FUELS, FILTERS, AND FUNGI

BY

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The microbial spoilage of materials was well known in ancient times. The book of Leviticus in the Old Testament* warns: 'When there is a stain of mould, whether in a garment of wool or linen, or in the warp or weft of linen or wool, or in a skin or anything made of skin, if the stain is greenish or reddish in the garment or skin it is a stain of mould which must be shown to the Priest'. The passage then describes how the Priest should diagnose the growth and, should it prove 'unclean', how he should adopt the somewhat drastic treatment of destroying it by fire.

If such treatment were still mandatory there would be a great number of awe-inspiring blazes from kerosene fraction fuels infected with the micro-fungus *Cladosporium resinae* (Lindau) de Vries. This now infamous fungus has been found in jet aircraft fuels such as Avcat and Avtur, diesel fuels of many specifications, hydrocarbon-based hydraulic fluids and lubricating oils; and there is little doubt of its ability to grow in quantity in any kerosene fraction, or formulation containing such a fraction, should contaminating water be present in fuel tank or system. It has been shown to have caused blockages of fuel feeds and filters, to have interfered with coalescer filter function, and to have been the cause of the corrosion of aluminium alloys in certain instances. Kerosene fraction fuels, kerosene-based lubricating oils, hydraulic fluids, etc., would seem inhospitable for any living organism; but a good number of micro-organisms (bacteria, fungi, and yeasts) are able to multiply in this situation—none of them quite as successfully as *Cladosporium resinae*. This paper attempts an explanation for the success of *Cladosporium resinae* by describing its natural history; it outlines the problems caused by its growth in kerosenes and gives some account of remedial measures.

Natural History

Cladosporium resinae was first discovered growing on the resinous exudates from pine trees, hence the descriptive epithet 'resinae'. Its role in nature is most probably to initiate the decay of such material and to assist in the breakdown of the resinous fraction of pine litter. Its unusual adaptability soon brought it fame. It was discovered to be able to grow on telegraph poles, railway sleepers, and other creosoted timbers where weathering had reduced the content of the preservative to below 3%. At this concentration of creosote most other micro-organisms are either killed or are unable to grow, and *Cladosporium resinae* had found itself an enviable ecological niche with no competitors. It was soon dubbed the 'creosote fungus'.

These original habitats give clues to its abilities. Pine resins contain terpenes which are hydrocarbons, and creosote itself has a hydrocarbon content so that 'wild' strains of the organism are most probably well used to attacking hydrocarbon molecules and gaining from them the carbon essential to their

*'New English' translation.

growth. This view is supported by work done by Douglas Parbery[1] and others with strains isolated from natural sources. Parbery used matchsticks soaked in low concentrations of creosote, dried them, and placed them in strategic positions in the litter of the pine forest floor. Recovery after a few weeks showed that the matchsticks were colonized by *Cladosporium resinae* and from these it was relatively easy to isolate many 'wild' strains. Many of these freshly isolated strains were able to grow in fuel/water cultures as readily as those isolated from fuel samples. Some, disturbingly, grew more strongly!

Brief History of Microbial Growth in Fuels

Early in the 1950s jet aircraft proliferated for both civil and military use. Stocks of the highly refined kerosene needed to fuel the jet engine began to build up and, as they did, reports of slimes and sludges in fuel samples began to cause concern. These slimes and sludges were always associated with fuel contaminated with water, with their greatest mass appearing at the interface between fuel and water. The slimes were often a shade of brown or black, felt-like in consistency, with a tendency to adhere together in 'mat' form unless vigorously agitated. Examination showed that the bulk of such material was biological, containing micro-fungi, yeasts, and bacteria.

Preliminary investigation by microbiologists showed a great number of differing micro-organisms to be present, many types of bacteria, yeast, and fungi being isolated. Contamination from air-borne dust, tankers, bowsers, and pumps used during fuel transfers added to the variety grown on the investigators' culture plates. Many of these microbes were unable to grow to any significant amount in fuel/water mixtures whilst others were only able to multiply because their brethren, able to gain nutrient directly from the fuel, provided waste products which they, in turn, could assimilate.

The lists of microbes isolated soon contained the name *Cladosporium resinae* with regularity and this organism was recognized as constituting the bulk of the 'bio-mass' in many samples. The remarkable ability of *Cladosporium resinae* to use kerosene as a food source is shared only by a few other fungi, bacteria and yeasts, and none of these ever seem to proliferate to the same extent; nor do they so ably colonize even the smallest droplet of condensation on the walls of the fuel tanks. The fungus was soon awarded another soubriquet—that of 'kerosene fungus'.

