounts. Most of the samples were cultured on Czapek-Dox medium (pH 6.8) at 25 C for 14-16 days. The culture filtrates were treated with activated charcoal to obtain the toxin concentrates.

Some of the Fusaria were cultured directly on moistened rice grains at 25 C for 10-12 days. The molded rice was then blended with 50% aqueous methanol and the mixture was filtered to obtain the liquid toxic extract. These were vacuum dried and then re-dissolved in n-hexane and chloroform to further purify. The toxins cultured on the Rice converted over the time of culture from one type of trichothecene to another. This transformation is believed to be a regular occurrence in storage grains.

Some cultures taken from the outbreak of ATA in the Soviet Union in 1947 were preserved for decades by maintaining the cultures in sterile soil and sub-culturing on Potato Dextrose Agar at 3 C. The strains were tested in the 1970's by inoculating them into wheat and millet at 5 C, 12 C, and 29 C for 10, 21 and 45 days. The toxins were then extracted and isolated from the infected grains and the extracts tested on rabbit skin. The most active extracts were those cultured at 12 and 5 C. The extracts were made in a kitchen blender using ethyl alcohol. A total of 4.2 grams of T-2 toxin was recovered from 1 Kg of infected millet. Testing in cats produced reduced white blood cell counts, vomiting, hemorrhage, and neurological disturbances.

It was found that sharp fluctuations in temperature affected the toxin production and resulting effects on the animal dermal tests. The following chart shows the temperature and conditions of fungi growth –

ACCUMULATION OF TOXIN IN FUNGI DUE TO SHARP TEMPERATURE FLUCTUATIONS

	Toxin accumulation[a]								
	Fusarium poae			*Cladosporium epiphyllum*			*Fusarium* + *Cladosporium*		
Conditions	L	O	H	L	O	H	L	O	H
Room temperature (18°C)	–	+	–	+	+	–	–	–	–
In snow	+	+	++	+	++	–	–	+++	+
Room temperature-in snow	–	–	–	–	–	–	+++	+	–
In snow-room temperature	–	–	–	–	–	–	–	+	++
Room temperature-freezing-room temperature	–	+++	+++	+	+++	–	–	–	–
In snow-freezing (−15°C)	–	+++	+++	++	+	–	–	–	–
Room temperature-freezing-in snow	–	–	–	–	–	–	–	+++	–
In snow-freezing-in snow	–	–	–	–	–	–	–	+++	–
In snow-freezing-room temperature	–	–	–	–	–	–	–	+++	++
Alternating room temperature-freezing	–	–	–	–	–	–	–	+++	+
Alternating in snow-freezing	–	–	–	–	–	–	–	++	–

[a]Reactions: L = leukocytic; O = edematous; H = hemorrhagic. Degree of toxicity: + = mildly toxic; ++ = toxic; +++ = very toxic.

Subsequent tests of Fusarium demonstrate that toxin production is at its peak at temperatures of –2 to –7 C and during abundant sporulation, or also just prior to sporulation at –7 C to –10 C. Extracts at advanced stages of senescence were considerably less toxic. Heating the filtrates at 100 C for 30 minutes did not reduce the toxicity of the filtrates. Overall, the highest toxicity is associated with abundant spore production. It was also discovered that the liquid filtrate produces stronger test reactions on test animals than the mycelium film. This means that the toxin is excreted into the surrounding medium and act as exotoxins.

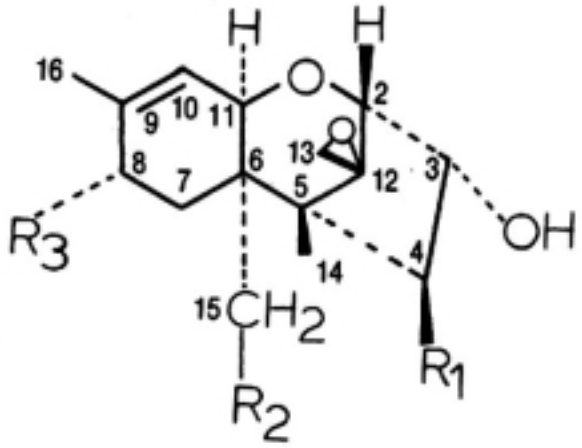
In some of the ATA tests from stored samples it was found that samples could still be highly toxic when there were no longer living fungi present in them. This means that the toxin was excreted and persisted in the samples for years while the Fusaria died off.

During the laboratory discovery of the trichothecenes, most of the initial toxins were produced in submerged culture fermentation at 25 C with stirring and aeration. Most used corn steep liquor and/or malt or yeast extracts and peptone supplemented with mineral salts and glucose. Several were grown directly on sterile rice. T-2 toxin, HT-2 toxin and diacetoxyscirpenol were grown for 2-4 weeks on solid media at 25 C and 8 C for peak production for T-2 toxin.

D) Toxin Separation & Purification

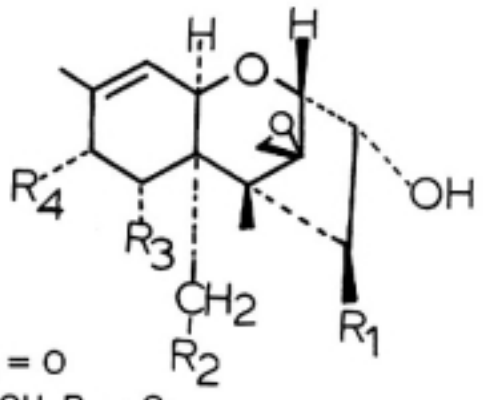
The Fusarium produce toxins that are divided chemically into two groups according to their structures –

Structure of trichothecenes included in Group A:



T-2 toxin $R_1 = R_2 = CH_3COO$-, $R_3 = (CH_3)_2CHCH_2COO$-;
HT-2 toxin $R_1 = OH$, $R_2 = CH_3COO$-, $R_3 = (CH_3)_2CHCH_2COO$-;
Neosolaniol $R_1 = CH_3COO$-, $R_3 = OH$, $R_2 = CH_3COO$-;
Diacetoxyscirpenol $R_1 = R_2 = CH_3COO$-, $R_3 = H$;
Monoacetoxyscirpenol $R_1 = OH$, $R_2 = CH_3COO$-, $R_3 = H$.

Structure of thrichothecenes included in Group B:



T-2 tetraol $R_1 = R_2 = R_4 = OH$, $R_3 = H$
Scirpentriol $R_1 = R_2 = OH$, $R_3 = R_4 = H$;
Deoxynivalenol $R_1 = H$, $R_2 = R_3 = OH$, $R_4 = O$
Fusarenone-X $R_1 = CH_3COO$-, $R_2 = R_3 = OH$, $R_4 = O$;
Nivalenol $R_1 = R_2 = R_3 = OH$, $R_4 = O$.

The Group A toxins have similar polarity and are highly soluble in aprotic solvents like ethyl acetate, acetone, chloroform, methylene chloride, and diethyl ether.

These are usually used in extracting the toxins from cultures and samples. These toxins are generally insoluble in water.

The Group B toxins are highly hydroxylated and are relatively polar making them soluble in very polar solvents or protic solvents such as methanol, ethanol, acetonitrile and water mixtures. Group B can also be extracted with water.

Extracting the toxins from different media often requires different solvents for recovering the largest quantity of toxin. The following charts describe solvents used in toxin recovery in research.

Extraction of Trichothecenes in Different Solvents

Solvent	Toxin	Substrate
$CHCl_3$	Diacetoxyscirpenol	Culture Filtrate
$CHCl_3$	T-2 Toxin	Corn
CCl_4	Trichothecin	Culture Filtrate
EtOAc	T-2, HT-2 Toxins	Culture Filtrate
EtOAc	Monoacetoxyscirpenol	Rice Culture
EtOAc	Scirpentriol	Corn Culture
EtOAc	Diacetoxyscirpenol	Corn
EtOAc	Diacetoxyscirpenol	Mixed Feed
50% Aq. EtOH	Deoxynivalenol	Barley
40% Aq. MeOH	Deoxynivalenol	Corn
EtOEt	T-2 Toxin, Neosolaniol, T-2 Tetraol	Corn and Millet Cultures
EtOH	T-2 Tetraol	Rice Culture
CH_3CN-KC1 (4%) 9:1	T-2 Toxin, Diacetoxyscirpenol	Mixed Feedstuff

Recoveries of Trichothecenes from Mixed Feeds Using Ethyl Acetate and Acetonitrile

Toxin	Ethyl Acetate Recovery %	Acetonitrile Recovery %
T-2 Toxin	87.0	80.0
Diacetoxyscirpenol	99>	97.6
Monoacetoxyscirpenol	97>	92.8
Scirpentriol	115.2	83.2

Purification of extracts containing trichothecenes by liquid/liquid partition.

Partition System	Toxin Extracted
Acetonitrile/Pet. ether (60-70°) 50:50	Monoacetoxyscirpenol Diacetoxyscirpenol T-2 Toxin
Aq. MeOH (80%) /Pet. ether (60-70°)	T-2 Toxin
H_2SO_4 (0.8 N) /Ethyl acetate	Diacetoxyscirpenol
50% Aq. MeOH/Ethyl acetate:chloroform 1:1 (v/v)	T-2 Toxin
MeOH-Acetone/Aq. $NaHCO_3$ (pH 8 - 8.5)	Deoxynivalenol

Trichothecenes will also absorb onto silica gel and charcoal and these have been used in liquid extracts to absorb, remove and purify the toxins. Charcoal is the most frequently used solid extractant following water extraction of moldy grains. The liquid is poured through the charcoal which absorbs the toxin. Methanol or other solvents are then used to re-dissolve the toxins and separate them from the solid (charcoal).

Some of the cultures grown on solid media are ground to a powder, freeze dried and then extracted with ethyl acetate. The material handling properties using this technique were improved.

In the red mold disease epidemic in Japan in 1970, moldy (suspect) barley was ground to a powder and suspended overnight in cold water. The suspension was then homogenized with ethanol and then filtered by suction. The filtrate was evaporated by vacuum to remove the ethanol. The remaining aqueous solution was then treated with active charcoal to absorb the toxins. The absorbent charcoal was then eluted with methanol. The methanol was then evaporated leaving behind the crude toxin concentrate.

In the case of moldy rice, the rice powder is first extracted with acetone to remove unwanted pigments. The residue is then extracted with 50% aqueous methanol or ethanol. In liquid broth's, charcoal is used to absorb the toxins directly, then the charcoal is eluted with methanol or ethanol and evaporated to yield a concentrate. Chloroform is then used to dissolve the toxin and concentrate it further.

The extracted and purified tricothecenes are colorless, crystalline, optically active solids which are generally soluble in moderately polar solvents and are very slightly soluble in water (increasing with temperature). The parent alcohols are less lipid soluble and are more soluble in polar solvents and water and are difficult to crystallize. As a class, the compounds are quite stable over long periods and are not destroyed by heat (or cooking).

E) Toxicology

Common symptoms for most of this family of mold toxins include emesis, vomiting, inflammation, feed refusal, diarrhea, abortion, hemorrhage, loss of white blood cells, necrotic angina, nervous system disturbances and destruction of bone marrow. These symptoms have been demonstrated with all the toxins in mice, rats, guinea pigs, rabbits, cats, dogs, horses and poultry. The younger the test animal, the more susceptible they are to the toxins. In dogs, cats and ducklings, vomiting occurs shortly after dosing by all routes of administration and temporal leukocytosis is noted. Cats intoxicated with T-2 toxin suffer hemorrhage in the intestine, lung and brain and destruction of bone marrow. A second dosing in cats results in marked decrease of circulating white blood cells and this response is common among most test animals with almost all of the trichothecenes.

The hemorrhage of the brain and bleeding in the lungs and intestines is very similar to the symptoms observed in the humans who consumed over-wintered millet in the Soviet Union in the outbreaks described at the start of this chapter.

LD_{50} values (mg/Kg) of trichothecene mycotoxins[1]

Type	Trichothecenes	Mice				Rats				Guinea pigs		
		i.v.	i.p.	s.c.	p.o.	i.v.	i.p.	s.c.	p.o.	i.p.	s.c.	p.o.
A	T-2 toxin		5.2		10.5				5.2			ca. 2
	HT-2 toxin		9.0									
	Diacetoxyscirpenol	12	23.0			1.3	0.75		7.3			
	Neosolaniol		14.5									
	Monoacetoxyscirpenol							0.725				
	Scirpenol						0.81					
B	Nivalenol		4.1									
	Fusarenon-X	3.4	3.4	4.2	4.5				4.4	<0.5	<0.1	
	Diacetylnivalenol		9.6									
	Rd toxin		70		46							
	Rc toxin		49		34							
	Trichothecin	~300						<250				
C	Roridin A	1.0										
	Verrucarin A	1.5	0.5–0.75			0.87						
	Verrucarin B	7.0										
	Verrucarin J		0.5–0.75									

Type	Trichothecenes	Chicks	Trouts	Cats	Dogs		Ducklings	Rabbits
		p.o.	p.o.	s.c.	i.v.	s.c.	s.c.	i.v.
A	T-2 toxin	3.6	6.1	<0.5				
	HT-2 toxin							
	Diacetoxyscirpenol				ca. 1.1			1.0
	Neosolaniol							
	Monoacetoxyscirpenol							
	Scirpenol							
B	Nivalenol							
	Fusarenon-X			<5.0			ca. 2.0	
	Diacetylnivalenol							
	Rd toxin					>3.8	27	
	Rc toxin					>1.0	37	
	Trichothecin							
C	Roridin A							
	Verrucarin A							0.54
	Verrucarin B							
	Verrucarin J							

Subcutaneous LD_{50} values of trichothecene mycotoxins in new-born mice.[1]

Type	Trichothecenes	LD_{50} (mg/Kg)
A	T-2 toxin	0.15
	Diacetoxyscirpenol	0.17
B	Nivalenol	0.14
	Fusarenon-X	0.23

[1] Mice of the ddyS strain were administered with the mycotoxins within 24 hours after birth.

All the trichothecene compounds induce skin necrotization. These lesions can be induced orally in feeding trials and involve the digestive tract as well. The skin injuries resemble those of the military poison gas “nitrogen mustard”.

A skin assay for detecting trichothecenes was first developed in 1965. The trichothecenes cause dermal irritation and injury that is detectable in very tiny amounts when placed on the skin of guinea pigs, rats and humans. In the USSR, the toxicity was often determined by applying the diethyl ether extract of the moldy grain to shaved skin of the rabbit. These preparations caused edema, hemorrhage, and in large doses were fatal. At levels of .1 mcg, T-2 toxin would produce detectable effects.

These tests were repeated in the USA with the cereal grains infected with the organisms grown for 25-70 days at –5 to +8 C. They were also subjected to successive freezing and thawing. This seemed to increase the toxin yields. Ether or alcohol were used to extract the toxin from the fungus infected media and the extracts were tested on the skin of rabbits. Rabbits with non pigmented skin were used with the skin shaved in areas of 3x3 to 4x5 cm on each side. The extract was applied to the skin of the rabbit with a platinum loop twice at intervals of 48 hours. The reaction was recorded at 48 hours and observations made for 6-8 days. Each rabbit was then treated with a control of uninfected grain extract.

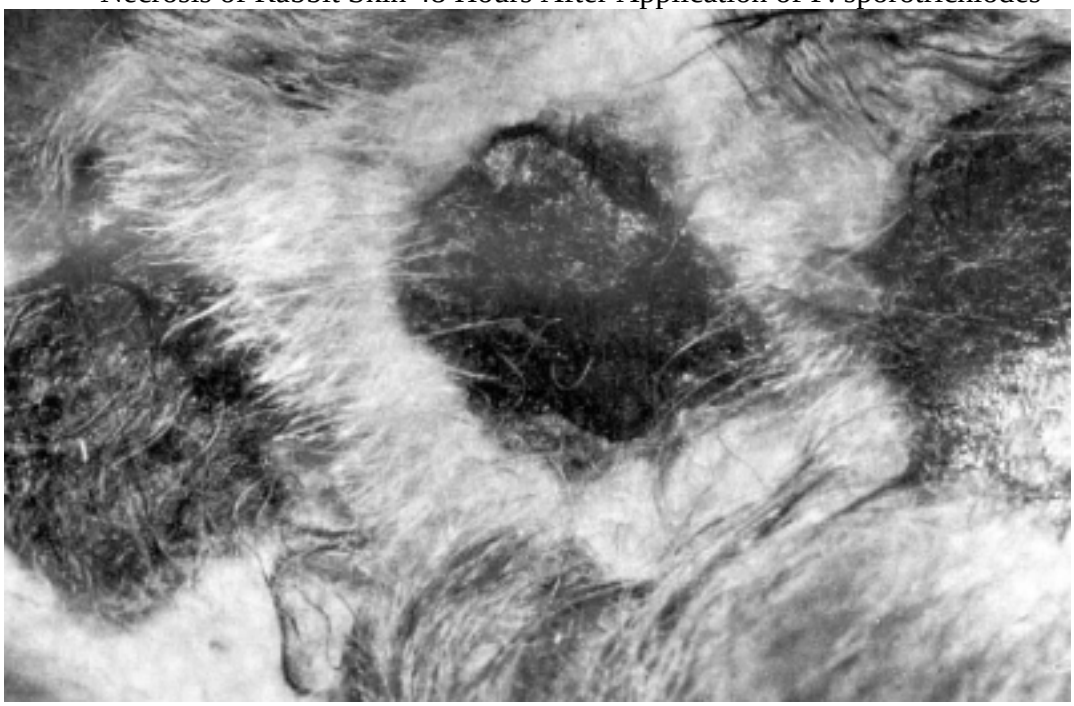
The skin reactions were either 1) leukocytic in which skin surface turns whitish with an easily detachable film which contains a mass of leukocytes in the horny layer of the epithelium. 2) involves an acute edema, hemorrhage and necrosis where there is no lykocytic film. The intensity of the necrosis is recorded on the 8^{th} day while the other components are recorded on the 3^{rd} day. The presence of edema, hemorrhage and necrosis was regarded as marked toxicity of the fungus. Different volumes of fungus were tested to determine the toxic levels in the samples. The toxic extracts were also tested on the eyes of rabbits the skin of cats, sheep, dogs, cows and horses.

Dermal responses are graded as follows –

0	No observable skin reaction
1	Just noticeable skin reddening followed by formation of a slightly dry or crusty area
2	Appreciable edema or inflammation
3	Severe edema plus spreading of the affected area and heavy scab formation
4	Like 3 but accompanied by a marked sub-dermal hemorrhage in the affected zone
5	Death of the animal with or without any of the above skin responses

The first signs of response usually appear at 12-24 hours. With high levels of toxin in the sample, deaths are usually observed within 48 hours, sometimes before any noticeable skin reaction has occurred. T-2, HT-2 and diacetoxyscirpenol at .25 mg usually produces a 3-4 response while .4 mg or more is usually fatal. A 1-2 are recorded at .5-1 mg or less. This method has been used for rapid screening of large numbers of mold culture extracts.

Necrosis of Rabbit Skin 48 Hours After Application of F. sporotrichiodes



A rat skin assay was developed in 1971 to detect and quantify trichothecenes in culture and moldy corn extracts. The tested samples were dissolved in a small volume of the desired solvent and applied in a single dose to the shaved back of 21 day old rats. The skin response is noted every day for five days. Levels of .05-1 mcg of T-2 toxin produces a detectable effect yielding reddish weals with nearly white centers.

Subsequent tests have shown that the guinea pig is most sensitive to the dermal effects of the tricothecenes with levels of .2 mcg producing results from both group A and Group B toxins. Dermal tests usually use .5ml or 25 grams of extract for the base application and then the results are measured and future doses adjusted tenfold either direction accordingly.

Skin-necrotization induced by trichothecene mycotoxins on the back of guinea pigs, mice, and rabbits (Ueno *et al.* 1970 a).

Type	Trichothecenes	MED[1] (μg/spot)		
		Guinea pigs	Mice	Rabbits
A	T-2 toxin	0.2	1	
	HT-2 toxin	0.2	1	
	Diacetoxyscirpenol	0.2	10	1
	Neosolaniol	1	10	
B	Nivalenol	10	100	10
	Fusarenon-X	1	10	10
	Diacetylnivalenol	1	10	

[1] Minimum effective dose

The dermal necrosis also occurs in the intestines. The mucosal epithelium of the stomach and small intestine erode with accompanying hemorrhaging and eventual death in sufficient doses.

Brine shrimp have also been used to measure trichothecene toxins. Levels of .5 mcg of T-2 toxin will kill 50% (LC50) of the shrimp and as little as 125 ng/5ml causes noticeable effects. Chick embryos also die at levels of .7-5 mcg depending on the individual Fusarium toxin. The same procedures for testing aflatoxin in chicken eggs can also be applied here. The chick embryos can be injected before incubation or several days after when a live embryo can be assured. The extract can be injected in either the yolk sac or air sac of the fertile egg.

These mycotoxins also cause vomiting at tiny doses in ducklings, cats and dogs when given orally or by subcutaneous injection and the vomiting lasts for an hour. Feed refusal and vomiting are clearly associated with vomitoxin, T-2 toxin and diacetoxyscirpenol. One day old ducklings are usually used for this lab bioassay.

Induction of vomiting by trichothecene mycotoxins in ducklings and cats.

Type	Trichothecenes	MED[2] (mg/Kg s.c.)	
		Ducklings	Cats
A	T-2 toxin	0.1	0.1–0.2
	HT-2 toxin	0.1	
	Diacetoxyscirpenol	0.2	
	Neosolaniol	0.1	
B	Nivalenol	1.0	
	Fusarenon-X	0.4	1–2
	Diacetylnivalenol	0.4	
	Rd toxin	13.5[2]	
	Rc toxin	13.5[2]	

[1] Minimum effective dose.

Feed refusal is another common symptom among farm animals exposed to tricothecenes. This can also be observed in mice, cats and rats shortly after dosing.

Retarded growth rate and abortions are also observed in farm animals exposed to moldy feeds and induced in test animals with a single dose

In 1976, crude extracts from F. poae and F. sporotrichiodes were tested in long term in very low doses in mice and rats. They caused depletion of the lymphoid tissues which caused subsequent widespread infection in the animals. The suggest that the toxins produce an immunosuppresive action that may be useful in combination weapons designs.

One of the most effective methods for testing the presence and amount of trichothecene toxin is the “pea seed” test. These toxins inhibit germination and growth of higher plants.

It has been found that .5 mcg of T-2 toxin inhibits germination of the pea seed by 50% when the seeds are soaked overnight in a solution of the toxin. Severe wilting of plants occur in 24 hours when roots are immersed with complete necrosis in 72 hours. This assay can detect as little as 1 ppm of T-2 toxin (or less).

Early perfection pea seedlings 72 hours after immersion of the roots in aqueous solutions of T-2 toxin for 20 minutes. Rows of five seedlings from left to right: 0.0, 1.0, 2.5, 5.0, 10.0, and 100.0 ppm of T-2 toxin.

Chronic feeding studies were conducted in 1974. Rats and were fed rice, molded with Fusaria. More than half the animals suffered bone marrow damage, intestinal injuries, and atrophic or hypoplastic changes in the thymus, spleen, bone marrow and testicles. The most frequent cause of death was chronic bronchitis and bronchopneumonia. This indicates that the toxins have an accumulative debilitating effect on the animals and also makes them more susceptible to other infections due to immuno-suppression. Autopsy indicates this was due to damage to the lymphoid tissue in the thymus, spleen and bone marrow. It may also have been due to immunological exhaustion due to chronic infection.

In test animals that were injected instead of fed the mold substances, loss of hair at the injection sites was noted in addition to similar symptoms. A few unusual tumors were also observed although the incidence was so small that the toxins were not considered a carcinogenic threat. Acute leukopenia was observed when the animals were fed 50 mcg/day (20 ppm). At 200 mcg, they all died within two weeks. It was also difficult to get the animals to ingest these amounts in the trials.

Leukopenia induced by trichothecene mycotoxins

Type	Trichothecenes	Animals	Routs	Dose (mg/Kg/day)	Duration (weeks)	References
A	T-2 toxin	Cat	s.c.	0.05	1	Sato *et al.* (1975)
	"	"	"	0.1	0.5	"
	"	Hen	p.o.	0.9[1]	3	Wyatt *et al.* (1975)
	Diacetoxyscirpenol	Rat	i.v.	0.15–0.3	1–5	Stahelin *et al.* (1968)
	"	Dog	"	0.05–0.15	4–5	"
C	Verrucarin A	Rat	i.v.	0.25	4	Rusch *et al.* (1965)
	"	Dog	"	0.08–0.15	2–4	"
	"	Guinea Pig	"	0.08–0.15	2–4	"
	"	Monkey	"	0.08–0.15	2–4	"

[1] Calculated from consumption of dietary T-2 toxin (20 μg/g) by the present authors.

In tissue cultures, human cells (HeLa) are inhibited from growing in concentrations of only 1 mcg/ml of toxins. The tricothecenes are considered to be the strongest protein synthesis inhibitors (cytotoxicity to eukaryotes) known to man.

Cytotoxicity of trichothecenes to cultured cells (Ueno, 1983)

Types	Trichothecenes	LD_{50} ($\mu g\ ml^{-1}$) HeLa	HEK	HL
A	Trichodermol	5.0	3.0	2.0
	Monoacetoxyscirpenol	0.1	0.1	0.3
	Diacetoxyscirpenol (DAS)	0.01	0.01	0.001>
	Neosolaniol	0.1	0.06	0.05
	Acetylneosolaniol	0.3	1.0	0.1
	7,8-dihydroxy-DAS	0.3	0.2	0.3
	T-2 toxin	0.01	0.01	0.003>
	HT-2 toxin	1.0	0.1	0.01
	Acetyl T-2 toxin	1.0	0.8	0.03
	Calonectrin	3.0	0.8	0.03
	Deactylcalonectrin	7.0	0.0	1.0
B	Nivalenol	0.3	1.0	0.3
	Fusarenon-X	0.1	1.0	0.3
	Deoxynivalenol	1.0	3.0	0.5
	Monoacetyldeoxynivalenol	10.0	10.0	10.0<
	Trichothecin	0.1	0.1	0.1
	Tetraacetylnivalenol	10.9<	10.0<	10.0
C	Crotocin	0.5	0.6	2.0
D	Verrucarin A	0.005	0.002	0.0003>
	Roridin A	0.0003	0.0003	0.0003>

In 1974, tests were conducted using doses of both T-2 toxin and aflatoxin B1. It was found that when both were administered together, they were app. 4 times as toxic as either administered by themselves in the same dosage. Ochratoxin A and T-2 administered together were fatal to young chicks at doses of only 20 mcg/g of T-2 and 8 mcg of Ochratoxin A. The conclusion is that combination mold toxins are synergistic and their combined use enhances the potential of weapons of this type.

In tests conducted in 1975 and 1978, T-2 toxin was found to increase lethaility in rats and mice that were subsequently challenged with pathogenic organisms. The effects on immune system suppression have already been described. Autopsy on the test animals indicated that the lymphoid tissues, bone marrow, and spleen were all depleted after exposure at subclinical levels of T-2 toxin. Thymic evolution has been observed in poultry. These tests suggest that immunodeficiency can be induced in combination biological weapons using T-2 and other tricothecene toxins. Studies in calves indicate that a dose of .3 mg/kg of body weight would produce significantly decreased immunoglobulin levels which is considered a serious immune deficiency.

All the trichothecenes also have fungistatic properties which inhibit competing fungi and give their species an advantage in the soil and plant tissues. Some of the Fusarium species produce non-trichothecene toxins in the water soluble fractions (in addition to the toxins) that affects bone formation, causes hemorrhage, and reduces hatchability when extracts are fed to laying hens.

The conclusions of the tests indicate that trichothecenes are growth inhibitors in test animals and lower their resistance to infection at 3.5-7 ppm of the diet. They are, at best, weak carcinogens. They are also mutagenic to bacteria at high concentrations. They also cause cumulative skin injuries similar to those of nitrogen mustard or nuclear radiation. It has also been concluded that the trichothecenes dermal injury and other effects can be recovered from (completely in many cases) if the exposure is not acute, sustained or insulted with further infection or injury.

Although no human testing has been done, a 1985 test performed on cynomolgus monkeys indicates that the LD50 for T-2 toxin is .8mg/kg of body weight which is similar to that of the rat. The monkeys were dosed with the toxin in ethanol at levels of .25 to 6 mg/kg. The minimum lethal dose was .31 mg/kg or 39% of the LD50. The monkeys also responded to dermal doses of 8 mg/kg without death indicating that less than 10% of the toxin is absorbed dermally which suggests low or slow skin permeability or skin metabolism for absorption in this primate model. Erythema was noted at 200 ng/spot in the skin test. The dermal samples were painted on in solutions of ethanol or DMSO.

Ingestion of .25 mg/kg caused food refusal, listlessness, diarrhea and emesis in 1-2 hours. The animals which died showed severe hypothermia which suggests a central temperature regulation affect that appeared to correlate well with T-2 dosing. The surviving monkeys regained appetite and normal body temperature in 2-7 days.

A final note will be addressed regarding the use of these toxins by the Soviet Union in Afghanistan in the early 1980's. The Soviets have had a substantial and large scale experience with exposure to these toxins. They observed many fatalities for almost a century and were very familiar with the culturing and production of these toxins.

The US experience in human exposure to concentrations of the extracted toxins is primarily the result of a laboratory accident in 1969, where two laboratory workers were handling crude ethyl acetate extracts containing T-2 toxin (at 200 mg/liter). The extract accidentally spilled to the inside of the protective plastic gloves they were wearing. The hands were thoroughly washed with a mild detergent within two minutes after contact. In about four hours, the workers reported a burning sensation on the exposed skin tissues which increased in intensity to about eight hours after contact. By 24 hours, the burning disappeared and the fingers were numb. At three days all sensation was lost in all exposed fingers and at 4-5 days, the skin turned hard and white. In the second wek, the skin peeled off in large pieces of 1-2mm in thickness. Afterwards, new skin grew and by day 18, normal sensitivity had been regained with complete recovery.

The United States alleged that the Soviets produced and used Tricothecenes in the Afghanistan war in 1980-84. This was based on the recovery of unusual concentrations of tricothecenes in samples taken at the attack sites. The topical and inhalation effects studied in victims in the aftermath by physicians appeared to be more consistent with *Stachybotryotoxicosis* (covered later in this book) which is caused by macrocyclic tricothecenes rather than the normocyclic tricothecenes described in this chapter. Preliminary and unpublished studies in the aftermath of the attacks indicate that if a suitable carrier were used, the tricothecenes described in this chapter might well produce the effects observed in the attacks. There are no mycotoxicoses or related disease processes that exist in that part of Asia that could otherwise account for the effects on humans, plants and animals that were described in the aftermath.

F) Other Fusarium Toxins

Zearalenone
Moniliformin
Buteniolide (Tall Fescue Toxin)
Fusarins
Stachybotryotoxicosis

Zearalenone

This toxin is mentioned here as a matter of academic interest. It is an estrogenic toxin, which means that it affects female reproductive organs in animals and man. Symtoms include swelling and reddening of the vulva, increased secretion in the vagina, keratinization of the pavement cells, prolapse of the vagina, metrorrhagia, increase in secretion and size of the uterus, abortions, infertility, growth and lactation of mammary glands even in immature and ovariectomized animals and even castrated males.

The major toxin producer is *Fusarium roseum (graminearium)* and possibly other *Gibberella Zeae*. It is not common on corn at harvest, but when corn is stored in the crib and exposed to the weather, spores from these and other fungi invade the grain. The toxin is produced only at low temperature (12 C) and is not produced at 25 C. It usually occurs in the highest dosages in weather of alternating moderate to low temperatures. It requires 23% moisture for propagation.

Early researchers of this toxin recovered the F. graminearium from suspected corn samples stored in cribs and exposed to the weather. They grew the fungus at 25 C for 2-3 weeks for good mold growth and then lowered the temperature to 12 C for several weeks to produce the toxin. They fed the toxic corn to swine and produced the symptoms described above within 4 days. The isolated toxin was called F-2 and was found to act on the body as a sex hormone. It was the first sex hormone acting substance to be discovered that was not a steroid. (It has a phenol structure).

When mixed species of Fusarium are grown simultaneously, there is little F-2 toxin produced, but if the production has already started with good mycelial growth, the additional species interfere very little with production.

The F-2 toxin fluoresces blue-green under long wavelength ultraviolet light and intensely green under short wavelength radiation (260nm). The toxin turns to alight green color and then quickly to yellow when it is sprayed with 50% sulfuric acid 50% methanol solution.

Artificial mediums were found to be unsuitable for growth of F-2 toxin. Copious toxin production was achieved when growing the fungi on sterile solid rice or corn grain with added glucose (1-6%). The highest and quickest yields were obtained with autoclaved parboiled polished rice. The yields were as high as 30-60,000 ppm with the

added glucose. The toxin could not be produced in liquid cultures and was recovered only using solid substrates.

Laboratory production of F-2 is accomplished by seeding autoclaved rice or corn in quart bottles with a soil suspension of F. graminearum spores. The corn is adjusted to 45% moisture and the rice to 60% before seeding for best results. The culture is incubated for 1-2 weeks at 24-27 C and then at 12-14 C for 4-6 weeks with added glucose for maximum yields. The enzymes which allow for F-2 production are activated at 12 C in about 1-2 weeks after which the temperature can be raised to 25 C and this will speed production of the toxin.

About ½ of the Fusarium species tested could produce F-2 toxin and it is believed to act a sex hormone in these and other fungi species. The toxin is insoluble in water, carbon disulfide and carbon tetrachloride. It is soluble in aqueous alkali, ether, benzene, chloroform, and alcohols. The grain is usually extracted with ethanol, dried, dissolved in ether and then transferred into .25% sodium hydroxide. It is then acidified and re-extracted with ether to remove impurities.

Estrogenic effects were recorded in mice dosed orally at 20-650 mcg administered over 7 days. It also induced abortion in swine when 50% of the fed corn was invaded with F-2 producing Fusarium species. It will also cause infertility in most species. [an interesting potential large scale weapon that would be hard to detect at sub-clinical levels that could effectively sterilize large populations covertly?]. The oral LD50 in mice is >40g/Kg.

Zearalenone has also tested positive for carcinogenic and mutagenic activity (measured by DNA attacking ability)..

Moniliformin & Fusarins

Moniliformin is a toxin produced by F. monilliforme which infects a wide range of plant hosts and is widely distributed in corn worldwide. It causes equine leukoencephalomalacia, human esophageal cancer, abnormal bone development and sweet potato toxicosis. This same organism also produces Fusarins, Fusaric acid, zearalenone and other toxic substances. These are extracted using the same techniques as for tricothecenes.

The sweet potato toxicosis is attributed to a toxin found in mold damaged sweet potatoes. F. solani was isolated from the damage site and it is believed that the mold causes the potato to produce the toxin in its presence at injury sites. In affected lung in cattle fed the moldy potato's, the disease was progressive and fatal to 69 of 275 animals. The toxin appears to cause the cells lining the alveolar wall to proliferate as much as ten times preventing gas exchange. This provides a means for producing the toxin in the potato itself. [Many molds cause the plants they infect to produce toxins that can be deadly to humans and animals.]

Buteniolide (Tall Fescue Toxin)

In January 1967, a farmer placed a herd of cattle on a pasture of tall fescue. In one month, several of the animals were lame in the hindquarters. The herd was moved to another pasture but 11 of the cows were so lame that they had to be cared for separately. The animals were very thin, had rough hair coats, and showed cracks at the junction of hoofs and skin on their rear legs. The rear hoofs of one of the animals fell off. The pregnant cows could not produce enough milk to feed their calves.

This pattern has been repeated on tall fescue pastures up to the end of March making it a winter pasture disease. The disease causes a reduction of blood flow to the extremities of grazing animals. In cold weather, cessation of blood flow to the extremities causes dry gangrene and eventual sloughing of the affected limbs. The clinical signs of fescue foot resemble ergot poisoning. Alkaloids extracted from the tall fescue were tested and found to not be the cause.

To isolate a possible fungal cause of the tall fescue disease, scientists placed samples of moldy or suspect tall fescue hay onto the skin of rabbits. Those that produced a reaction were then examined for fungi. These molds were cultured, extracted, and then tested on the skin of rabbits. Nine of twenty four isolates caused erythema to necrosis and the most toxic of these were fatal when injected intreperitoneally (IP) in mice.

In the pasture case mentioned above, scientists took samples of grass, leaves, and stems and placed them on potato dextrose agar, fescue infusion agar and 2% plain agar plates. They were incubated at room temperature and 200 isoaltes were selected and grown on any medium that produced sporulation. They were incubated at 15 C to avoid loss of toxin producing ability. Each plate was grown in duplicate with one plate extracted with aqueous ethanol and the other with dichloromethane. The solvent was then removed and the samples tested by suspending the extract in propylene glycol or ringers solution and then injected into mice (.1ml per mouse).

Over 13% of the 200 extracts tested were toxic to the mice. All but one of the toxic species were Fusarium and about 50% of the Fusarium species in this group produced toxins. One of the most toxic of these was an F. tricinctum. It was grown in liquid Sabaroud's Maltose medium at 3 C in the dark for 20-30 weeks and three different toxins were recovered. The first and primary toxin identified is called *Butenolide* which accounts for 87% of the toxins by weight. T-2 toxin and an unknown third toxin were also studied. In subsequent tests, the Butenolide was produced at 7-15 C but not at room temperature.

Butenolide is sparingly soluble in dichloromethane and crystallizes when the solvent is being removed. It is then re-crystallized from ethyl acetate, chloroform, or acetone. It is soluble in water but will eventually hydrolize in H2O and it is insoluble in carbon tetrachloride. Large scale laboratory production is accomplished by growing the F. tricinctum on hay infusion agar slants at 25C for 3-5 days and then stored at 7C until ready for use in fermentation batches. Isolates from the parent cultures occasionally lose

their ability to produce toxin and this is usually accompanied by a change in pigmentation from yellow and red to dark reddish brown. The final toxin can also be produced on Sabaroud's dextrose agar at 15C with the petri dishes periodically extracted and the extracts evaporated.

Butenolide extracts applied at 30mg in three applications to the backs of rabbits turned the skin white and slightly puffy with small red spots. After nine applications, a hemorrhagic reaction is produced. The effects were magnified by 10 times when the toxin was applied in di-methylsulfoxide instead of olive oil. The oral LD50 in mice is 275mg/Kg and 43.6mg/Kg (by IP).

Tall fescue hay fermented with F. tricinctum (with added glucose, salts and peptone) and extracted with 80% ethanol was toxic to cattle. The extract from 1 ¾ # of hay was fatal in 24 hours. When applied at low levels by IM to a heifer for 90 days, the tip of its tail became necrotic and dropped off.

Stachybotryotoxicosis

This disease has caused the deaths of thousands of horses and affects swine, cattle, sheep and human beings. It was originally associated with the mold *Stachybotrys atra* and is associated with tricohtocene producers, with the symptoms reported similar to that of the tricothecenes already described. The mold prefers cellulose type substrates such as straw, oats, beans and hay. The entire length of the digestive tract will hemorrhage and the toxin extracts also irritate the skin and mucus membranes. The disease symptoms include leukopenia, shock, stomatitis, dermal necrosis, thrombocytopenia and nervous disorders.

The toxin is extracted with ether and is heat stable. They have been labeled Stachybotryotoxin A & B and Satratoxin C, D, F, and G. In hay or straw, they produce a dark, sooty layer, especially around the nodes, and the mold can be recovered from these dark samples. It is easy to isolate from these substrates by using wet filter paper to grow them. The isolation of S. atra is enhanced by using ultraviolet light which strongly inhibits the growth of other fungi while mildly affecting this species.

A toxin is inactivated by alkali and chlorine. It is soluble in organic solvents. B toxin is less soluble in organic solvents and is less toxic. It is extracted with ether and evaporated to form toxin crystals. The dermal irritation test on rabbits is a common screening method for these toxins.

Several types of stachybotryotixcosis are classified into these four main groups –

1. Dermal where the skin and mucus membranes are affected
2. General toxicosis with changes in the blood and blood forming organs prevailing
3. Nervous form

4. Abortions

In horses, when large amounts of toxic hay are ingested, the animals die suddenly in 1-3 days, with disturbances in the nervous system and circulatory organs. When smaller amounts are ingested over a prolonged period, the eyes and mouth are affected first. The mucus membranes are irritated and then become hyperemic, oedematic and later necrotic. Sores and cracks appear on the lips and the corners of the mouth. Salivation and runny nose ensue with the head and eyelids swelling producing a "Hippopotamus Head". Necrotic sores appear on the tongue and tonsils and cause reduced feed intake. This first stage may last only a few days or several weeks.

The next stage is characterized by changes in the blood and general toxicosis. The number of leukocytes increase and then decrease sharply. Thrombocytes also drop significantly and blood coagulation is disturbed. This lasts for 15-20 days usually.

Next, the temperature rises to 41+C and the leukopenia gets worse. Blood coagulation is lost, appetite is poor and digestion is disturbed. Secondary infections and septicemia are common. The bone marrow turns to jelly and the liver, kidneys and myocardium are degenerated. This lasts 1-6 days and is fatal.

Cattle and sheep experience similar symptoms as the horses while swine suffer necrotic tissue on skin with suckling piglets and sows (especially those nesting on straw). The legs and bellies are also affected. Vomiting, muscular tremors and sudden death are also common. Abortions have also been reported.

In farm workers affected by handling moldy hay and forage, symptoms include conjunctivitis, cough, rhinitis, burning in the nose and nasal passages, cutaneous irritation at the points of contact, nose bleeds, fever, leukopenia in a few cases, pharyngitis, and laryngitis.