The Organism

Colonies of common micro-fungi consist of a fluffy or slimy mass of tiny threads averaging from one to three microns in width. From this mass spring tiny specialized stalks which bear the microscopic spores in huge numbers. The microscope reveals that each of these threads in the colony is, in fact, a tube enabling the organism to absorb and distribute food throughout the colony. These tubes are called 'hyphae' and the stalks which bear the spores or 'conidia' are referred to as the 'conidiophores'.

Cladosporium resinae has a basic structure similar to thousands of other species of micro-fungi. (Differences between species are in the type of colony produced, the foods which are favoured, and details of their structure as seen under a high power microscope.) The hyphae of *Cladosporium resinae* are a mere two or three microns wide, its spores no wider and perhaps five microns long. The spores of micro-fungi being small and light are carried easily by air currents or by water to new sites where, if conditions are favourable, new colonies will be produced. Most micro-fungi growing on wet or moist substrates produce their spores into the air to aid dissemination. *Cladosporium resinae* growing in water beneath the fuel produces its spores in the less dense medium above. The spores are thus disseminated by the fuel itself, being freed from the parent colony by agitation, currents set up by addition or removal of fuel from the tank, and even convection currents set up within a tank by temperature change. When these freed spores contact other contaminating water in fuel systems or tanks they will germinate and produce new colonies (FIG. 1).

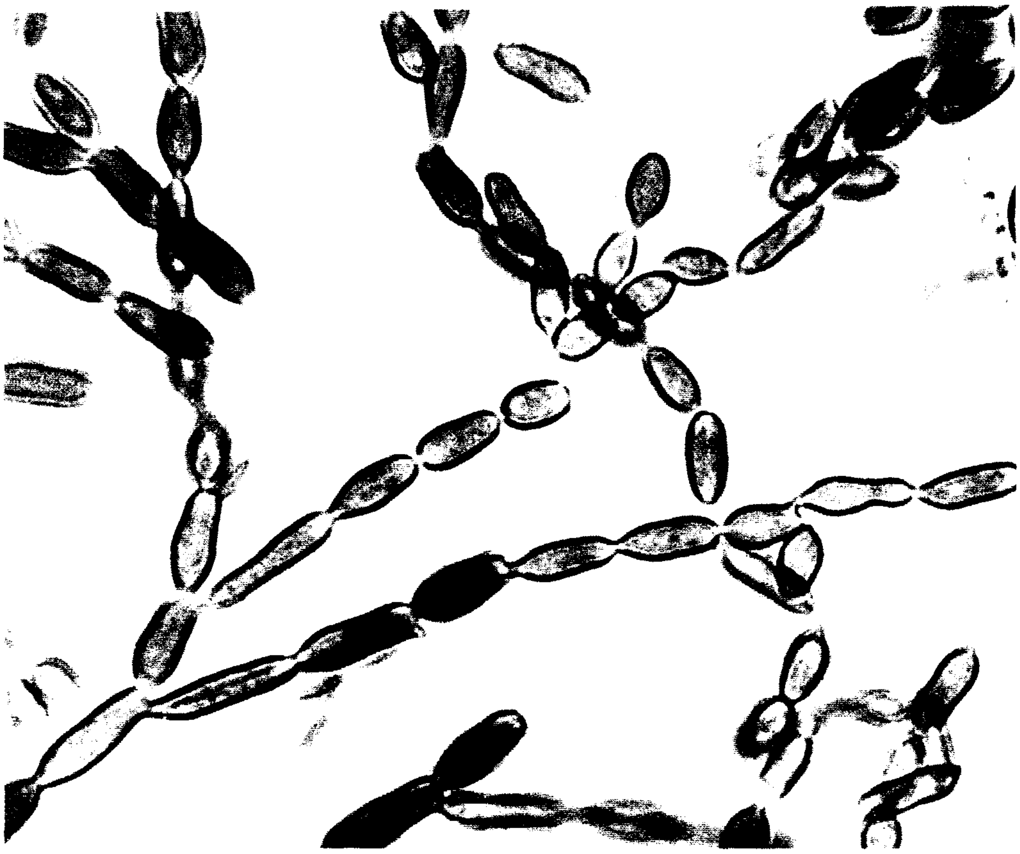

FIG. 1—*CLADOSPORIUM RESINAE* (×2500), SHOWING THE CHARACTERISTIC BRANCHING SPORE CHAINS AND SPORE SHAPES OF THIS MICROFUNGUS

Fungi feed by producing digestive enzymes which break down complex substances to acceptable molecules. The enzymes can act inside the fungal cell but often they are extruded on to or into the material and the fungus absorbs its food pre-digested. Carbon is a prime requirement for all micro-fungi and, given a supply of this and traces of other essential nutrients such as nitrates and phosphates, surprising amounts of growth can occur. In the fuel tank *Cladosporium resinae* has a more than ample carbon source from the fuel itself and the limitation to its food supply is the presence of the other necessary nutrients in the far from pure contaminating water. The concentration of these other essential foods is clearly a factor limiting the amount of growth in a given sample. No growth at all will occur if water is absent as all organisms need water in order to grow.