Chapter 10

The Ergot Alkaloids

Ergot, also known as ergotism, is a name of the mold and disease caused by species of *Clavieps*, the best known of which is Claviceps purpurea. These species produce alklaloids that are poisonous when ingested in modest quantities. When ergot grain was mixed into flour or bread, epidemics occurred with wide ranging effects. It caused gangrene, hallucinations, and convulsions on enormous scale in Europe for many centuries. The early stage of poisoning was often accompanied by a sensation of "ants running around underneath the skin". Ergot has long been known as a powerful constrictor of blood vessels. By the 19th century it changed from a feared poison to a starting material for important remedies and chemicals.

The middle ages records are littered with instances of "holy fire" or "St. Anthony's Fire" in those areas where rye was used for bread. Inflammation consumed the limbs which turned black from necrosis and gangrene before they detached. Screams from violent burning pain, rotting flesh and death were common. The worst epidemics occurred when rains alternated with hot spells and the mildew was heavy. About 100 grams of ergot over several days in the grain and bread was enough to cause gangrene and death. The grains were estimated to be 25% ergot in the worst epidemics. In Russian epidemics, wheat flour containing 7% ergot would cause gangrene and death. Most European countries have set limits of .1-.2% ergot in flour in the 20th century.

In 1582, its first recorded use as a drug was described and there are remarks of its use by women as a pupil dilator. By 1764, Ergot was finally recognized as a fungus attached to rye rather than being a diseased rye grain. The first crystalline alkaloid was isolated in 1875 and finally, in 1943, LSD was synthesized from a degradation product of the ergot alkaloids. It is interesting that Ergot became associated with witchcraft and that its sufferers were possessed by demons. It has even been suggested to be the cause of the bewitched girls behavior at the Salem witch trials.

Rye plants become infected when a Claviceps species ascospores invades the inflorescence of the grain. Germinating spores grow around the ovary and enter it at its base. The hyphae then spread out. It only develops in the female sex organs of grasses such as rye. The hyphae form a neck-like ring for the attachment of sclerotia at the base of the stalk. This also serves as a supply of water for fruiting body. The conditions for germinating ergot sclerotia include chilling the plant for 3-4 weeks at –1-3 C after which they start to germinate over the next two months at 10-15 C. Asexual spores or conidia are produced and are spread to other flowers by honeydew produced by the host plant.

Various races of ergot react differently to the cold, and their pigmentation varies considerably. After the chilling and pre-germination periods, a large increase in water uptake and respiration takes place. The conidiophores produce hyphae. The addition of

honeydew promotes germination of the spores. The density of spore suspensions also influence the germination rate.

There are many species of Claviceps and many of these produce various alklaoids. Claviceps purpurea is the best known and it produces a treasure house of pharmaceuticals. The best known of these in medicine and toxicology are known as alkaloids.

One group of alkaloids called the “clavine” alkloids, are found in the sclerotia of saprophytic cultures of Claviceps that parasitize wild grasses in the Far East and Africa.

Peptide alkaloids are those found in C. purpurea and on hydrolysis, they decompose to lysergic acid or isolysergic acid. C. purpurea also infects barley, wheat, and more than 100 grasses. The ergot of corn in humid parts of Mexico will grow to 8cm long x 5 cm thick.

There is an enormous volume of literature on the alkaloids that can be extracted an synthesized from ergot. We will not attempt a comprehensive review here but will cover the main properties.

Most of the alkaloids will turn intense blue when brought into contact with sulfuric acid which allows a test for their presence. The alkaloids are extracted as water soluble and the as water insoluble fractions using chloroform and methanol (90%/10%) mixtures. Ethyl acetate (85%), ethanol (10%), and di-methylformamide (5%) are used to separate individual clavine alklaoids. Ergoline alkaloids fluoresce in UV light which permits detection in extracted samples.

The sclerotia of C. purpurea contain a number of yellow and red-violet pigments that have been isolated since 1877.

Production of the ergot alkaloids first came from collecting samples from crops and fields. By the 1800’s, it has been produced by infecting rye plants with suitable strains. One crop a year is obtained and yields are dependent on the weather. The rye plants are typically injected (inoculated with spore suspensions) into the rye spikes 2-3 weeks before flowering and through flowering. Six to seven weeks after inoculation, the harvest begins, usually by hand picking. Yields are usually 50-100 Kg per acre.

Ergot fungi can also be cultivated in surface cultures and under submerged conditions. Strains isolated from wild grasses were grown on a medium of mannitol (5%), ammonium succinate (.8%), potassium dihydrate phosphate (.1%), magnesium sulfate (.03%) and tap water adjusted to pH 5.2. It has had good success with saprophytic cultures of Claviceps and produced yields of 600 mg/liter after 30-40 days at 26-27 C. Improvements in yields have been obtained by replacing mannitol with 10% sucrose and trace minerals.

Other cultures used Mannitol at 6.5% and glucose at 1% from the carbon sources. Submerged culture yields are also good with a medium based on glycerol at 10% and peptone at 2%. A sporulation medium was described in 1964 for C. paspali using 250ml of corn steep liquor and 500 ml beer wort per liter in agar slants. Using liquid fermentation media, texts described rapid growth and good yields with yeast extract and glucose.

Media with high osmotic pressure containing 20-30% mannitol or sucrose favor peptide alkaloid formation in submerged cultures reaching their peak at 10-12 days. Strain degeneration occurs in some strains accompanied by changes in colony morphology. These strains can be returned to high production by passage through the host plant.

Entire textbooks have been written on the pharmacology and biological effects of the ergot alkaloids. Thousands of scientific papers have been written on LSD alone describing its potent hallucinogenic effects. The classic use of ergot has been dilation of pupils of women as a beauty aid, and contraction of the uterus for childbirth.

Chapter 11

Penicillium Molds & Toxins

This chapter will cover the large array of toxins produced by Penicillium species. It will be broken down into the following areas –

1. Overview & History
2 P. viridicatum & Ochratoxin
3 P. patulin & Patulin toxin
4 P. cyclopium & Penicillic acid
5 Yellowed Rice Toxins
6 P. roquefortii & PR toxin
7 P. rubrum & the Rubratoxins
8 Cyclopiazonic Acid & related toxins
9 Miscellaneous Penicillium Toxins

1) Overview & History

The genus *Penicillium* is well known for its role in producing antibiotics (penicillin from P. chrysogenum) and fermented foods (blue cheese). P. roquefortii and P. cambembertii are used to make mold ripened cheese. They add a distinctive flavor to a wide range of cheese products and are even used in meat products.

They are also responsible for the majority of human food spoilage. Most of the spoilage problems are associated with moldy bread, flour, confectionery, cheese, dairy products and meat products. When these items are refrigerated, the predominant spoilage microflora has been determined to be penicillia with aspergilli the leader in non-refrigerated mold spoiled products. In cheese products, the molds growing on the outside are typically washed off prior to final coating and sale, removing most of the mold and any toxic products that may have been accumulated on the outside. The striking, smallish blue-green color of the mycelium and spores make these molds familiar to most people who have seen them on bread, oranges, cheese and other foods and fruit preserves.

Like most other fungi, penicillia can produce various mycotoxins in foods, feedstuffs, and culture media. Some of these are well known like citrnin, penicillic acid and the already described ochratoxins. They also produce tremorgenic toxins, PR toxin and a variety of other acid toxin materials. Many surveys of the incidence of penicillia have been made in cereals and other agricultural products but those in foods have been mostly limited to spoiled cheese.

In a 1973 study, spoiled foods were selected in the UK and Australia and plated on Czapek-Dox agar and malt extract agar. Isolated colonies were then plated for examination. These isolates were cultured for 7 days at 25C in Czapek solution and in

media with yeast extract (20 g/liter) and sucrose (40-200g/liter). These cultures were then extracted sequentially with hexane, chloroform and ethyl acetate. These were concentrated in vacuum, pooled and tested against baby hamster kidney cells, brine shrimps and germinated pea seedlings (see the tricothecenes pea seedling testing).

Penicillia were isolated from over 50% of 215 moldy food samples from six major classes of foods. A total of 413 fungal isolates were obtained in which 219 were penicillia (53%), 56 were aspergilli (14%), 65 were zygomycetes (16%), and 36 were cladiospora (9%).

Frequency of isolation of fungi from spoiled U.K. foods—numbers (and percentages)[a] of fungi

Genus	Meat products (n=51)	Cheese (n=32)	Bakery products (n=46)	Fruit and vegetable products (n=50)	Nuts (n=11)	Misc. (n=25)	Total (n=215)
Penicillium	52 (52)	51 (89)	58 (56)	33 (44)	6 (24)	19 (37)	219 (53)
Aspergillus	2 (2)	2 (4)	29 (28)	10 (13)	7 (28)	6 (12)	56 (14)
Cladosporium	25 (25)	1 (2)	2 (2)	3 (4)	0 (0)	5 (10)	36 (9)
Mucor	13 (13)	1 (2)	3 (2)	14 (18)	5 (20)	6 (12)	42 (10)
Rhizopus	7 (7)	1 (2)	3 (3)	3 (4)	4 (16)	5 (10)	23 (6)
Other	1 (1)	1 (2)	9 (9)	12 (16)	3 (12)	11 (21)	37 (9)
Total	100	57	104	75	25	52	413

[a] Rounded to nearest whole number.

Most of the Penicillium isolates belong to the sub-genus viridicata and expansa. The following tables show the identification of those organisms isolated from the moldy foods –

Sources of identified U.K. isolates belonging to genus *Penicillium* according to series

Series	Meat products	Cheese	Bakery products	Fruit and vegetable products	Nuts	Misc.	Total
Expansa	8	6	14	8	3	4	43 (20)[a]
Viridicata	33	43	31	14	2	8	131 (62)
Urticicola	4	0	7	6	0	1	18 (8)
Cylindrospora	1	0	0	1	0	1	3 (1)
Furcatum	0	0	4	0	0	1	5 (2)
Divaricatum	0	2	0	0	0	1	3 (1)
Aspergilloides	1	0	1	3	0	3	8 (4)
Biverticillium	0	0	0	1	0	0	1 (1)
Eupenicillium	0	0	0	0	0	0	0 (0)

[a] Figures in parentheses indicate percentage of total penicillia to nearest whole number.

Number and percentage of *Penicillia* isolated from foods

Subgenus	Species (after Pitt, 1979a)	No. and (percentage) U.K. isolates	Australian isolates
Penicillium	*P. aurantiogriseum*	42 (20)	6 (6)
	Near *P. aurantiogriseum*	11 (5)	0 (0)
	P. crustosum	35 (16)	16 (15)
	P. chrysogenum	24 (11)	10 (9)
	P. expansum	19 (9)	6 (6)
	P. roquefortii	19 (9)	6 (6)
	P. brevicompactum	13 (6)	3 (3)
	P. echinulatum	10 (5)	4 (4)
	P. hirsutum	7 (3)	0 (0)
	P. verrucosum	5 (2)	5 (5)
	P. viridicatum	4 (2)	0 (0)
	P. italicum	2 (1)	1 (1)
	*P. palitans**	2 (1)	0 (0)
	P. camembertii	1 (0.5)	0 (0)
	P. digitatum	1 (0.5)	0 (0)
	P. olsonii	0 (0)	1 (1)
	unplaceables	2 (1)	0 (0)
Furcatum	*P. corylophilum*	4 (2)	7 (7)
	P. griseoroseum	2 (1)	1 (1)
	P. citrinum	1 (0.5)	6 (6)
	P. janthinellum	0 (0)	2 (2)
	P. miczynskii	1 (0.5)	2 (2)
	P. canescens	0 (0)	1 (1)
Aspergilloides	*P. glabrum*	0 (0)	3 (3)
	P. thomii	0 (0)	1 (1)
	P. implicatum	0 (0)	1 (1)
	P. spinulosum	8 (4)	4 (4)
Biverticillium	*P. minioluteum*	1 (0.5)	2 (2)
	P. pinophilum	0 (0)	4 (4)
	P. islandicum	0 (0)	2 (2)
	P. purpurogenum	0 (0)	1 (1)
Eupenicillium	*E. ludwigii*	0 (0)	3 (3)
	E. erubescens	0 (0)	4 (4)

* Identified according to Raper and Thom (1949).

Sixty of the moldy food samples were extracted and tested for toxins. Thirty five of these tested positive with the following breakdown –

Ochratoxin	14
Patulin	8
Citrnin	16
Aflatoxin	6

Zearalonone and trichothecenes were also detected in some samples. The following table shows the association between the different penicillia and the toxins that were studied –

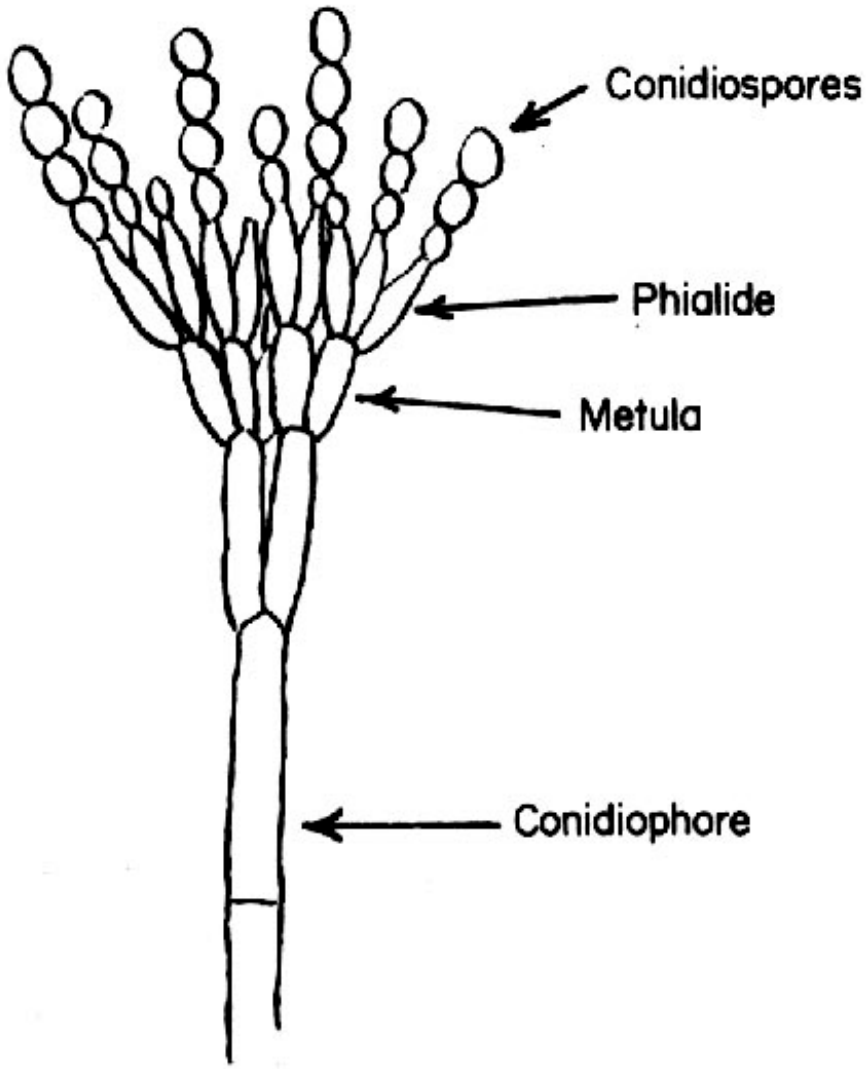
Association of fungal isolates with specific toxins detected in various foods

Isolate	Toxin	Food
P. roquefortii	Patulin	Cheese
A. flavus	Aflatoxin	Bread
P. citrinum	Citrinin	Cornish pasty
P. expansum	Citrinin	Cheese (three samples)
P. viridicatum	Ochratoxin	Cheese

All the toxins were tested positive in one or more of the pea seedling (all of them tested positive by this method), brine shrimp, and BHK cells. The high proportion of Penicillia recovered from the chilled foods was unexpected at the time.

Separate tests in cereal grains in 1968 showed that up to 44% of corn seeds are contaminated with penicillium species before harvest. Twenty two species recovered in these tests caused death of ducklings within 14 days of being fed a corn meal infected with pure cultures of the organisms. Of these, seven were very toxic, six less toxic, and the remaining were non-toxic to mice. The most deadly species were P. oxalicum and P. viridicatum, the latter which produced a variety of liver lesions. The P. oxalicum yielded a toxic pigment (secalonic acid D) that produces birth defects and respiratory disease in mice.

Penicillium is one of the most frequently encountered molds and is characterized by the production of small, dry, single celled, air dispersed conidiophores from phialides arranged as a brush-like structure at the ends of aerial conidiophores. This is what they look like under the microscope –



Structure of the conidiophore of *Penicillium*

The identification of individual species of penicillium requires careful examination of color, texture and size of the colonies growing under defined conditions as well as being able to examine the spore producing structures under the microscope. The most important species with the foods and common rots they produce are listed in the next table –

The more important toxigenic species of *Penicillium* on cereals and other foods

Species	Toxins	Comments
P. citrinum	Citrinin	Common biodeteriogen, worldwide on foods, decaying plant materials, textiles
P. cyclopium	Penitrem A Cyclopiazonic acid Penicillic acid Ochratoxin A	(=*P. aurantiogriseum*). Common on cereals and other foods
P. expansum	Patulin Citrinin	Predominantly from rotting apples and pears, but also other fruits
P. islandicum	Luteoskyrin Islanditoxin Cyclochlorotine	Cereals, particularly in the tropics
P. purpurogenum	Rubratoxins	(=*P. rubrum*). Primarily a soil fungus associated with the decay of many substrates
P. roquefortii	P.R. toxin Roquefortine	Blue cheeses, also cool stored products
P. viridicatum	Ochratoxins Citrinin Viridicatin Xanthomegnin Viomellein	Worldwide, cereals and cereal products

In Japan in the 1800's and 1900's, it was discovered that rice which turned yellow was moldy and caused a number of serious health problems. In 1938, one of the causative molds was finally isolated and tested (P. citreo-viride also called P. toxicarium). It was the primary cause of cardiac beri-beri. Other pigmented penicillia species have since been identified and associated with disease in "yellowed rice" in Japan –

Species of *Penicillium* and their metabolites associated with different aspects of 'yellow rice' toxicoses

Species	Toxins	Disease
P. citreo-viride	Citreoviridin	Cardiac beri-beri
P. islandicum	Luteoskyrin Cyclochlorotine	Hepatotoxicity
P. citrinum	Citrinin	Nephrotoxicity

2. P. viridicatum and Ochratoxin

P. viridicatum species is one of the most frequently occurring molds found in foods and feed. They are often found on decaying vegetation in the soil and have been recovered from moldy grains in storage and wheat paste. Along with P. expansum, it is the most frequently encountered species in mold ripened sausages and ham. There is some difficulty reported by lab workers and scientists in differentiating isolates of P. viridicatum, P. cyclopium, P. palitans, and P. crustosum. Some scientific papers have suggested that all blue-green and yellow-green strains of these species with similar morphology be designated as the P. cyclopium-viridicatum series.

A toxin designated as viridicatin was first isolated in 1953 from the mycelium of P. viridicatum grown on Czapek-Dox solution. It was obtained with a chloroform extraction although ethanol could be used as well although it yielded mannitol in the extractant. The cultures were incubated at 25 C for 3 weeks at which time the glucose in the solution was nearly depleted. The harvested mycelium was dried and ground to permit continuous extraction with chloroform. The toxin was obtained by recrystallizing from ethanol, and yielded lustrous needles with a melting point of 268 C. The colorless needles give an intense green color when reacted with ferric chloride and show a violet fluorescence under UV light.

In 1954, another toxin called cyclopenin was recovered. In 1960, viridicatic acid was extracted from culture filtrates grown for 7 days and extracted 4 times at a pH of 2.0 with ethyl ether. The acidic fraction of this extract was separated into saturated aqueous sodium bicarbonate solution which was then acidified and extracted with ethyl ether. Crude crystals were obtained in this extraction.

In 1968, researchers fed artificially contaminated corn (with P. viridicatum) to mice and produced liver damage, bile ductile cell hyperplasia, cholangitis, and periductular fibrosis. Other tests have implicated the mold with chronic kidney degeneration in pigs and rats eating infected barley. Japanese scientists (1972) grew the mold on rice, wheat, flour, beans, and seaweed and produced kidney, liver and nerve toxicosis in rats fed these infected diets. Long term chronic feeding to mice produced pulmonary adenomas and adenocarcinomas.

From the above history, it is clear that P. viridicatum produces numerous toxins and different strains will yield widely different arrays of metabolites. The mycotoxins that cause liver damage is different than that which injured the kidneys. A total of 12 toxins have been extracted and isolated to date. Of these, Ochratoxin A seems to have been partly responsible for the recorded kidney damage along with oxalic acid (the same toxic substance found in rhubarb plants). Its LD50 in rats is 22mg/Kg. Ochratoxin has already been described in the aflatoxin chapter and will not be reviewed here.