The Problems

. The obvious problems with fungal growths in fuel tanks and systems are those of blockage or restriction of fuel flow. These will occur particularly where fungal growth in storage or ready-use tank has produced copious growths in the mat form at the fuel/water interface. Similar problems in kerosene fraction based hydraulic fluids are often exacerbated by the compacting action of the pressurized hydraulic system.

In aircraft fuel systems a further complication can ensue. Where *Cladosporium resinae* encloses small amounts of water with its mesh of hyphae or produces extensive mats on the floors of tanks, a semi-permeable membrane is approximated between water and fuel. Differing electrical charges between fuel and water and the presence of this membrane encourage the electrolytic corrosion of aluminium alloys. Further corrosion will occur when the organisms produce corrosive organic substances as by-products. Actual perforation of aircraft wing tanks has occurred and bends in thin gauge fuel feeds have been shown to be especially vulnerable.

Norman Hendey[2] in the early 1960s was able to show perforation of aluminium baking foil placed in contact with pure cultures of *Cladosporium resinae*. Hendey's further experiments placed strips of baking foil in fuel/water cultures so that the interface, and therefore growth, occurred midstrip. These showed conclusively that growth of the fungus was conducive to corrosion. Perforation of the baking foil at the midstrip point was clearly seen after four weeks.

Growth on the interior of coalescer filter elements causes blockage and flow restriction, whilst growth on the external cotton sleeving provides a more complex problem. Surface growth on the stockinette sleeving alters the coalescing properties of the material (Fig. 2). This is due to the fact that slimes produced by microbial growth are often polysaccharides and these substances act as drag-reducing agents so that water droplets will be prematurely released and, being too small, will be carried forward in the fuel flow rather than falling to the bottom of the coalescer vessel. Some organisms will produce substances which act as surfactants, and coalescing problems with such compounds are well documented.

Fig. 2—The cotton sleeving of a coalescer filter element showing colonies of *Cladosporium resinae*

Such surface growths are avoidable if filter elements are in an uncontaminated coalescer vessel, and have not been contaminated during installation or while being changed. The filters will of course remove spores and fragments of fungal hyphae from contaminated fuel prior to its reaching the stockinette. The difficulty is to avoid contamination, for one spore will produce a colony and, hence, thousands of other spores. The coalescer vessel itself or its elements can be infected very easily during maintenance, element changes, or even by airborne spores. During use, with the coalescing process continually flushing the element with fuel/water mixtures, growth is less likely, as no quiescent fuel/water interfaces would be available. During rest periods however, with multiplicity of more stable interfaces available, growth may well occur. The adherence of colonies of *Cladosporium resinae* on to surfaces is surprisingly strong and once its hyphae have penetrated the stockinette, colonies are very difficult to remove. Such colonies are ideally placed to disseminate spores into the system downstream of the coalescers.

Examination of Samples

Ideally, fuel tanks are inspected *in situ* by a microbiologist who can then take selected samples and possibly express an opinion or suggest early remedial treatment. However, this ideal is by no means always practicable and the following notes are for the guidance of those forwarding samples to a microbiological laboratory.

As *Cladosporium resinae* will grow in a sample jar as in a fuel tank, samples should be forwarded to the microbiologist as quickly as possible or kept at temperatures below 10°C, to delay growth.

A sterilized sampling bottle is advisable; but an ultra-cleansed one, washed in water as hot as possible and dried in heat, will suffice in an emergency. Filter elements may be enclosed in unused polythene bags but it should be remembered that the damp atmosphere inside these bags is very conducive to the growth of many species of micro-fungi and that rapid transit is therefore even more highly desirable.

Samples of fuel without a portion of the water bottom and visible growth, if this is present, are likely to be misleading. Such samples may be filtered and cultures made from the deposits and the numbers of spores or fragments of fungal hyphae able to produce colonies estimated. This may or may not give a result comparable with the amount of actual growth present in the tank, for such techniques really indicate only the number of spores able to develop if conditions are favourable. However, these samples do give an indication of the potential growth capability in the fuel.