Citrinin is also produced by this mold and and many other asperigillus and penicillium species. The LD50 for Citrinin in mice is 35 mg/Kg subcutaneously and by IP. Death is caused by kidney damage. When fed to swine it caused a nephropathy.

Penicillic acid is another substance produced by this fungi and its LD50 in mice is 110 mg/Kg by sc (subcutaneous) injection. It also produced malignant umors in rats at the site of injection.

A cardiotoxic substance has also been isolated called *viridicatumtoxin* which has an oral LD50 of 122 mg/Kg in mice and causes death by degeneration of the myocardium (heart disease) and renal tubular necrosis.

Strains of P. viridicatum grown from Denmark seem to produce toxin combinations that target the kidneys while strains cultured from mold samples in Indiana affect the liver primarily. The mold also produces red, yellow, orange, and purple pigments which were tested and found to be toxic in only in large amounts in brine shrimp.

3) P. patulin and Patulin toxin

Patulin and penicillic acid are metabolites produced by several species of Penicillium and Asperigillus that have similar biological effects. Patulin has been called claviform, clavacin, clavatin, expansin, leucopin, myocin C, penicidin, and tercinin in previous studies. It belongs to a class of chemicals known as carcinogenc lactones.

Patulin was discovered in 1943 and synthesized in 1949. Patulin producing strains are widely spread in food as contaminants. It has been found in commercial batches of apple juice in Canada and the US (1975) at levels of 9-150 mg/liter. It originated from cider mills where decayed apples are not sorted out or are stored in large bins for long periods. The storage rots of a number of fruits are caused by patulin producing species such as P. patulin and P. expansum. It has also been recovered from mold fermented sausages.

At one time (1944), it was believed that Patulin was seen as a possible cure for the common cold. It has since been recognized that it is useless in that regard and to be very toxic. It inhibits both gram positive and gram negative bacteria and is one of the most potent antibiotics known. It is not used for this purpose because of its high toxicity and its teratogenicity. It inhibits the respiration of bacteria and plants. It is also very effective at inhibiting the growth of tissue cultures and inactivates viruses (bacteriophages) in many bacteria. Patulin also inhibits seed germination and causes plant wilting. It also inhibits many fungi.

Its LD50 in mice is 5.7 mg/Kg. When fed to mice at 100 mcg/day it drastically reduced the lymphocyte count in the blood but did not affect granulocytes. Patulin also increases vascular permeability causing serious edema. It also suppresses urine formation in mice with an accompanying increase in blood sugars. Patulins effects on cells include inhibiting fission in bacteria resulting in giant cells, and production of binucleate cells in corn and onion roots. A huge range of other effects suggest strong mutagenic, teratogenic and cancer causing properties. Injected twice weekly in rats (sc) for 15 months produces 100% tumors at the injection sites.

Patulin and penicillic acid contain five membered rings that are carcinogenic. Because of this, these compounds are considered carcinogens. Patulin toxin is unstable in alkali and loses biological activity but is stable in acid.

Growth of P. patulin is accomplished by placing spores into a suspension media of .1% agar or on Czapek agar and grown at 26C. [Mycelium starter cultures are very slow and difficult to grow.] Colonies are first seen in 2 days after inoculation. Toxin is extracted with a mixture of 88% acetonitrile, 10% sulfuric acid and 2% potassium chloride. After filtration, distilled water is added to the filtrate (33%). This mixture is extracted twice with chloroform and then evaporated to near dryness. Patulin production peaks at 5-9 days after colony appearance and averages 45 mg/40ml in liquid cultures.

Patulin can also be extracted and concentrated with chloroform and methanol followed by drying in vacuo. The extracts can then be dissolved in propylene glycol or olive oil and injected into the air cell of pre-incubated fertile eggs. Very toxic extracts result in no survival of the embryos (amounts and procedures described in the aflatoxin chapter).

4) P. cyclopium and Penicillic acid

Penicillic acid was first isolated in 1913 from P. puberulum grown on corn. The extract of the culture was found to be toxic to lab animals. This culture could not produce large amounts necessary for testing chemically and in farm animals. In 1936, researchers found a strain of P. cyclopium that could produce large amounts for study. It has also been recovered from a large number of other Penicillium and Asperigillus species since then.

In 1970, scientists isolated several strains of P. martensii from high moisture corn stored at 5 C and described as blue-eye diseased. Feeding the moldy corn to mice killed the test animals. The toxin was isolated, crystallized, and identified as penicillic acid. It was produced in culture on the grain at 5-32 C with maximum production at 15-20 C. Penicillic acid producing strains have been recovered from cured ham (P. verrucoum) and European salami, frankfurters, country cured ham and fermented sausages (P. cyclopium). Both Patulin and Penicillic acid could be produced in the sausages when inoculated with the organism and ripened at 15 C. There was no toxin production at 25 C which was believed due to the pH of 5.5 at 15 C and 7.1 or higher when maintained at 25 C.

Penicillic acid crystallizes as anhydrous needles from pentane, hexane or benzene with a melting point of 83-84.8 C. Its monohydrate form crystallizes from water as large transparent, monoclinic or triclinic, rhombic crystals. It is moderately soluble (2%) in cold water and cold benzene, and highly soluble in hot water, alcohol, ether and chloroform. It is insoluble in pentane-hexane. This allows it to be extracted with hot water and precipitated by chilling. Penicillic acid also sublimes at 80-90 C.

Penicillic acid reacts with hydroxylamine in strong alkali to give a red color which gives a good test for its presence in mold samples. It also forms a stable reddish-purple complex with ammonia. It is stable if stored under refrigeration for 15 days.

Penicillic acid has strong antibiotic properties and in many ways mirrors the other properties of Patulin. Its toxicity prevents its use in therapy. Its LD50 in mice is 100mg/Kg. When injected at levels of 1 mg twice weekly for 64 weeks, it produces transplantable tumors in all rats surviving treatment. At levels of only .1 mg it would initiate tumor development as well.

P.cyclopium reportedly does not produce penicillic acid on Czapek-Dox medium with glucose as the sole carbon source and sodium nitrate as the nitrogen source. It does produce considerable acid on Raulin-Thom medium. A. ochraceus growing on sucrose-glutamic acid-salts medium produced .7 gm/liter of penicillic acid.

In moldy meat and meat product tests, over 42% of all the isolates tested were P. cyclopium which produced both penicillic acid and cyclopiazonic acid.

5) Yellowed Rice Toxins

Shortly after World War Two, mold metabolites from “yellowed rice” were studied and found to be able to induce liver tumors in test animals and humans. Fungi present in stored rice was studied from Burma, Thailand, Egypt, Spain, Italy and the US. Some of the shipments were found to be contaminated with strains of P. islandicum, which produced highly toxic metabolites. When ingested by animals they caused severe liver damage at low doses.

There have been more than 15 types of fungi that have been identified as causing moldy or yellowed rice since the 1940’s. One of these P. toxicarium (also known as P. citreo-viride) produces a powerful toxin in the rice that causes an ascending paralysis as well as circulatory and respiratory disturbances that resemble beri-beri in man.

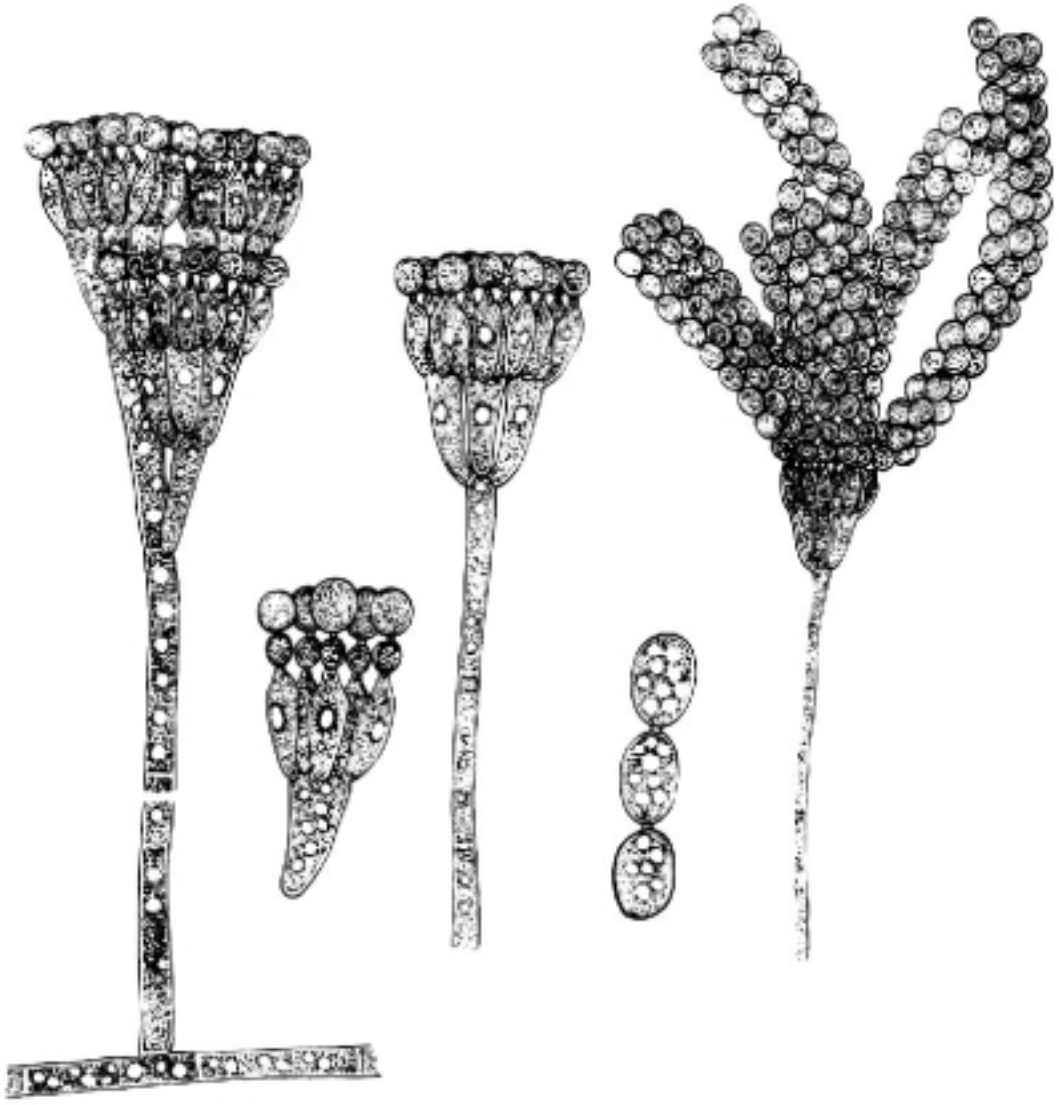
P. citrinum was also isolated from rice imported from Thailand in 1953 and was found to produce citrinin. When this moldy rice was fed to animals it caused renal damage.

P. rugulosum produces a toxin called rugulosin that causes necrosis in liver cells in experimental animals and was recovered as a storage fungi in rice produced in Japan.

A survey of polished rice in Japan took place in the 1950’s and 1960’s with Asperigillus as the most frequently recovered followed by Penicillium species. About 10% of the isolates recovered produced strong toxins. The most influential factors in the mold growth in the grains were temperature and moisture content of the grain. When stored below 14% no mold growth occurred for one year. A. glaucus and P. citreo-viride

begin to grow in stored rice at 14-15% moisture. Most other storage fungi grew at 15-17% moisture. Above 18%, bacteria would begin to grow and compete in the grain with the molds.

Penicillium islandicum, when grown on malt agar produces rapid and flat colonies, more or less flocose with zones of pigmented mycelium. It is heavily sporulating with a slightly aromatic odor. It is deep red at the center on the reverse side of the agar plate. Conidiophores arise from tailing or aerial mycelium, are yellow-green, walls are encrusted with pigmented material. Conidia are elliptical and smooth bearing short chains.



Penicillium islandicum

P. regulosum forms colonies on Czapeks agar that are restricted, velvety, with tough mycelium irregularly folded and wrinkled. The colony is dark green, and the reverse is yellow-orange-red. Conidiophores arise from surface hyphae with smooth walls. Penicilli are biverticillate-symmetrical. Conidia are elliptical, blue-green, smooth and bearing tangled chains.

Many tests have been conducted on P. islandicum. Early ones were done with methanol extracts of the fungus mats cultured in Czapeks solution at 33 C for 14 days. When fed orally to rats, they produced marked liver damage. Virtually all acute and chronic feeding trials of the mycelium mat or contaminated rice grains produced acute injuries in the livers as well as tumors. The injuries correlated directly with the amounts and lengths of the feeding trials.

Rice grains were directly cultured with inoculum of P. islandicum at 33 C for 7 days in other tests. These could be inoculated into sterile grain and fed diluted to measure the lower chronic levels of exposure. The intake of moldy grain is listed in the chart below along with the severity of liver damage. The acute atrophy resulted in animal death following a prolonged comatose state similar to hepatic coma in humans.

YELLOWED RICE TOXINS

LIVER DAMAGE WITH DAILY INTAKE OF TOXIC MATERIAL[a]

	Moldy rice			Fungus mat		
By autopsy	Mice	Survival time (days)	Intake (gm/day)	Mice	Survival time (days)	Intake (mg/day)
Acute atrophy	18	3-8	2-4	22	2- 10	2.3-25
Subacute atrophy	7	36-64	0.5-1.5	9	19- 48	0.6 - 6
Subchronic atrophy	6	72-96	0.5-1.5	7	29-211	0.25- 6
Diffuse atrophy	9	205-566	0.05	45	30-618	0.08- 6
Liver cirrhosis	18	49-594	0.05-4			
Total	58	3-594	0.05-4	83	2-618	0.08-25

In all tests, hepatomas (tumors) appear even when very low doses of the toxin are fed to mice and rats. Most of these are not malignant but cellular changes are observed in those cells not without cirrhosis.

The rabbit is the most sensitive to hepatotoxic agents and when fed 1-5% (of the total grains) of moldy rice mixed with bean curd residue, all died within a few days. At 1-2.5% moldy rice, some of the rabbits survived to 90 days with severe liver necrosis.

Acute liver damage has been induce in rhesus monkeys fed infected moldy rice grains but these animals did not experience cirrhosis or tumors. Other organs affected by the moldy grains included atrophy of the thymus, spleen and fat tissue (in rats).

Strains of P. islandicum that produce yellow or brown rice typically produce "luteoskyrin" although other strains without pigments or producing yellow-orange rice have also produced similar toxins.

Luteoskyrin was first isolated from the fungus mat of P. islandicum cultured on Czapek medium. It was one of several toxic pigments eventually recovered. It was extracted with methanol and purified with systematic fractionation. To obtain pure leuteoskyrin, the methanol extract was chromatographed on a carbon- Sodium Sulfate (1:20) column with acetone. The elution product was re-crystallized from acetone and methanol.

Rugulosin has been isolated by many Penicillium species found in domestic Japanese rice. This pigment is extracted from P. rugulosum fungus mat with light petroleum and then ether. The ether soluble fraction of the de-fatted mycelium was extracted with 5% sodium bi-carbonate. On acidification of the bicarbonate soluble fraction, a considerable amount of rugulosin was precipitated. Repeated re-crystallization from ethanol produced large yellow prism like crystals.

In 1971, another scientist isolated the pigment by culturing the fungi on Czapek medium at 27 C for 2 weeks and the acetone soluble fraction of the pigments is chromatographed with charcoal as adsorbent and acetone as developer. Re-crystallization with acetone or methanol gives rods or fine needles. The yield is about 1.5-1.8 grams per 100 gm of dried mycelium.

The LD 50 of luteoskyrin in mice is –

IV	6.65 mg/Kg
IP	40.8
SC	147
PO	221

With repeated administrations of less than $1/10^{th}$ the LD50, the same lethal effects occur as in the single large dose. This means that much lower doses administered over time is more effectively lethal. Liver changes are seen in 24 hours. Its color changes to cloudy yellow, becomes soft and is diffusely dotted with minute red spots.

Feeding moldy rice (50%) infected with lutoeoskyrin or rugulosin producing strains caused 100% mortality in mice in 9-23 days. At 25% all died in 24-30 days. :iver cirrhosis occurred in those mice fed 10% moldy rice. Rats fed dried mycelium (from P. rugulosum or P. tardum) with regular feed at 25-50 grams/Kg died of nephrosis-like injuries. Luteoskyrin is also highly cytotoxic with as little as 1 mcg/ml lethal to tissue culture cells.

In 1955, another liver toxic compound was isolated from a culture filtrate of P. islandicum. It was water soluble with a melting point of 251 C. The yield was quite small, only 20-40 mg/50 liters. It has also been recovered from grain and mycelium mat filtrates. The effects on the liver were markedly different than that of luteoskyrin with levels of micrograms producing significant changes within minutes of administration. This new toxin was called islanditoxin.

Another toxic pigment isolated from P. islandicum on moldy rice imported from Spain, is called *erythroskyrine*. It is extracted from the benzene soluble fraction of the mycelia. It is soluble in chloroform, methanol, benzene, acetic acid, and pyridine. It is less soluble in ether, hexane, and petrol ether. It reacts in sulfuric acid turning blue violet. Purified crystals are orange-red.

When injected into mice at 60 mg/Kg, 50% were killed. At 600 mg, all died. After administration of the pigment, most of the mice were paralyzed, became comatose and died. All suffered liver damage, and cellular injuries of the lymph nodes, spleen and thymus.

Yellowish colored rice imported from Thailand to Japan in 1951 was contaminated with the mold P. citrinum. Subsequent studies showed that the mold was distributed worldwide in rice producing areas. It has since been isolated in rice from Burma, Italy, Egypt, the US, Red China and in Japanese polished rice. It prefers polished rice and causes the stored grains to turn yellow on the surface. The colored areas fluoresce under UV radiation.

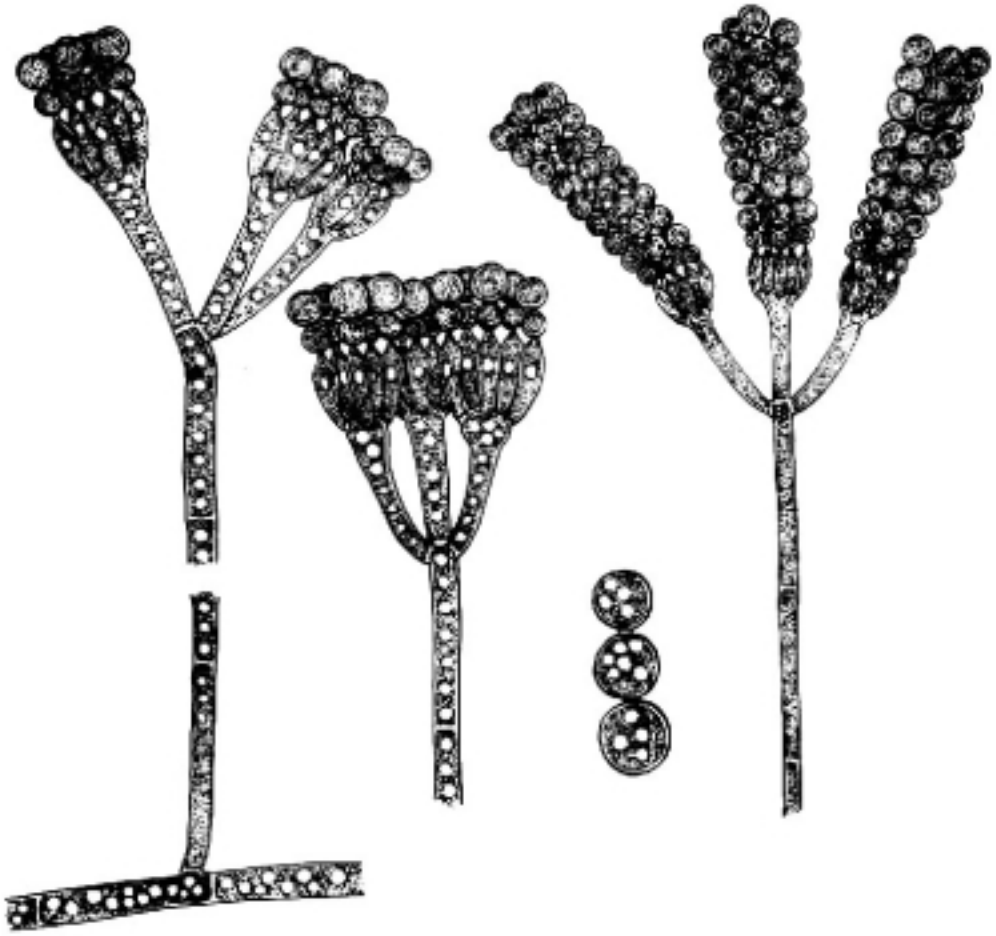
P. citrinum conidiophores are smooth, penicilli are biverticillate and assymetric, and stigmata in 6-10 verticils. The conidia are globose, smooth and conidial chains are parallel in divergent columns. There is no growth at 37 C.

The colonies have a deep bluish-green velvety appearance with a brown central area containing yellow pigment (citrinin). The pigment is partilly crystallized out on the reverse side of the colony. It shows a citrinin reaction with hydrochloric acid and Lugol's solution to give a deep reddish-brown reaction.

Many Penicillium and Asperigillus species produce citrinin. Ether or chloroform extracts of culture filtrates are made slightly acidic and Congo red by the addition of hydrochloric acid and the precipitate is obtained. The extract fraction of the acidic broth can be purified by re-crystallization from absolute ethanol or from benzene-cyclohexane solution. Citrinin was obtained as lemon-yellow needles with a melting point of 172 C.

In a 3 week feeding study using rice infected with P. citrinum, rats developed enlarged kidneys. A 1955 test used unpolished rice grains inoculated with the fungi and cultured for 48 hours at 25 C. Rats were fed the grain at 10% and 100% of their diets. All kidneys were enlarged and gray-white in color. Many cellular and other pathological changes occurred in the kidneys and the rats suffered renal damage and growth retardation as well.

The mycelium of the fungus was non-toxic in mice but the toxin is present in the culture filtrates and in the infected rice.



Penicillium citrinum

The LD50 of citrinin in the mouse is 35 mg/Kg by SC and IP. When given orally to rats it increases urinary output indicating that water re-absorption is inhibited by this pigment. In addition to the renal toxicity, studies have also revealed pharmacological effects including vasodilation, bronchoconstriction, and increase in muscle tonus.

Citreovirdin is another yellow rice toxin extracted from P. toxicarium, (P. citreo-viride) and P. ochrosalmoneum. The first extracts were obtained in 1940. Scientists observed that the yellow rice fungus would start to grow on the rice in storage shortly after harvest. A scratch on the surface of unpolished grains, especially around the embryo bud, allows the fungus to enter the interior. Germination and infection begins when grain moisture reaches 14.6%. At about 15.6% moisture, many other fungi begin to grow and

overwhelm this species so this one is found only in this narrow moisture range in moldy grain samples. This fungus and its toxic orange and yellow pigments are widely distributed but occurs in greatest frequency in the northern part of the main island of Japan.

Those grains infested with P. citreo-viride is more toxic to mice when it is incubated at a lower temperature. When given by IP, SC or PO it causes typical acute poisoning an all mammals tested with progressive ascending paralysis beginning in the hindlegs and flank, vomiting or convulsions and gradual respiratory disorders. Severe cases in primates include neurological symptoms, with those surviving experiencing residual paralysis and blindness. Death usually comes from paralysis of the thorax and diaphragm. These symptoms were consistent with acute cardiac beri-beri in Japan and Asia where the toxin is frequently encountered.

Intermittent low level exposures also cause sub-acute intoxication with the test animals more sensitized to the toxin. Human fatalities of acute beri-beri usually experience violent and malignant symptoms like those described above. In epidemics from the 1700's and up to and including World War 2, the mortality rates were very high.

The toxin citreoviridin was first extracted in 1947 using re-crystallization with methanol. More modern methods involve extracting from rice cultures (infested and incubated for a month) ground to a powder using n-hexane which removes other materials and leaves behind a residue containing the toxin. This residue is extracted with ether which dissolves the toxin and is filtered off.

Citreoviridin is soluble in acetone, chloroform, benzene and ethanol (and other alcohol) and is insoluble in n-hexane and water. It forms dark yellow, crystalline needles which melt at 100-111 C. The LD50 reported for mice is .2 mg/10gm body weight. The crude extracts fluoresce a glittering golden yellow or brilliant cadmium orange under ultraviolet light. Its toxicity disappears when the fluorescence disappears which can be caused by exposure to sunlight. Fluorescent measurements inside of animals tested indicate the toxin distributes widely in the body including the brains, kidneys, liver, and almost all other tissues. In surviving animals, it is quickly eliminated from the body and is found in the urine, bile, milk and vomitus.

Since the 1970's, more than 29 other toxins have been isolated from various strains of P. islandicum. Some of these are potent hepatotoxic carcinogens including luteoskyrin. Some of these toxins were much more toxic (.5 mg/Kg or less) in test animals when purified, but could not be produce in the laboratory in large enough amounts for large scale study. To produce these toxins, the fungus was cultured on Czapek medium (both liquid fermantation and plated) that was enriched with .5% yeast extract and .5% casamino acids. Spores (10ml) were used to inoculate the medium which was incubated at 30 C for two weeks. The grain fermentations used 300 grams of grain moistened with 150 ml of water. Most of the toxins were extracted with acetone and/or water. Rice, wheat and corn were the best producers of the most toxic extracts.

6) P. roquefortti and PR toxin

P. roquefortii is used in the fermentation of blue cheeses since AD500 and is frequently found in cool stored food products and is known for the PR toxin it produces. It is found in all blue cheeses samples from all countries tested. It produces a toxin called roquefortine that is produced with high yields when the fungus is grown on medium containing15% sucrose and 2% yeast extract for 16 days at 25 C. Other toxins have also been extracted. Roquefortine has also been recovered from P. oxalicum.

The toxic extract causes severe tremor and convulsions in test animals. The PR toxin is lethal to mice and rats by all routes of administration. It increases capillary permeability which leads to severe systematic dehydration and a decrease in blood volume. It directly damages the lungs, heart, liver and kidneys. The LD50 ranges from 1-14 mg/Kg when injected and 70-115 mg/Kg by oral routes. It is soluble in DMSO.

7) P. rubrum and the Rubratoxins

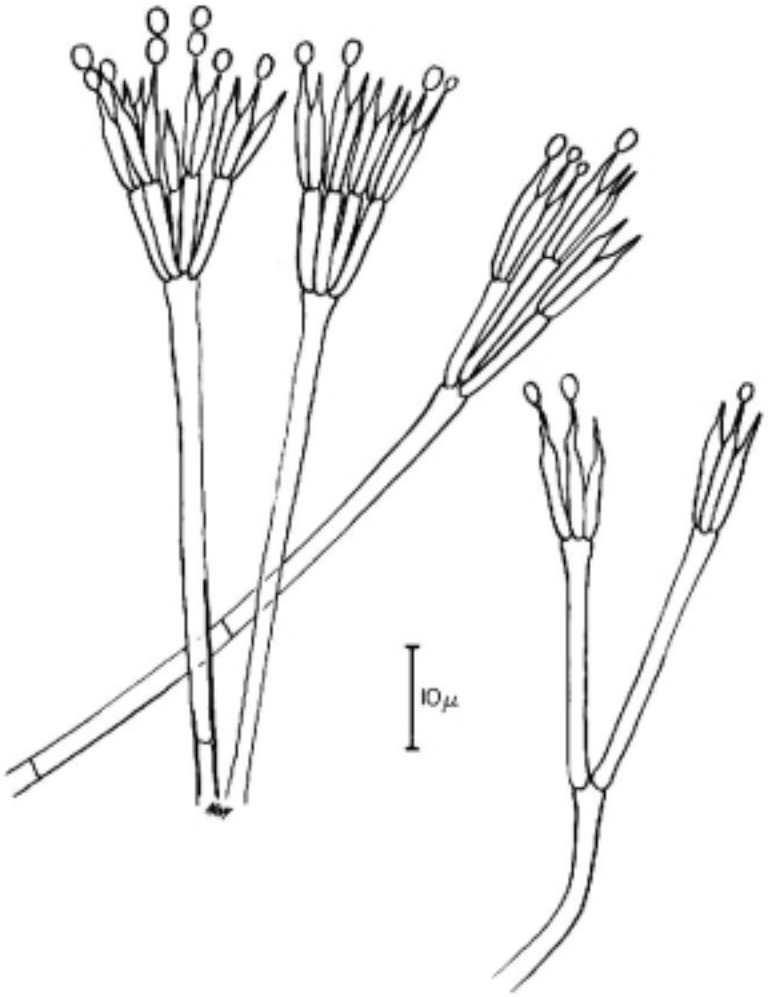
In 1953, scientists examined moldy corn that caused disease and death in pigs and cattle. They grew 13 cultures of fungi from the moldy samples and found only two which yielded toxic extracts. These were A. flavus and P. rubrum. They contaminated fresh corn with both isolates and found that the P. rubrum infected corn was far more toxic. A single dose of only ½ # of moldy corn was sufficient to kill pigs in a single day where A. flavus required 7-8# and 4-5 days for mortality.

P. rubrum belongs to a group of penicillia that produces pigments ranging from yellow, orange, deep red, and purple-red. The speices is widespread in nature and is found in soil and decaying organic matter, especially dead plant material. It has been isolated from cereal grains, legumes, peanut pods, sunflower seeds and bran. The cultures can show wide variation when sub-cultured for any length of time on laboratory media. On Czapek media, cultures vary from thin gray-green and heavily sporing to thick colonies with a dense felt of irregularly pigmented aerial mycelium and only localized areas of sparse sporulation. The pigment on the underside can also vary from one isolate to another. This can range from intensely deep red to almost black.

The colonies have smooth conidiophores which end in a verticil of 3-5 metulae. Each in turn has a verticil of a similar number of very lanceolate sterigmata. The uniformly smooth walled conidia vary in dimensions and be subglobose or even strongly elliptical.

P. rubrum does not grow or produce toxin well on many media. Good early results were reported using cracked hard corn moistened with 1% sucrose. Recovery of up to 1 gram of toxin per liter were reported in 21 days on stationary cultures. The culture extracts were able to produce symptoms in animals similar to that of natural outbreaks of moldy corn toxicosis. Medium containing yeast extracts and malt extract plus zinc also enhance growth and production. Toxin production peaks at 7-21 days and declines therafter. Good extractions are produced with di-ethyl ether soaking the sample for 10-12

hours in a continuous solvent-liquid extractor. As the toxin concentrates in the solvent it eventually crystallizes as long needles.



Normal and abnormal penicilli of *P. rubrum*

The toxin is excreted outside the mycelium and not retained. The washed and dried mycelium is not toxic to the test animals. During the culture growth, the pH drops rapidly and remains at 2.5-2.7 until the culture begins to autolyze. The colony first produces orange pigment on the surface and then orange at the base of the mycelial felt. This occurs only when logarithmic growth has begun to subside. Other color pigments occur from toxic metabolites as they are produced. Toxin producing isolates often lose their toxin producing ability when routinely sub-cultured.

Pure rubratoxins are not very soluble in water, are fairly soluble in alcohols and esters and very soluble in acetone. They are completely insoluble in non-polar solvents such as chloroform. Pure rubratoxin B crystallizes from diethyl ether as rosettes of needles and from mixtures of benzene and ethyl acetate as long lathes. It may crystallize from amyl acetate as regular hexagonal plates. Rubratoxin A is much more soluble in ethyl alcohol than Rubratoxin A. They both decompose on melting. Rubratoxin A is

unstable in alkaline solution and the solution slowly develops a yellow color and slightly pungent smell.

The LD50 of Rubratoxin A & B is 3.0-6.6 mg/Kg in mice using propylene glycol as the carrier. When DMSO (Di-methyl Sulfoxide) is used as the carrier, the LD50 (IP) is .37 mg/Kg. When sub-lethal doses are used, the toxicity is limited primarily to liver related damage. Toxic levels cause hemorrhaging and congestion of the visceral organs, especially the liver and kidneys. Some combination tests seem to indicate that the rubratoxins have a potentiating effect on other more deadly toxins such as aflatoxin. Rubratoxin and aflatoxin B act synergistically when administered to rats simultaneously. They increase the acute toxicity of either toxin but have no effect on the carcinogenic of aflatoxin. The toxins are also is teratogenic.

Fresh corn samples inoculated with P. rubrum and A. flavus separately, showed the P. rubrum to yield greater acute toxicity in the test animals that A. flavus indicating that the rubratoxins are more toxic directly than the aflatoxins in corn fed field conditions. A subsequent test in which aflatoxin B was added to rubratoxin at only .01 mg/ day showed significant increase in toxicity.

Rubratoxin has also been isolated from P. purpurogenum grown on Czapek-Dox medium supplemented with malt extract and yeast extract at 25 C for 21 days. Yields of 3 gm/liter were reported.

Sterile soy whey fortified with malt extract and incubated with P. rubrum for 28 days at 28 C yielded both rubratoxins while cultures at 40 C produced no toxin.

8) Cyclopiazonic acid (CPA) and related toxins

Certain hay, grains and feeds containing P. cyclopium were implicated in the 1960's in moldy corn toxicosis in farm animals. The organism is frequently encountered in stored grains and foodstuffs intended for human consumption. In 1968, P. cyclopium was isolated from groundnuts which caused acute toxicosis in ducklings and rats. It was grown on corn meal for large scale cultivation and the toxins were extracted with chloroform-methanol. A fraction soluble in sodium bicarbonate solution was then obtained that contained a new toxin called cyclopiazonic acid. It was found to be the main cause of toxicity of the fungus. A flavus (26% of isolates) has also been found to produce CPA.

Cyclopiazonic acid is a colorless crystalline solid which melts at 246 C. Grown in Czapek-Dox medium, peak production rates of 4.2 gm/liter were achieved using sodium nitrate as the nitrogen source. Initial pH of 5.5-8 in the medium gave the best results. About 2/3rd of the acid was found in the mycelium and 1/3rd in the medium. The cultures were grown at 25C and shaken during growth. The seed for the culture could be either spores or mycelium transferred to the medium.

When injected into rats (IP) at 2.3 mg/Kg, toxic convulsions were observed followed by death in 20-100 minutes which indicted it was a potent neurotoxin. Oral dosage required two days before mortality without the convulsions but with coma. This suggests slow absorption from the intestinal tract in the rat. The oral LD50 in male rats was 36 mg/kg and 63 mg/kg in female rats. Chicks and ducklings died with single oral doses in 20-100 minutes.

Autopsy revealed cellular changes in the liver, kidneys, pancreas and spleen. These included necrosis and hyaline degeneration of the myocardium. CPA is insoluble in aqueous solvents with a pH below 7.0. This suggests that when the toxin reacts with stomach acids, it becomes insoluble which accounts for its low oral toxicity.

P. cyclopium has been recovered from fermented sausage, raw ham, frankfurters and country cured ham. These strains all produced toxic acid when grown in special media for 21-28 days at 25 C. Substrates high in carbohydrates seemed to increase toxin production. They would grow luxuriantly on the sausages without producing toxin over 4-5 weeks but would begin forming toxin after 5-6 weeks at 15C.

A tremorgenic toxin has also been isolated from P. cyclopium which do not produce the toxic acid. It was formed in the mycelial mat on grown on various aqueous food products. The toxin was extracted with ethyl acetate. IP injection in rats produced marked tremors at only 250 mcg/Kg which lasted for several hours. At doses of 2.5 mg/Kg, the tremors soon progressed to clonic and tetanic convulsions followed by death.

P. granulatum and P. crustososum also produce the tremorgenic toxin. In 1969, another toxic alkaloid called cyclopiamine was isolated from P. cyclopium in moldy groundnuts.

Since 1968, more than 15 tremorgenic toxins have been isolated from fungi. These toxins consistently produce sustained tremors in lab animals. Some are produced in the soil which may be ingested by grazing animals.

9) Miscellaneous Penicillium toxins

The genus of Penicillium, along with Fusarium and Asperigillus contains large numbers of toxin producing species. These delicate paintbrush-like organisms are found worldwide in which they decompose plant matter, spoil food and infect other species. We will mention a few more Penicillium species and toxins in this section that may be useful as weapons.

Mycophenolic acid is a strong antibiotic substance that was purified and crystallized in 1896, although its properties were unknown at the time. It was isolated from moldy corn and when it is mixed with ferric chloride it yields a blue color. Several strains since then have been discovered which produce this substance including P. stoloniferum, P. glaucum, P. brevi-compactum, P, viridicatum, and P. bialowiezense.

Medium used to produce mycophenolic acid were Czapeks modified with either 2% malt extract and corn steep liquor which are indispensable for production. Antibiotic activity against bacteria was detected from cultures in 7 days and peaked at 14 days (at 24 C). The acid was first extracted in 1913 using hot sodium bicarbonate on both the culture medium and the mycelium followed by acidification of the filtered alkaline solution using hydrochloric acid. Adding the acid precipitated clusters of needles along with a dark impure material. Hot toluene dissolved most of the crystalline material which re-crystallized on cooling. The needles still retained some color which was removed with charcoal. Another procedure was to form the potassium salt of the acid which is insoluble in ethanol. The ethanol readily dissolves the colored impurities. It was dissolved in hot water and precipitated with hydrochloric acid. The melting point is app. 140 C.

The crystals give an intense blue color in solution when ferric chloride is added. They are nearly insoluble in cold water but can be re-crystallized from hot water solutions. It is stable in hot acids and alkaline solutions. Its potassium salt is soluble in water but insoluble in ethanol while its other metal salts are insoluble in water.

Studies in 1946 found that mycophenolic acid is a strong antibiotic, inhibiting most gram positive and some gram negative bacteria. It was also found to inhibit anthrax bacilli. Many strains of fungi that are pathogenic to man and plants are also susceptible to the acid. They also found that it killed leukocytes immediately at concentrations of 1:200 and in three hours at concentrations of 1:500.

Mice injected (IV) with 2 mg of the sodium salt of the acid became ill. At 5 mg, there was a prolonged illness and a 10 mg dose was lethal in 2 hours. Topical applications were not toxic. The acid acted as a spleen enlarging agent (immunosuppressant) in mice infected with mouse sarcoma virus.

Decumbin is a toxin produced by P. decumbens. Colorless, odorless short needles which melt at 204 C were obtained from the culture filtrate of this species which was found growing on moldy corn in 1958. It was grown in a broth containing potato extract plus 2% glucose. A heavy spore inoculum provided complete growth in 6 days at room temperature. Large needles of decumbin formed on the sides of the bottle as early as the eighth day.

The fungal mycelium was extracted with hot methanol and the remaining broth was chilled to 10 C to precipitate the decumbin. This was then collected and extracted with hot methanol. The hot methanol is then chilled, filtered, diluted with water and then concentrated under vacuum until all the methanol has evaporated away. Decumbin crystals form during concentration. The crystals melt at 278 C and the maximum yields were 278 mg/liter of culture. Completely pure crystals were obtained by washing the crude crystals with petroleum ether followed by re-crystallization from 50% aqueous methanol and ethyl acetate.

Pure decumbin has low solubility in water and is insoluble in non-polar organic solvents. It is fairly soluble in ethanol. It has some antibiotic properties but is lethal to rats at doses of 200 mg/Kg in 24 hours. It is also toxic to goldfish and wheat seeds.

Beta-Nitropropanoic Acid (BNPA) was first obtained in 1958 from P. atrovenetum. The organism was grown in Czapek-Dox medium with glucose as the sole carbon source and sodium nitrate as the sole nitrogen source. Stationary incubation was at 24 C in the dark for variable periods.

The culture broth containing the BNPA was separated from the mycelium and extracted three times with ½ volumes of ethyl ether. The extract residue was subjected to exhaustive sublimation in high vacuum at 60-65 C to obtain colorless needles which melted at 65-69 C. Average yields of 660 mg/liter were obtained by the seventh to tenth days. A peak of 880 mg/liter was obtained by day 12.

BNPA is soluble in water and several polar organic solvents. It is also produced by A. flavus and in several fungi infected plant species. Crude BNPA has an LD50 of app. 250 mg/Kg in mice in 40 minutes to 24 hours.

In 1968, a flock of prized sheep in Tennessee was decimated by feeding corn heavily contaminated with the P. crustosum. The sheep showed loss of appetite, depression, humping of the back, diarrhea, slobbering, generalized weakness, convulsions and death. One of the few surviving sheep was called “dummy” for not responding to various stimuli.

This and other stock cultures from the US Army Natick laboratories were used to culture the mold and isolate the toxin. The toxin is produced on several natural substrates (moistened foods). The most common laboratory substrate used was dehydrated mashed potatoes, 2% skim milk solids and 2% granulated sugar (sucrose). Potato usually acrries a spore forming, heat resistant bacteria that will also grow in cultures so antibiotics were added to this medium. Large scale surface production was obtained using 11” diameter pans covered with aluminum foil.

The 5/8” thick medium was inoculated with a heavy spore suspension in distilled water. Growth was rapid at 25 C and the surface soon becomes covered with a greenish gray mat which thickens and becomes convoluted after seven days. Toxin, which is confined to the mycelium mat ca be detected on day 7 and peaks by day 14.

The mat is removed from the culture and macerated in a blender. Water is added and the suspension is filtered through a fiber glass filter. The filtrate is discarded and the compacted solids are chopped and dried in an oven at 80 C until all moisture is evaporated. The friable dry pieces are then ground to a powder and extracted for three hours with anhydrous ethyl ether which yields a yellow solution containing the toxin concentrate.

The resulting toxin is a cyclopium tremorgen which is very soluble in polar organic solvents (especially methanol) and somewhat soluble in non-polar hydrocarbons. It is insoluble in water and slightly soluble in dilute hydrochloric acid and sodium hydroxide. When the toxin is in solution in chloroform and certain other chlorinated hydrocarbons, the solution will change color from grown to green to dark blue when exposed to light. This represents degradation of the toxin into several other compounds. The toxin crystals do not melt but turn brown and black starting at 180 C.

The effects on animals with this toxin are classified as tremorgenic-diuretic. All animals tested were susceptible to the neurotoxic properties. Because of its water insolubility, the toxin is dissolved in ethanol or propylene glycol and then dispersed in saline for administration. Within 5-30 minutes, tremors are induced in mice at dosages of 250 mcg/Kg. Larger doses elicit irritability, limb weakness, and marked tremors. At 2.5 mg/Kg, convulsions begin usually followed by rigor mortis and death. Survivors recover in about 4 days. Rats also exhibit persistent vertigo. The hamster was the most resistant of all the laboratory animals to the toxin. Oral dosing produced marked diuretic effects.

P. puberulum is a frequent food contaminant that produces an antibiotic when grown on several moistened foods such as millet, oats, wheat and corn. Toxin was detected in wheat and whole grain cultures in as little as 10 days after inoculation with spores and as late as 29 days of growth. Toxin yields were very low on other grains and synthetic media.

Day old ducklings fed the toxin by stomach tube became uncoordinated in 15-30 minutes which lasted for 24 hours in the surviving ducklings. Large doses caused death in a few hours. Fatal doses in mice caused a cyanotic coloring of the nose, feet and tail with a “wagging” of the head, difficulty in walking, exaggerated stepping motions, apnea, brief convulsions, apnea and death. Symptoms lasted for more than a day in surviving animals.

Toxin extraction appears best using methanol for 6 hours of the undried, contaminated whole grain wheat cultures followed by removal of the extracted lipids with petroleum ether. The methanol-water solution is adjusted to pH of 2 and extracted with ethyl ether yielding a yellow solution. The solvent is evaporated and the residue is dissolved in a small amount of methanol and added to an aqueous 5% sodium hydroxide solution in order to form the water soluble sodium salt of the toxin. The salt solution is acidified with concentrated hydrochloric acid and the toxin begins to precipitate out at pH of 4.7 and is completed at a pH of 3.0. The flocculated toxin is tan colored and is filtered immediately and then washed with slightly acidified water and then dissolved in ethyl ether which gives a yellow solution. Slow evaporation yields microcrystals that are nearly colorless and rectangular in shape.

About 1-2 mg of crystals were recovered for each Kg of wheat culture. The crystals melt at 236 C to give a black melt which yields larger lance shaped crystals which do not melt through 350 C. Injection (IP) of 25 gm mice with .5 g caused severe

reactions, 2 mg caused prolonged, severe illness and larger doses were fatal. Several other toxins are also produced by P. puberulum.

Griseofulvin is an unusual fungicidal substance that was once used in human medicine but has proven too toxic for modern use. It has been obtained from cultures of P. griseofulvum, P. janczewski and nine other Penicillium species. Commercial production of the toxin were obtained using submerged fermentations in a corn steep medium with intermittent additions of glucose (to 8%), 2.5% sodium nitrate, and aeration. Using a 10% actively growing inoculum at 30 C, yields of 6 gm/liter were recovered at 9 days.

The mycelium is separated, washed with water and dried at 50 C. Thdried material was finely ground and extracted with petroleum ether for 3 days. This was followed by a 4 day extraction with ethyl ether which yields a solid residue. This residue contained griseofulvin and a nitrogen bearing compound called mycelianamide. This residue was extracted using boiling benzene and on cooling forms crystals of the mycelianamide. Gradual evaporation of the benzene solution gives successive yields of griseofulvin which were then purified by crystallization from ethanol.

Griseofulvin precipitates as large colorless rhombic crystals from ethanol. They have a melting point of 218 C. They are sparingly soluble in chloroform, ethyl acetate, benzene, toluene, alcohol, acetone and are insoluble in water.

Griseofulvin has proven itself to be an effective fungicide when absorbed into plant tissues or ingested by animals and humans. Many dermatophytic fungi are very susceptible to this toxin and when given daily orally, in guinea pigs, at 60 mg/Kg over 10 days, it eliminated induced fungal infection of hair follicles. The base of the hair tips became free of infection first while the tips continued for a time to show fluorescence caused by the fungal invasion.

The keratin of skin, nails, hair, body fat, liver and skeletal muscle in humans all contain concentrations of the toxin after oral dosing. Its use has been restricted due to toxicity and side effects including erythema, vesicular and macular eruptions, photosensitivity, blurred vision, headaches, vertigo, anorexia, vomiting and other effects. The LD50 for rats is 400 mg/Kg. Sublethal doses caused liver and bone marrow damage.

Xanthocillin X is an unusual isocyanide which was first obtained in 1950 from P. notatum. It has also been isolated from A. chevalieri in 1966 when it was discovered to be hepatotoxic in experimental animals.

Xanthocillin X is produced using a 5% inoculum of P. notatum to a culture broth which is agitated and aerated for 8 days at 24-30 C or until the mycelium is autolyzed. The salt of the compound is obtained by adjusting the pH to 11-13 with either sodium or potassium hydroxide.

The compound is obtained as yellow clusters of needles from alcohol or yellow rhombic prisms from ethyl acetate. Both of these char without melting at 200 C. They are slightly soluble in alcohol, ethyl ether, and dilute sodium hydroxide. They are insoluble in water, benzene, and chloroform but a di-potassium salt may be formed which is soluble in water.

Xanthocillin inhibits several gram positive and gram negative bacteria at low concentrations making it a potential antibiotic. It is absorbed poorly by oral and parental dosing. It was tested on 20,000 patients for local infections in 1953 and found to be effective. Its use at low doses with sulfonamides increased its effectiveness many times and no resistance was developed on the part of bacteria populations at that time.

The LD50 of aqueous suspensions was 60 mg/Kg (IP) and 150 mg/Kg (SC) for the guinea pig. For the white mouse, they were 25 mg/Kg (IM), 35 mg/Kg (IP) and 40 mg/Kg perorally.

One overall principle of mycotoxin production of penicillia species is that high carbohydrate substrates (especially on meats) favors mycotoxin production.

Chapter 12

Blue-Green Algal Toxins

Algae do not fall into the classification of molds directly but for convenience purposes your author has decided to include them in this volume.

Toxic algae are found in marine, brackish and freshwater habitats throughout the world. They form dense uni-algal growths called blooms or tides. These blooms are responsible for large scale mortality of fish, livestock, waterfowl and humans. It has been observed for centuries that livestock which watered on ponds with extensive algal growth often became ill and died quickly. In 1878, a scientist reported that a thick scum of algae growth on Lake Alexandria in Australia was responsible for the deaths of sheep, horses, pigs and dogs.

In Minnesota in 1886, winds concentrated algae into thick windrows along lee shores of various lakes. Animals drinking the water died quickly. Since then, tens of thousands of livestocks deaths have been reported from similar causes throughout the world. In the fall of 1952, at Storm Lake, Iowa an outbreak of algal poisoning killed over 5,000 gulls, 500 ducks, 400 coots, and numerous pheasants, squirrels and muskrats.

A number of outbreaks occurred in the 1930's where dense growths of algae in municipal water supplies were responsible for gastroenteritis. Numerous accounts of gastrointestinal, dermatological, respiratory and allergic responses have been due to algal growths in human water supplies since then.

Algal growth on lakes occurs at high rates and cell densities. It was almost impossible to reproduce this type of growth in artificial culture or recover toxin until 1958 when scientists finally developed a systematic approach to the investigation of the problem.

Unialgal cultures were obtained by using capillary pipettes to isolate and wash individual colonies or cells from the site of blooms. They are then transferred to sterile medium. The blue-green algae have a mucopolypeptide sheath which often harbors both bacterial and algal contaminants. Some of these can be removed by rolling filaments or groups of cells over the surface of .8% sterile agar. The washed algae material is then transferred to suitable culture media and incubated at 20-25 C with shaking or aeration and 750-3000 lux (light).

Nutrient levels in natural outbreak sites is usually much lower than those in artificial medium. Cells transferred to the higher ionic strength medium often fail to grow or lyse without suitable adaptation. Cells are usually transferred to diluted versions of the final mediums as an intermediate step to adapt them to the new nutrients. The diluent can be filtered-sterilized lake water or distilled water (1:1 ratio).

Adding 50mg/liter of actidione retards the growth of eukaryotic cells while not affecting prokaryotic cells. This helps to keep chlorophyll utilizing species from contaminating the culture or outgrowing the desired algae. A soil extract of 2-5% has also been used to enrich the medium.

The most widely used medium for the culture of toxic blue-green algae is the ASM medium which uses the following formula –

Substance	Micromole/liter
NaNO3	1,000
MgSO4	200
MgCl2	200
CaCl2	100
K2HPO4	100
FeCl3	2
H3BO3	10
MnCl2	7
ZnCl2	.8
CoCl2	.02
CuCl2	.0002
Na2EDTA	20

When 2-5 ml of isolate is transferred into this medium or a partially diluted version of it, there is consistently good survival and growth rates. None of the freshwater blue-green algae have been found to require vitamins, but some of the marine species do require vitamins, especially B12.

Microcystis aeruginosa was first isolated, cultured and purified in 1951. The optimum thermal growth occurred at 32.5 C with slightly reduced rates at 25 and 28 C and significant reduction at 35 C. Toxin production was best at 25 C and 60% lower at 28 C. In cultures grown at 25 C, toxin production declined markedly at 4-5 days of growth which coincided with peak biomass. The interaction of light, aeration and temperature determined toxin production rates. Temperature optimum was definitely 25 C. At an aeration rate of 100cc/minute and 2,200 lux toxin production was constant from 20-30 C. At 16,000 lux, toxin production decreased with increasing temperature between 20 and 35 C.

The LD100 for white mice was constant during the first four days of growth of 2-7 x 100,000,000 cells per ml. A marked increase in toxin per cell occurred at days 5-6 (LD100 = 80 mg/Kg) with cell densities of 9 x 100,000,000. Cell lysis began to occur on the 3rd day.

The toxin FDF (fast death factor) is soluble in water, methanol and ethanol and insoluble in non-polar solvents such as acetone, ether, chloroform, and benzene. The toxin diffuses through collodion, cellophane and animal membranes. It is heat stable at neutral pH, non volatile, and is irreversibly absorbed onto charcoal.

Cells incubated at 37 C from cultured M. aeruginosa that were disrupted by freezing or sonication killed mice in 30-60 minutes at much lower dosages than fresh cells. The fresh cells usually required 24-48 hours to produce death in the test animals. Testing at this time period also established the presence of bacteria in algal blooms that produce some toxins (slow death factor).

To extract and analyze the toxin, lyophilized cells were aqueously extracted at a pH of 7.0-10.0 in sodium bicarbonate solution. They were centrifuged and concentrated in vacuum. The concentrate was extracted with n-butanol, evaporated to dryness and dissolved in water. This water was washed with ethyl acetate, dialyzed at 4 C and the dialyzate extracted with n-butanol. The concentrated toxin was then extracted with 95% ethanol and stored at 5 C. The LD 100 of the free acid and its sodium salt was 2.0 mg/Kg.

The species M. toxica produces a similar toxin that is extracted almost identically that produced an LD100 of .1mg/Kg (IP).

Anabaena flos-aquae has produced some of the deadliest cases of algal poisoning recorded. Lab studies show that the survival times of this species blooms is much shorter than that of Microcystis-FDF. The minimum lethal dose for lab animals usually caused death in 2-10 minutes with no detectable abnormalities on autopsy.

The best culture conditions occurred at a pH of 7.5, light saturation at 3,000 lux and temperature of 22 C. Elevating iron concentrations or reducing manganese enhanced filament coiling in cultures while deficiency in iron and elevated manganese resulted in trichome straightening.

The toxin (VFDF-very fast death factor) is water and ethanol soluble and insoluble in chloroform, acetone and ether. Extraction was achieved using hot absolute ethanol.

Aphanizomenom flos-aquae has been incriminated in many livestock and fish mortalities. Attempts to control a toxic bloom in 1964 with copper on Lake Winnesquam, New Hampshire produced a toxic fish kill. A similar effort in 1966 on Kezar Lake, also in New Hampshire resulted in the death of more than 6 tons of fish.

In 1968, the first sample of the toxin producing strain was successfully cultured. Its initial growth was slow with changes in their spindle shaped fascicles. In a second aerated culture, the fascicles decreased in size until they disappeared and the growth of the resulting trichomes was rapid. Mass cultures were toxic to fish with a minimum lethal dose in white mice of 10 mg of culture/Kg and death occurring within 5 minutes.

Toxicity in the cell samples peaked at light intensities less than 5,000 lux at 26 C. Toxin production was halved at 20 C and almost non-existent at 30 C. The toxin is soluble in water and methanol, and less soluble in ethanol. Extraction with acid, neutral or basic chloroform was unsuccessful. It is insoluble in non-polar solvents such as acetone, ether and benzene. Stored at 5 C, the toxin is stable at pH of 1.0-7.0 with slight

loss of activity at 11.0. At greater than 20 C, the toxin is labile at a pH over 5.0. When the toxin was finally purified and characterized, it was found to be similar chemically to saxitoxin and would kill white mice at 1.5-2.0 mcg making it one of the deadliest pure substances known.

Peridinium polonicum is a toxic freshwater dinoflagellate. In the fall of 1962, mass mortalities of fish were observed with an extensive water bloom of this dinoflagellate in Lake Sagani, Tokyo. Annual developments of the organism occur during September and October of each year in the areas of tributaries where water reaches temperatures of 20-23 C. Cell densities reach 4-7,000 cells/ml in the upper one meter of the lake water. Mortalities were confined to late afternoon when lake waters were saturated with dissolved oxygen. The pH at this time was 8.7-9.2 from the photosynthesis of the phytoplankton.

Biochemical studies were performed on the natural blooms that were harvested, lyophilized, and stored at 20 C. Toxin activity was pH dependent and peaked at 8.0-9.5. The MLD for 20 gm mice was 250 mg/Kg with death occurring in 2 minutes.

The toxin was initially extracted from the cells with either water, dilute acids, methanol, ethanol or acetone. Using pH adjustments, non-polar solvents were effective when used with aqueous extracts in concentrating the toxin.

Summary

After blue green algal toxins are injected (IP) there is a latent period where the animal acts normal. The FDF latent period is usually 30 minutes to an hour while VFDF is usually 1-2 minutes. After this, the animal undergoes alternating periods of restlessness and quiescence. This is accompanied by changes in peripheral circulation as seen by the pallor of the ears and tail and a change in eye color from red to pink. There is then a loss of equilibrium and a dragging of the hindquarters often punctuated with spasmodic leaping. Next are convulsive contractions of the thorax and a gaping mouth followed by death.

Autopsy reveals an engorged liver, normal lungs, reduced peripheral blood supply, and continued beating of the heart. Death is due to asphyxiation. Its chemical similarity to saxitoxin suggests that a peripheral paralysis is induced by the toxin.

Algal blooms are generally described as unusually excessive growth of a single algal species. They are usually seen in late summer in many lakes and ponds. They usually occur where the water is rich in excess nutrients. The blue green algae begin growing in the spring, reach dominance by early summer and a succession of blue green algae species takes place during July to September. Temperatures rise to 18-25 C. During the midday peak of photosynthetic activity, dissolved oxygen reaches or exceeds 10 mg/liter with the pH reaching 9.5. Because of the algal respiration, pH will drop to 6.5 and dissolved oxygen to 1.2 mg/liter by late evening.

Each algae produces substances that inhibit or accelerate the growth of other algal species which is why there is a succession of single species that predominate during each phase of the bloom.

The simplest way to recover potential toxin producing species from algal blooms is to take samples from each phase of the "scum" or bloom. The cells are lyophillized and then extracted and the dried extract is then injected at various doses into mice. The toxicity of each bloom can be measured with the cultures from the deadliest retained for further artificial culture. The bloom itself may be harvested on a large scale if desired, especially since some of the toxins are among the most potent known to man. The following charts give an interesting comparison of the toxicity of various deadly substances –

Toxin	Minimum Lethal Dose-Mice(mcg/Kg)	Source
Botulinum Toxin A	.00003	Bacteria
Tetanus Toxin	.0001	Bacteria
Ricin	.02	Castor Bean
Diphtheria Toxin	.3	Bacteria
Cobra Neurotoxin	20	Snake
Crotalus Toxin	60	Rattlesnake
Kokoi venom	2.7	Frog
Tarichatoxin	8	Newt
Tetrodotoxin	8-20	Fish
Saxitoxin	9	Shellfish
FDF	50-100	Algae *
VFDF	250	Algae *
Bufotoxin	390	Toad
Curare	500	Plant
Strychnine	500	Plant
Muscarin	1,100	Mushroom
Samandarin	1,100	Salamander
DFP-Nerve Agent (Diisopropyl-fluorophosphate)	3,000	Synthetic-Nerve gas
Sodium Cyanide	10,000	Chemical

Chapter 13

Mold Mutation and Strain Modification

Mycotoxins are poisons produced by molds that cause disease in plants and animals. These mycotoxins can be mass produced in artificial cultures and used as weapons against humans as well.

The molds that produce these toxins are characterized by production of a mass that is visible to the naked eye. This mass usually consists of fine filaments called a mycelium. Each “thread” of this filament is a fine, tiny tube called a hyphae. Much of the mold structures cannot be seen with the naked eye such as individual cells and spores.

One of the biological processes that we did not discuss yet in this book is what happens when different strains of molds, even of the same species, come together in nature or on a culture plate. When different molds grow together which each other, adjacent hyphae can fuse with each other. This process is called *anastomose*, in which the cellular contents of the hyphae can intermingle and in this manner they may exchange the ability to produce certain physical properties and characteristics.

Inside the tubes of the hyphae, cellular structures called nuclei, mitochondria and other sub-cellular organelles can move from place to place along with the food and water that is transported to the growing front of the hyphael tip. Enzymes are produced by these cellular structures which are excreted outside the hyphae and then break down (digest) surrounding materials. These digested substances are then absorbed back into the hyphae through the wall membranes and used as food to produce more cellular structures and hyphae. Mycotoxins are also produced in this process.

When molds grow together and anastomose, their cellular structures intermingle and each new hyphael structure with the new nuclei will begin to produce different types of pigments, spore structures, and toxins. This is why some colonies lose the ability to produce toxins in the laboratory or in the field. This loss of ability to produce toxins, antibiotics and other substances is called strain degeneration.

Studies of molds over the last 200 years have indicated that they spontaneously change at a frequency greater than that of the mutation rates for normal populations. These changes include both sexual and asexual sporulation, formation of aerial mycelia, pigmentation, virulence, mating type, and toxin production. These variants are often seen in the lab cultures from a single growing inoluclum with the changes observed in different parts of the radially growing colony. Terms used by scientists to describe this includes *strain variation, saltation, mutation, strain degeneration* and *pleomorphism*. This is one of the reasons that it is difficult to describe some fungi as belonging to one species. Molds can be grown and then cultured later and thought to belong to a different species due to these changes.

Observations in the early 1900's led researchers to realize that the variability in fungi could not be explained on the basis of Mendelian genetics. The genetic instability in filamentous fungi caused them to finally be viewed as molds existing in nature as combinations of distinct genetic entities. In fact, many genetically dissimilar nuclei can exist in the same mycelium in many fungi, and this type of mycelium has been given the name **heterokaryotic**. Homokaryotic colonies contain genetically identical nuclei.

Molds can be grown in the lab like bacteria, which means they grow in pure cultures, produce colonies on agar and artificial media, have simple triggers in the media to trigger sporulation and so on. Unlike bacteria, fungi are not single cells, but spores or mycelial fragments that give rise to hyphae that extend by apical growth at the hyphael tips. A mycelial mass contains young and old cells, and structures at various stages of development. This means that phrases that biologists use to describe bacterial growth of single cell populations such as *lag phase, exponential or log phase,* and *stationary phase* do not apply to fungal populations.

New terms have been developed for mold growth. These include *tropophase* or feeding phase where nutrients are taken in and dry weight of the colony increases, and *idiophase* or peculiar phase where nutrients are depleted, dry weight levels off and this is usually the time where toxins are produced. These phrases are used in the antibiotics industry by scientists to describe submerged batch fermentations.

When describing *variant* strains, they usually mean that the strains arise through gradual change from normal members of an identifiable species. The new character of a variant is generally not stable but subject to continued change and further variation. Variants frequently appear as colony sectors, overgrowths or other localized areas of changed appearance. When isolated in pure culture, they may or may not retain their distinguishing characteristics.

The term *mutant* strains apply to those strains that show abrupt, marked, and persistent differences from the known parent or culture.

Fusarium is considered to be the most variable fungi, although all of the toxin producers described in this book will be described in other texts as having considerable strain variability. It is common for wild strains to change radically in appearance when subcultured in a laboratory. These changes usually involve loss of aerial mycelium and an increase in macroconidia. An increase in pigmentation is also observed occasionally.

Variation in strains has been observed to increase in some strains as the temperature increases. The rate of mutation observed accelerates from 25 C to 37 C. By irradiating condiophores with ultraviolet light (in solvents or dry), greatly increased rates of mutations have been produced. By growing colonies together and allowing the mycelia to fuse, certain properties can be combined producing a new mutant or variant that may not have existed before in nature.

[The purpose of this chapter is to introduce the reader to the possibilities of genetic modification for weapons production. The best example using the above information that the author can think of would be the fusing of the mycelia of highly infectious C. immitis or even athlete's foot species with aflatoxin or trichothecene producing species. This would yield a new and particularly insidious weapon that ordinary citizens could own and use invisibly as a protection against their governments.]

Many fungi also contain viruses, especially those affecting RNA. They were first discovered by their association with interferon-inducing properties of Penicillium species. Some viruses are the genetic determinants of "killer proteins" while others cause fungal diseases.

The ability to modify microorganisms such as molds, bacteria and viruses may be covered in a later volume. If the reader is interested in learning how to modify fungi before then, the author will refer you to –

Handbook of Applied Mycology
Volume 4 Fungal Biotechnology

Chapter 14

Industrial Mycology

In order to use fungi effectively as weapons in war, you must be able to produce them in sufficient quantity to be useful. The purpose of this chapter is to acquaint the reader with a variety of methods for mass producing molds and their metabolites that have been practiced by individuals and industries.

We have already described in detail the nutrient requirements for growth, spore production and conditions of ideal toxin production for many of the candidate weapons. We will focus on the mass feeding of these fungi to maximize the amounts of colony mass and toxins. There are two main techniques of mass production, and these are –

1. Growing the mold on a solid substrate
2. Growing it in a liquid submerged medium

Growing molds on solid substrates is the simplest and requires very little, if any, equipment or monitoring. Liquid cultures require a holding tank and considerable agitation and aeration to keep nutrients mixed and to maintain enough dissolved oxygen to support growth. Oxygen has a low solubility in water and is absolutely necessary to maintain growing colonies so it must constantly be added to the liquid. It usually takes about 100 parts of oxygen pumped into a medium in tiny bubbles for one part to become dissolved. The advantages of the liquid medium is that the temperature, pH and nutrients of the medium can be monitored and maintained for best production,.

Culture Improvement

The cultures used in producing weapons are usually selected from pre-screened samples obtained from nature. This can be as simple as taking samples of corn kernels from the ends of ears (or from the soil under the grain) and growing them on pans with wet paper towels underneath. The colony growth is observed and then in a few days, when the toxin production starts, the molds can be examined with an ultraviolet lamp. Very large numbers of candidates (thousands) can be screened in a few days using this method. The best growers and toxin (blue fluorescing in the case of aflatoxin) are selected for production. In the case of trichothecenes, the samples can be graded for dermal injuries on mice or guinea pigs. This method is called **strain selection**.

Improvements in selected strains can be made by **mutation** as described in the last chapter. Those methods used in commercial mold industrial settings include exposing spores and other parts to ionizing (ultraviolet) radiation in diepoxybutane, and chemical mutagens such as N-methyl-N'-nitro-N-nitrosoguanidine (NTG), nitrous acid phenethyl nitrogen mustards and ethylene-imino pyrimidines. Mutation frequencies are increased over natural occurrence rates by 1,000-10,000 times using these materials. The Aspergilli happen to be especially sensitive to solutions of .2% sodium nitrite which yields many

morphological mutants. Improvement programs generally mutate and screen thousands of strains daily in military and industrial organizations. Screening could include getting the high toxin producing strain to grow on a selected grain or substrate rapidly, or it might be to combine properties of two species for infectious properties and use of a toxin producing pathway that it did not have previously.

Genetic recombination by patented processes can allow the true hybridization of fungal species. A combination of a T-2 toxin producer with an infectious species of fungi such as C. immitis or one of the athletes foot and jock itch families can create weapons with entirely new properties. This example combined with bacteria based weapons in combination, would find enormous value on a battlefield. Inhalation weapons that included such a fungi combined with Clostridium cocktails would likely yield fatal weapons that would cause gangrene in the lungs and that would persist beyond the use of antibiotics. It would also be effective in overcoming immune system defenses by causing injuries in the lungs which would physically block lymphocytes, immunoglobulins and other protective substances. Some could also be designed to counter the effects of antibiotics or use antibiotics as food.

Early Fungal Fermentations

The earliest records of using molds to produce fermented foods go back about a thousand years to the south of France where mycelial fungi were used to make Roquefort cheese. It was only in the 20th century that the effects and use of the molds were actually well understood. P. roqueforti turned out to be one of the few molds that would grow under the limited oxygen in the narrow spaces of the curd. The growth of molds used to flavor cheeses include Brie, Camembert and other varieties. In these, the molds P. caseicolum and P. camemberti are surface growers.

The earliest mushrooms were produced in Japan and China where “shitake” spores were inoculated into specially prepared wooden logs and left to incubate for months before the sporophores are harvested and eaten. It is estimated that the practice may be 2,000 years old. Agaricus campestris, the most well known mushroom in Europe has been cultivated in caves since the 18th century. This technique applies only to this particular mushroom which is one of the problems with applying one technique of fungal culture to other fungi species.

Rice Koji in Japan has been prepared since about the 8th century. A moldy wheat bran or rice is grown in trays in a semi solid culture after being inoculated with a culture of Asperigillus oryzae. The rice koji is mixed with steamed rice, water and yeast and then fermented to make “saki”. Soybeans, salt and rice koji are also used to make soy sauce and a semi-solid cheese like food called “miso”. Large scale production of the Koji process began when the manufacturers switched from using bran or rice to wheat in 1914. This process first involved moistening and then steaming the bran which both sterilized and then gelatanized the starch. This was then cooled and inoculated with spores and then spread onto the floor of a room (in winrows) or on trays with wire netting bottom that improved the aeration. The room was heated at first to start the culture and then as it

heated up and the fermentation became exothermic, the room would be switched to air conditioning to keep it cool. Temperature is kept below 28 C. After 5-8 days the product is air dried in the room so it could be preserved without contamination. The scientist in charge modified the strains of fungi so they would be tolerant of added antiseptics that could be added during production.

In later improvements, 4,800# slowly rotating drums would be used for factory productions. The koji was then extracted with water and precipitated with alcohol for a much more concentrated and purer enzyme that could be used in food processing. In experiments at Iowa State University in 1939, aluminum pots were used in which holes were drilled into the bottom and air blown through them to provide aeration. They duplicated the process of koji using wheat bran with this technique and applied it to alcohol fermentations.

Another process for preparing similar enzymes around this time period involved using corn starch or other grain starch and gelatinizing them in a pressure cooker with a small amount of sulfuric or hydrochloric acid. This sterilized the grain mash which was then incubated at 40 C. Air was pumped with a hose (at 5# pressure) and air compressor into the submerged culture and was stirred. After 24 hours, the batch was cooled to 32 C and yeast or mold was added to begin alcohol manufacture.

In 1867, gall nuts were piled in heaps on a floor and moistened with water. Mold would grow naturally in the heaps and after about a month, gallic acid was leached out with water. The organism was later identified as Asperigillus niger. The gallic acid was used in tanning, printing and other industries.

In 1891, it was discovered that some growing molds would convert sugars into organic molecules such as oxalic, citric and fumaric acids. In 1913, a scientist developed a special formula of –

Sucrose	125-150 gms
Ammonium Nitrate	2-2.5 gms
Potassium dihydrogen phosphate	.75-1 gms
Magnesium sulfate heptahydrate	.2-.25 gms

These were mixed into a culture of black Aspergilli at a pH of 3.4-3.5 and then incubated to convert the sucrose to citric acid. This method was the basis for the first commercial fermentation of sugar by a mold into citric acid and it has been used ever since. It was also found that the yield of the citric acid was highest when the development of mycelium mass was restricted rather than stimulated.

In the old European process, the culture solution is dispersed in shallow pans. Up to 30 acres of pans were used in one large plant. The pans used were aluminum or stainless steel to prevent corrosion. The inoculum was spores spread on the surface of a liquid designed for spore production but unsuitable for citric acid production. After about nine days, the fragile sporulating pellicles are transferred to a sterile fermentation solution and mechanically dispersed. This medium is then distributed to the fermentation

pans that now contains a medium for citric acid production (above). The spores have also been blown over all the pans in the fermentation rooms to seed them. Beet molasses or cane sugar are the primary carbon sources. Acid is added to bring the pH down to 2.5-4.0 prior to inoculation.

Spores germinate in the first 24 hours and a thin white pellicle of mycelium covers the surface of the solution. Sterile humidified air is blown over the surface of the pans at 30 C. In 5-6 days, the mycelium is a crinkled or folded mat on the surface. The humid air is discontinued and in 8-10 days all the sugar is converted to citric acid. If the pH rises over 3.5, considerable oxalic and gluconic acids may be formed. Iron favors production of pigments, sporulation and oxalic acid. The sporulating mycelia produce little citric acid.

In 1924, it was found that certain strains of Asperigillus niger would produce gluconate on the surface of a liquid culture if the pH was kept near neutral. This led to the industry of using liquid cultures on trays with a sugar in the medium to grow the mold and make the gluconic acid.

In 1928, Fleming isolated Penicillin from P. notatum which he grew on a solid medium in surface culture. The first British company grew the mold on a liquid surface culture during WW2 to make the antibiotic commercially while the American companies used submerged fermentations. The British method involved using a bottling plant and filling individual flasks or milk bottles with medium and growing them at a slant to maximize the surface growth area.

[In one of the amazing secret stories from WW2, one of the original science papers on the process were read in Holland and certain enterprising Dutchmen found a culture of P. notatum in their national collection and used it to make their own penicillin and treat patients with it without the Nazi's ever finding out. This should offer a solid lesson on the potential of invisible and self reproducing bio-weapons that can be concealed, produced and used against police states.]

X-rays and ultraviolet light was used to mutate strains of penicillin producing fungi obtained from a moldy melon bought in a Peoria, Illinois store in 1945. Out of more than 85,000 mutants screened, 398 were auxotrophs, one of which yielded the commercial quantities under special growth conditions that finally provided this antibiotic to the masses.

Mushrooms were first cultivated in caves in France in 1683-1715. Manure obtained from horse stables was (and still is) used for medium because of its self heating properties. It was stacked into ridge beds in rows on the cave floors. The beds were then inoculated with soil that contained the mycelium of the desired mushroom species (Agaricus bisporus) that grew near the horse manure heaps in nature or horse pastures. Anywhere the horses stood in which the mushrooms grew was considered suitable.

Modern production involves the use of trays or shelves on which to incubate and grow the mycelium. Tunnels, caves, sheds and basements were commonly used to provide the cool, humid conditions necessary to promote fruiting body formation.

Early 1900's methods of producing mushrooms involve taking the spores directly from the mushrooms and culturing them under suitable conditions in artificial media. Milk bottles are filled with manure, plugged with cotton wool and sterilized to kill contaminating molds, insects and bacteria. This bottle is then inoculated with the spores or mycelium at about 21 C. The bottle is then used to seed the trays in large scale production.

In modern culture, chalk and water are added to grain (rye is the most popular but wheat, millet or sorghum are also used) which is used to grow the mycelium and form the grain "spawn" that is sold today. The nutrients available in the grain accelerates the growth of the mycelium in the beds and its granular nature makes it easy to handle. Mixtures of straw and horse manure form composts that are seeded with spawn. The spawn will develop mycelium in unfermented horse manure but does not develop without the heat generated by the straw mix.

A shortage of horse manure in the 20th century led to the development of alternative compost which usually consists of corn cobs, legume hay, gypsum, ammonium nitrate, muriate of potash, and dried brewers grain. Straw and dried blood or blood meal have also been used successfully with minor ingredients added. Other animal manure has also been used as a substitute in straw based composts.

Making good compost requires keeping the stack at 50-60 C and well aerated inside and out. In practice, straw and manure are mixed together and water plus nitrogen sources are added to start the fermentation. The stacks are regularly turned to maintain aeration and ensure thorough mixing of the ingredients. The stacks begin to heat up from microbial activity. The stacks are transferred to trays and these are placed into the buildings (heat rooms). Gypsum is added to the mix to help it retain moisture and encourage mycelium growth. Microbes grow in the compost using many of the nutrients. In the end, the microbes themselves become food for the mushrooms. The better the mix of microbes in the final compost, the more fruiting bodies are formed.

General Operating Ideas

Maintenance of effective producing strains is a problem and one of the most effective ways of maintaining good seed stock is to place the cultures into sterilized soil samples and refrigerate or freeze them. They can also be maintained in solutions where part of the culture has lyophilized and provide nutrients for the remainder. They are drawn on as needed.

Spores are harvested in laboratories by brushing the surface with a sterile paint brush after the surface is treated with a tiny spray of a surfactant like Tween 80 or sodium mono-laurel sulfate.

Cereal grains such as cracked corn, barley, hard wheat bran and other listed for the particular organisms can be used for sporulation. Water is added to the grains and allowed to soak in before sterilizing and the atmosphere is maintained at 98% humidity. This is because most fungi nee a film of water around them to initiate mycelial growth. They can be grown in flasks, on trays or in any other suitable container. After 6 days of incubation at 25-28 C, the spores can usually be harvested. Certain Asperigillus and Penicillium species can be grown and mass produce spores on whole loaves of white bread.

In tray production, trays can be loaded with medium and spores and the trays stacked on top of each other for best use of the available space. Spores are separated from the rest of the medium and mycelium I some factories by dumping the tray contents into mixers with a detergent solution and water. The batch is vigorously agitated and then discharged as a slurry. The moldy grain and mycelium rapidly settle to the bottom while the spores remain in suspension for a very long time (many of them float). The suspension can be filtered through glass wool to recover most of the spores which pass through.

Production of toxins or other metabolites require the addition of nutrients which favor their formation. The nutrients are mixed into the medium and then the substrate is inoculated with spores.

When the toxin is water soluble and is also a protein, it is usually extracted with water and then the solution is saturated 10% at a time with sodium sulfate or ammonium sulfate. Proteins tend to precipitate out of strong salt-water solutions, especially at hydrogen ion concentrations close to their iso-electric points. Sodium sulfate can be added up to 40% and ammonium sulfate can be added to 70% saturation point.

Adding alcohol, acetone or methyl ethyl ketone to the water can also precipitate the toxins from the solution.

Chapter 15

Mold & Toxin Weapon Considerations

Deciding what molds or toxins to use as a weapon depends on several things. The most important is the availability of the source mold. Toxic mold spores are found virtually everywhere on earth and in practically every type of foodstuff. Even in a prison or POW setting a person with the knowledge can grow toxic molds and water extract toxins.

This chapter will cover the following areas –

1. Weapons purpose
2. Weapon recovery, growth, production and material handling
3. Weapons Form

1) Weapons Purpose

In almost any location on earth, most of the mold or related members of their groups can be recovered and cultured. This allows more flexibility in weapons choices. The most important feature of producing effective mold based weapons after availability is the weapons purpose. The weapons purpose generally falls into five broad categories of

a) Plant disease
b) Toxin attack
c) Infectious attack
d) Biochemical special effects

a) Plant Disease

Plants become infected with a wide range of fungal pathogens. Many of these are specific to the particular plant. Some of these cause illness in humans if the feedstuff is consumed or touched while many of these destroy the plants. A number of fungi also cause the plants to produce toxic components in combination with their own metabolites. These can cause illness and fatalities in livestock.

This author felt that a serious work on the use of plant disease was beyond the scope of this book and not practicable on a small scale. If practiced as part of national policy and using the scientific and financial resources of an entire country, it could be used to destroy an enemies food supply and cause widespread catastrophe in the areas affected.

The general operating principle in this type of warfare would be to identify the potential crop pathogens for the target area, recover them from the area and study all related papers to their cultivation and life cycle. Since these types of organisms tend to be

harmless to humans, all that is required after cultivation would be the direct mass dissemination of spores or mycelium into the target area.

B) Toxin Attack

The primary toxins described in this book that can easily be applied and used as weapons would be the aflatoxins and the trichothecenes. The other toxins would follow the same principles outlined here.

Aflatoxins are capable of killing or causing acute liver damage at small doses. At tiny (microgram) doses over longer periods of exposures, it can cause fatal mutagenic, teratogenic and carcinogenic illnesses. These properties allow its use in two broad categories of weapons. The first type of weapon is the direct exposure weapon. These weapons involve the mass growth and production of the toxins. The mycelium and medium can be used directly as a contact, inhalation or ingestion weapon. Alternatively, the mycelium and medium can be extracted with a liquid, and the liquid dried to concentrate the toxin to 10 times or more.

The physical production of the toxin and its extraction gives a substance that can cause harm or death. The toxin is then place in a form in which it may be used as a weapon. This can take the form of a liquid which may aid in sticking to the target (a surfactant or adhesive), may aid absorption through the skin (di-methyl sulf-oxide), or a simple aerosol that floods the air that touches the skin or is breathed in and ingested from accumulation on mucus membranes.

The liquid can be a concentrated form of the extract or a special substance with enhancing properties. The oil of poison ivy would be an example of an enhancement which would promote self inoculation of the toxin.

The toxins can be dried to a solid crystal or paste form and used a solid weapon. Solids can be used to form dust aerosols and the nature of the dust can be designed to allow for a huge range of properties. These can include using a silica based material such as asbestos or diatoms as a ship to carry the toxic cargo. These materials cannot be broken down chemically inside the body and therefore provide safe harbor to the toxins and any accompanying organisms that are inhaled. These types of mixtures make excellent combination weapons. The asbestos can act as a ship to carry the toxin inside. It may act like velcro making it impossible for the target to expel it as sputum from his lungs. The toxin can be T-2 which causes dermal necrosis.

This type of injury destroys surrounding tissues and turn it into food for microorganisms. The addition of infectious bacteria and/or mold spores allows species to germinate and grow in this new environment that is now rich in food. The necrosis can cut off surrounding blood supplies, inhibit air flow, and prevent the bodies immune system material from reaching the infection site. Without air, more tissues die and anaerobic species like Clostridium can now grow causing gangrene. A large supply of spores insures sustained and repeated infection at each dust particle if massive antibiotics

fight off the first infection. The fatality rate with this type of weapon should be very high with few treatment options.

The aflatoxins used in this example are capable of causing cancer at very low doses when exposure is sustained. Weapons designs which allow the mass distribution on carriers that dilute the toxin to these levels are the most effective. Sustaining the toxin and maintaining its low levels in the environment longer term require repeated delivery into the target area. The weapon can be designed to self deliver and distribute itself in small regular doses. Formulas that resemble feed blocks for cattle that are distributed as tiny granules may fit the bill. As the weather slowly degrades and liberates the granules layer by layer, the toxin is slowly and continuously released in the same place on a daily or weekly basis. Those in the area continue to receive the tiny doses without illness or awareness of their exposure until the liver disease and cancers finally begin show up in significant numbers at the hospitals.

By this time, entire cities or armies can be decimated and the cause completely disappeared from the environment. Micropellets can be designed to include immunosuppressant formulas so that whatever bacteria populations that the targets are exposed too become weapons as well.

b) Infectious attack

A number of fungi are capable of reproducing on human tissues. The most obvious are those that invade the skin tissues such as the athletes foot and jock itch causing species. The common requirements for growth are exposure to the organism in sufficient numbers (hyphae and/or spores) and moisture. Weapons design which would enhance the infectious ability would include a source of moisture that could exist inside the dust or as part of an aerosol without being consumed until it is needed. Solidified water such as methylcellulose and jelled formulas can be incorporated into the mix. Certain hydrated substances give up water when coming into contact with human skin (from body heat) which would aid in germination and infection. These skin invaders can be combined with other bacteria that would infect the newly exposed underlying tissues.

[These designs form part of the concept of a class of weapons called multiplier effects weapons. These weapons self grow, distribute into the environment, and can be formed into combinations with multiple effects.]

Some organisms will invade and cause lung disease resembling tuberculosis. This is because of the slow growth properties of the fungi. Typically, very large exposure is required to become infected. This is seen in agricultural areas in which species like Asperigillus produce spores in large numbers. Asperigillosis is a serious disease. The ability to artificially induce this disease as a result of a weapons use is based almost entirely on exposure rates. The greater the exposure the greater the rates of infection. The release of massive amounts of Asperigillus spores and mycelia would be practically undetectable and would not produce effects for weeks to months. Combined with

enhancements, these weapons offer the potential of a massive means of waging war that would go largely unnoticed for months.

The fungi C. immitis is highly infectious in its own right and can be cultured and added to any of the already mentioned designs as a direct effects weapon. In most cases, many humans can fight off these types of infections. When the human immune system is confronted with multiple insults, it eventually becomes exhausted and cannot defend the body. It is under these conditions that very high fatality rates can be obtained.

c) Biochemical special effects

In the 1960's several tests were conducted by the US military using spores. The Army radio-labeled fungal spores and released them on the first floor of a public building. Within 10 minutes, the spores were detected on the fourth floor at levels exceeding 1,000 spores per cubic foot. The purpose of the test was to measure the ability of the spores to quickly spread and enter into all parts of an environment they are carried into by tiny disturbances in the air.

During the 1960's, great strides were made into the study of the pigments produced by different species of fungi. Some of the toxins and pigments fluoresced at certain wavelengths and some could be used as dyes to stain human tissues as tattoos. Most of the fluorescing compounds have since been synthesized and produced in the laboratory. A few have been produced industrially. It soon becomes obvious that these fluorescing dyes could be used to immerse spores, such as those that were used in the distribution test. Such spores would saturate a local environment and stain, or tattoo all human skin in the exposed areas. The tattoo would be invisible in normal light and could only be seen under ultraviolet light. The spots caused by the tiny spores would be so small that they would be as invisible the spores themselves. They would only be able to be seen under conditions of massive amplification, such as those encountered in modern laboratories with chromatographic and spectroscopic equipment and in deep space telescopes and microscopes.

The uses of this spore tattoo concept and its permutations include –

KGB headquarters and training centers. In 1970 it was the height of the cold war. Special US-CIA and US Army operatives take heir special fluorescent labeled mushroom spores and drop them off upwind of these areas locations regularly. After a short period of time, these areas are super saturated with spores. The dye they use fluoresces red at a particular wavelength, say 260 nm. They provide a special detector like a video camera or space telescope which sees in ultraviolet and sends the signal to computer which amplifies this wavelength millions of times. Now, anyone who has come into contact with the invisible spores carries tiny, hugely amplified spots all over their face, hands, arms and the rest of their body. They cannot be seen with the naked eye since people do not see in the UV spectrum. They cannot be seen with UV equipment because the spots are still too small. Like the spores that carried them. With the super UV telescope

however, they can now be seen, just like the invisible stars and galaxies in the night sky are made visible with the Hubble telescope.

The CIA agents operating around the world can now monitor every airport, every embassy, every trade location and can see the invisible brands carried by those individuals who had been to the KGB headquarters or training centers or both if different wavelengths and colors had been used. They could now identify with absolute certainty all of the enemies agents without error. Even those of the Soviets allies who had sent agents there could now "glow in the dark". Science can reveal the invisible truth.

Imagine for a moment that an enemy decides in the year 2000 that they want to know all the US CIA, FBI, and military intelligence agents. They simply super saturate the upwind areas of Langley, Quantico, and FBI headquarters and regional offices with their own versions of these labeled spores. They can now separate the agents of these institutions from the legitimate tourists as they pass through the worlds airports and travel to the worlds hot spots. Unless you happen to have detection equipment that detects and amplifies that same exact frequency, color and possibly even chemical composition, you will never know that every single undercover agent you have employed and trained has been branded for life.

The US Navy launches a very special missile into Afghanistan in the middle of the night. Its target is the headquarters of a known terrorist named Bin Laden. The missile closes in on its target slowly. As it reaches the target area it does not descend into the mass of tents and temporary buildings. Instead it rises into the air to gain altitude. A small explosive detonates inside the warhead slightly upwind of the command tent where Bin Laden resides. In ordinary conditions, the small blast might have been seen from the ground. In this case, a huge cloud of invisible dust obscures all light that might have been seen. From an altitude of almost a mile, the cloud quickly disperses into the mild breeze until, as it finally reaches the ground, it diffuses into the air into invisibility, each of the particles far too small to be seen with the naked eye even in daylight. Each particle carries the selected dye. Within minutes, every single individual within several square miles is marked for life as an associate of Bin Ladens. Every visitor he has for the next few weeks is likewise marked. The US Navy periodically launches a booster marker missile to maintain the dust dye in the area. Whenever a Bin Laden branded associate travels through any foreign airport or seaport, the CIA and military can now see them glow in the dark with the permanent pigments branded into their skin. They know who to watch without any guesswork whatsoever. When Bin Laden finally shows up in disguise, he is no longer disguised from the multi-spectrum eyes of the secret governments.

Imagine now that you are a member of the KU Klux Klan. You are hidden by a white hood. You can go to a meeting feeling anonymous and safe from a possible undercover agent identifying you. An agent was at the location of your meeting a few hours before you were. He was planting bugs, he wasn't even going to be at the meeting. He simply dropped the spore packets onto the ground in some bushes on each side of the meeting area. Every single member of the Klan who attends the meeting is now marked for life with the airborne spore-dye, even under their hoods. Now, the FBI agents watch

people walk down the street using their special hidden cameras in a van. They see the banker, now not wearing a mask with a mottled appearing face. The see the local druggist with the invisible spots. They all walk down the street as the do every day, with not a single soul knowing where they secretly went the day before, except for the FBI agents who can now see them and the brand they left behind. The spores had drifted and floated around the site, marking their hands and faces under the hoods. They would forever wear the invisible brand for the day when the government wants to quickly sweep up all suspect Americans and intern them in times of upheaval.

The year is 2002. Every American criminal suspect is taken in or arrested, fingerprinted, photographed, and then a small piece of paper with a number is rolled across their forehead. The number washes off in a few days easily but it embarrasses the suspects not yet convicted of a crime. Before they go to court, the number has been washed off so no one can see it any longer. One of the suspects escapes and leaves the city for another location to live. Five years later, a secret video security camera pans the entrance to a football stadium. It sees an invisible number at a particular wavelength on a mans forehead. It matches the number of a man wanted for escaping from prison halfway across the country. The monitoring computer silently dials the local FBI number and informs them of the detection. The agents are dispatched with special detectors which quickly locate the suspect in the crowd at the football game. They walk up and arrest the fugitive who never knew that he had been marked for life.

A Jewish church group meets at a local church every week. The grounds on which they walk to the entrance has secretly been saturated a treated dust. A hate group with a special video camera attached to computer monitors the movements of all the members. They can see their invisible brand on the heads and hands of the targeted religious group even as they drive down the highway. They follow the cars with the marked drivers home. Then the terror begins.

The undercover agents of ATF, FBI, NSA and others routinely work the guns shows in the US to monitor firearms trafficking (which is legal as of this writing if such a right even exists to keep and bear arms). Many regular citizens and gun dealers also attend and work the shows. The NRA, or another of its more ambitious relatives decides that they need to know who is secretly working for the government at these shows. They decide to set up a 50 state labeling program. Every week, each gun show in every state will be labeled with the states invisible color and frequency. The harmless invisible dye is dropped into the trash cans at each show. The impact of the drop sends an invisible cloud of spores into the buildings. Every attendee is marked during their attendance. The detectors they use monitor every frequency and color set up in the spores. This time though, they don’t fluoresce. The government already knows about the dye system and has used it themselves so they are on the watch for it. This new system involves a special invisible colored ink that needs the detectors to be tuned only to a specific visible light wavelength and amplifies the tiny signal so they can be seen on every persons face.

They can now tell which individuals at the shows fly around the country and attend shows in multiple states. They can tell which states and how many shows were

attended from the densities of the markings. They can cross reference these with the license plate numbers of the cars they drive and the truth is quickly known. The day they try totake the guns away, the entire secret enforcement arm of the government is no longer secret because the visible light stain can be seen by any normal video camera. It can be hooked up to any computer which has the correct software and now every single agent can be seen and identified by any private citizen with the computer and video camera from Wal Mart.

The invisible, secret wars go on.

2) Weapons Recovery, Growth, Production and Material Handling

Recovery

In the home of almost every single citizen of the world, an entire arsenal can be found. It you can make a paper towel wet and place food, grain, or plant parts on it, within a few days, molds begin to grow. If you do not have knowledge of how to tell molds apart, there are several time tested methods for finding out which ones can produce weapons. Wheat flour, peanut products and almost all seed grains from the fields (especially the kernels at the tips of ear corn at harvest time) contain toxin producing molds.

The easiest and quickest way of screening thousands of candidate food samples is to use fluorescent (ultraviolet) light. The light bulb (black light) can be picked up at any Wal-Mart. Many of the toxins fluoresce such as aflatoxin B1 and G1 which fluoresce blue or green. Some fluorescences are not toxic however and so these must be checked to see if they are the real thing. Each toxin candidate can be sampled and tested with a tiny speck of the mycelium taken and fed to test mice.

The toxicity signs can show up in hours. In the case where the molds are grown over several weeks in the refrigerator, the samples can be placed onto the skin of the mice or guinea pigs to see if they cause necrosis. If so, you have a trichothecenes producer. In this way, any ordinary citizen can quickly acquire the primary toxin producing molds that he can build large scale weapons with. The spores from these molds can be sent to anyone, or everyone to quickly produce mold equipped citizen armies overnight. All a citizen has to do to produce the molds is to grow the spores on moistened and sterilized (baked in an oven) food or grain.

Growth and Production

The following chart shows a variety of foods and their moisture content as is and then when immersed in water for 4 hours so that the water soaks into the food and saturates its pores. These materials are then pressure cooked at high temperature to sterilize them. You can bake them and then soak them in sterilized or distilled water to create the same effect. What you want is to prepare them for growing your desired toxin

producing molds. In this way, many foods can be quickly tested with the same mold to see which one grows the easiest and quickest for you.

You can also use this method without the sterilizing step to start mold growth in the screening and recovery of toxin producing species without using the wet towels,

Kinds of foodstuffs and their moisture content

Food	Moisture content (%)	
	No Water added	After immersed in water for 4hr
Dried sweet potato	34.0	78.6
Salami sausage	18.0	40.0
Corn	13.1	34.0
Dried buckwheat noodle	13.2	40.0
Dried noodle	12.2	40.0
Dried slice radish	19.2	83.0
Soy bean	11.8	57.4
Sesame seed	4.0	40.0
Dried gourd shavings	27.4	80.0
Tangle	12.0	84.8
Azuki bean	14.0	26.2
Red pepper	13.0	69.2
Japanease pepper seed	13.2	64.6
Flakes of dried bonito	18.2	40.0
Dried persimmon	46.6	50.1
Dried mysid	28.0	70.5
Dried small sardine	10.0	40.0
Dried purple laver	6.0	40.0
Skim milk powder	5.0	40.0
Green tea leaf	8.8	40.0
Dried shrimp	21.5	71.0
Pepper	15.0	40.0
Roasted coffee bean	5.6	59.2
Mustard powder	7.0	40.0
Curry powder	14.8	40.0
Slice green garlic	22.8	26.3
Cinnamon	13.2	37.0

Excess water is drained off after the soaking period. When weapons are being produced, the food is inoculated with spores or mycelium and then incubated. This is usually done at 25 C except for the trichothecenes which are refrigerated and taken out periodically to stand at room temperature for a day about once a week. Many species produce the toxins and reach their peak at 8-12 days while a few may take up to 30 days.

The Trichothecenes can take up to 60-90 days in some circumstances. Dried sweet potato and salami are the most effective food substrate for producing mycotoxins, although they may not be the best for recovery and growth of the molds. The easiest materials to grow the molds on are dried noodles and cracked corn kernels. Antiseptics that kill bacteria and not fungi can be added in tiny amounts to help inhibit contaminants in the growth feedstock.

If the mold is the desired weapon or part of a combination weapon, it is taken in this stage for incorporation into the weapons design.

If the toxin or weapon is to be concentrated, then it is extracted at this time using water or other solvent such as alcohol (ethanol, methanol, isopropyl, liquor) or kerosene. The extracts that contain the toxic fractions will kill the test animals much more rapidly than the mold crude mold sample used in the screening process. The solvent can be evaporated to leave a paste or powder behind. If it is heat sensitive then it can be air dried or vacuum dried using a pressure cooker with a vacuum hose and pump attached. Water evaporates much more quickly (boils off) at room temperatures under vacuum.

If the toxin must be concentrated further and it is a protein, then it can be fractionated from the liquid by adding 10% ammonium sulfate at a time to the solvent, let it sit in the refrigerator for 24 hours and then filter it off. One or at most two of the fractions will contain the super concentrated toxin which can then be tested and then dried to a paste or powder. The advantage of a paste is that the contents stick together and there is little likelihood of creating an aerosol that will kill everyone in the area. This is how the Indians of South America prepare their incredibly deadly darts for use when hunting game or other tribes. They can use the deadly toxic plant extracts without fear of injuring themselves accidentally by handling it as a paste.

Material Handling

Producing small samples of molds that are not infectious can be done easily by anyone. In the screening samples, you can grow them on trays or in pans with the lids lifted off daily to provide fresh air. If done on a large scale, an air ventilation system should draw air from the growth area into a filter that can be incinerated. On small scale testing, it can be vented directly as it will be harmless when massively diluted into the atmosphere.

When mass producing large amounts of mycotoxins for use in weapons, several other methods are necessary to keep from killing yourselves. The easiest is to grow it in ziploc bags (double bagged) or in trays with lids having very tiny needle like holes for ventilation. An attachment for a hose or funnel should be prepared so that a solvent can be added to the fungal and toxin growth. Once the growth is under a solution, it stays there and does not form deadly aerosols unless agitated. The solution can be dried to a paste, or thickened with methylcellulose, gelatin or starch, or made sticky with a surfactant so that aerosols are inhibited and the toxin concentrated until ready to use in a weapon.

When handling dry growths that contain deadly toxins, a double or triple ziploc bag arrangement can be used to grow them. The bag must be ventilated so that the molds can continue to grow. An air filter can be used. A surfactant can also be used on the inside of the bags to catch most of the dust formed and reduce the hazard. This can be in a small sealed cup inside the bag. It can be released after growth to coat the growth or be used at the start to coat the lining surfaces. It can also be added in larger amounts at the end of the growth to suppress all dusts. Soybean and other liquid grain oils also work very well at suppressing dusts at this stage. The invisible aerosols of these fungi make incredibly deadly weapons when they are produced in volume. The dust from a single dried bag (8 oz) of aflatoxin can conceivably kill up to 1,000 people so care must be used to avoid producing visible dust aerosols (if you can see it, it will probably have already killed you).

Other large containers that are used to grow the weapons can be useful if the are going to act as the delivery system as well. The ziploc bags can be left in the ventilation systems of targeted buildings with a small amount of solvent (kerosene) added to dissolve the bags. The self biodegradable bags make the best weapons because they contain the weapon until they degrade and release their contents into the surrounding environment. These types of designs can make good time delay and booby trap weapons. They also release their contents more slowly making good low dose over long time weapons which are effective (such as when using aflatoxins as a cancer causing weapon).

Some weapons can be grown one bag at a time and accumulated over long periods, such as a year. Hundreds of bags grown in a day can easily leach enough toxin to kill its maker. One bag a day will not and its effects are often detoxified in the body (aflatoxin is an exception to this). This allows small exposure rates to be harmless while the mass produced and concentrated final weapon will be capable considerable damage.

A dust mask should always be worn when handling deadly toxins. A gas mask is used when the amounts handled and stored become large. Skin protection is essential if there is danger of an accidental spill. The mask should be removed only after a shower has diluted any toxins that may have gotten on the clothing or in the mask surfaces and filters. If you are handling infectious disease organisms, the shower method ill not work and can possibly make the exposure worse. Water and showers massively dilute and aerosolize the materials you have contacted. If the material is an infectious disease, the use of disinfectants, radiation and chemicals may be necessary. A series of showers and disinfecting are usually the best bet.

Liquid cultures can also be turned into jello with the addition and gelatin and refrigeration. Since jello melts at 78 F the toxins can be safely stored in a semi-solid until ready to use.

Weapons Form

The use of Jello has just been described. This can be a useful weapon by itself. When refrigerated as a semi-solid, it contains and holds in all the deadly toxic contents. It can be taken out on a cool night and distributed into the target area. By dawn, in summer, the heat reaches 78 F, the Jello melts and the toxin and mold is liberated to self dry and spread around the target area. A dust such as diatoms, asbestos or fine powdered clay can be used and mixed into the Jello during its creation so that the contents saturate these particles and as they dry they become the fine dust carriers which enhance the weapons.

The ziploc bags and other containers can also be used as weapons as long as there is a time release method of distributing their contents. The bags can be clandestinely place inside the ventilation of targeted buildings. It can actually be placed anywhere that it will not be seen and be able to release their contents. The tops of unused shelves, the roof of the entrance, taped to the bottom of desks. Anything that is invisible to the population will work.

When using a sticky or thickened liquid. The liquid contents can be used as a "paint" or coating to deliver the toxins. The underside of desks and chairs, the roofs of semi trucks, the tops of doors, and you can even dump the entire contents of a container behind a shelf that will go unnoticed. As the contents dry, they are released into the air and disseminate into the surroundings, attacking whoever breathes them in or has them descend on their skin. The formula can be adjusted for quick drying and release like paint using a solvent as the base, or it can be made with surfactants and slow drying solids for slow release.

If you intend to use spore as a carrier or as the weapon, there are a few facts that are useful to know. Measurements taken in grain elevators without ventilation have found over 1 billion viable spores of various fungi per cubic foot in the air from the grain dust. This level of concentration is nearly invisible and continuous exposure does cause some incidence of disease among elevator workers. These spores may represent over 100 species of grain infecting fungi. Most of these are harmless to humans and these as swell as bacteria dilute out the deadly spores to probably as low as a thousand/cu. Ft. Those spores that are capable of causing lung infections must be a size of 5 microns or less to enter the small air exchange sacs of the lungs where they are not as easily expelled.

The use of a carrier that dries as solid particles of 5 microns or less are the best carriers and spores individually are this small if they are not being carried on a larger dust particle. Diatoms are single celled organisms that died and left a silica shell that is that small. These are excellent carriers because they do not dissolve and dry as larger crystals like most other substances.

The use of combined weapons such as those which have a toxin that causes a necrosis (T-2 toxin) and several different species of fungi to infect the area injured by the toxin. When bacteria and molds are combined with toxins of each, the potential combinations of weapons designs run probably as high as 100,000. Bacteria and fungi

that might never infect human tissues can now grow and invade when the toxin is an immunosuppressant. This method leaves behind the mystery of why people fell ill and died.

In circumstances where a citizen population is invaded and oppressed, other weapons designs can be adapted. Instead of throwing Molotov cocktails at tanks, glass jars filled with sticky powders of bacteria and toxins can be thrown. When the tank crews try to leave their vehicles they become exposed. The use of washing systems to remove the dust creates aerosols that disseminate and kill everyone in the wash off area. They also work well for throwing into the path of oncoming troops. A simple garden hose and pump with a very fine shower head can be used to spray the weapons into the path of oncoming troops. You simply retreat and draw the troops into the contaminated area.

The use of fine humidifier sand ultrasonic misters can also be used to dispense the liquid weapons. If new citizens need to quickly produce these weapons, they can randomly grow them from feedlot manure, dead animals (deliberately killed and allowed to bloat) in culture. These blood based cultures for bacteria can be combined into dried fungal molds and then distributed. Random weapons such as these may be somewhat hit and miss and are not as concentrated as professional built bio-ordnance. The advantages are that anyone can grow them overnight from a single page of instructions with no biological training, they can be incorporated into all types of delivery constructions and because their contents are unknown even to their makers, the new combination weapons will have hard to defend against effects.

The mixing of a finished mold or toxin substance into a mix of blood, egg white and suitable other material (sodium bicarbonate for anthrax spores) such as a dust carrier allows a combination weapon to be grown in a day or two that self dries. The blood and egg white is consumed by the bacteria rapidly. Other microorganisms finish the job once it is distributed and the material is dried as a powder onto and into the carrier. As the powder dries and is carried into the wind, it is saturated with fungal toxin and bacterial and fungal organisms inside and out. They are capable of inflicting significant injury on any targeted areas.

The use of infectious and contagious organisms in these designs can quickly magnify their effects. The release of spores infected with plague on their surfaces from a biodegradable ziploc at an airport can quickly shut a nation down if all airports are saturated simultaneously. The bags need not pass any security and can even be dropped into the trash cans if their release or dissolving mechanisms act quickly. No one will know they were even there for several days. If all airports are affected, when the outbreaks are nationwide, it will disguise their initiating locations. Imagine the effect of doing this at every airport and train station all at the same day. Now combine this with random mailings of the disease to every zip code. One person using a judicious schedule could cripple a nation all by himself.

Now imagine this one person sending this information and the correct organism to every member of a particular targeted group that the government just offended on the

next day while they are still angry. A single one page letter of instructions and you have a ready made, angry and highly motivated army ready to wage war overnight. The targeted group could be gun owners who just had their firearms registered or confiscated. They could be environmentalists who were just hauled to jail en-masse by the police. They could be a labor union who were broken by governmental decree and made unemployed in huge numbers.

A person does not have to fight unjust government, he only has to arm everyone else to do it. As the government becomes more repressive, more people will be willing to fight. Instead of setting up cells to fight, one at a time, you set up army builders. Each one independent and each one capable of arming peoples and groups they do not even know. The army builders never have to fight or be seen. They never meet the people who do the fighting and are not associated with them. They need not even be in the same state or country. More will be said of this concept in the next volume of this series.