Samples with a water bottom and a portion of the suspected slime or sludge can be assessed by direct microscopy of the deposits. Estimation of the amount of biological material compared with other debris can be made, and the stage of growth and the amount of sporulation determined. Provision of a number of samples from the same tank increases the accuracy of these estimates. Cultures are necessary to confirm the indentity of the micro-organisms present and to show that those present are viable. Where suspected growths are seen on the walls of tanks or on the filter elements during use, a sterile 'throat swab' as commonly used by doctors may be used to 'wipe off' the materials. These swabs may be forwarded directly to the laboratory.

A maximum amount of data with the sample is desirable. Type of fuel, duration of storage, date of sampling, length of use of filter element, changes of fuel, etc., may supply important information for an accurate assessment of the findings.

Solutions to the problem

Solving the problem of the growth of *Cladosporium resinae* in fuels and hydraulic fluids may be divided into three areas: prevention of infection, 'good housekeeping', and the addition of suitable compounds which will act as *biocides* or prevent growth occurring.

Prevention of Infection

Although it is extremely unlikely that prevention of infection will ever be a practical means of combating the problem, it is worthy of discussion because it highlights some of the difficulties of dealing with microbial problems and indicates why some of the measures discussed under 'good housekeeping' are desirable.

The sources of infection for a given sample of fuel are from pine forests and creosoted timbers and also from other infected fuels, fuel tanks, and bowsers. It is not only unlikely that fuel stocks can escape infection but it is probable that very few uninfected stocks of kerosone fraction fuels exist in the world today. These will produce growth if they become contaminated with water containing essential nutrients and the rate of growth will depend on the prevailing ambient temperature. (*Cladosporium resinae* has a temperature range of 10°C to 35°C with an optimum varying strain by strain between 22°C and 30°C).

To keep fuel free from infection, it would be necessary to refine it, transport it, store it, and use it under conditions approximating those in an operating theatre. A standard of cleanliness somewhat less than this awe-inspring criterion can help prevent gross infection and, more importantly, mitigate against growth. This can be referred to as 'good housekeeping'.

Good Housekeeping

This means regular cleansing programmes for storage tanks, tankers, bowsers, coalescer vessles, ready-use tanks, fuel feeds, and delivery lines. Freeing these of water bottoms and slimes and sludges regardless of their nature will do much to prevent gross contamination and, incidentally, allow biocides to perform more efficiently if they are used.

The removal of water is perhaps the most important item in a good housekeeping programme as its complete removal would eliminate the problem. In practice, it is virtually impossible to remove it all: reducing the content is the best one can achieve. Rainfall via minor leaks, humid atmospheres causing condensation on the tank walls above the fuel, and rough weather during transport by sea can all add to the water content. The integral wing tanks of aircraft necessarily contain struts, stringers, and other obstructions where water will form pockets despite low point drainage, while high altitudes and the concomitant temperature extremes will add to condensation problems. Low point drainage in storage and other fuel tanks can be frustrated by similar problems. All tanks will suffer from the ability of *Cladosporium resinae* to enmesh water droplets and small puddles with its hyphae and then to adhere with some tenacity to wall, floor, or stringer complete with a captured water supply.

Even if high suction is used from storage tanks, vortices may well be created and water bottoms be mixed with the bulk fuel. Undoubtedly the most efficient method of freeing fuel from water is the coalescer filter, which, with porosity below 2 microns, will also remove fungal spores and fragments of hyphae. The cotton sleeving of new coalescer filter elements is often protected with a fungicide and if these are placed with care into a clean coalescer vessel they will be protected until the fungicide is leached away by the fuel/water mixture. The ideal fungicide for cotton sleeving, resistant to such leaching, has yet to be found. As previously stated, 'good housekeeping' with coalescer vessel and elements is of utmost importance.

Addition of Biocides

The criteria for the selection of a suitable biocide for use in fuels are formidable. Such a substance has to 'find' the water present and the addition of a biocide to the water alone would be a daunting task. It is desirable that such a substance be added to the fuel which is naturally in contact with all the water and that it will either suppress growth by virtue of its presence in the fuel alone, or be more soluble in water than in fuel, so that it will partition from fuel to water and build effective concentrations where it will be needed. Substances with the latter properties have been found to be most effective. Such substances must not significantly interfere with the fuel's function, must be compatible with the engine and the materials in the fuel system, not interfere with coalescer function, be of acceptable toxicity to humans, and its combustion products must be acceptable to the environment.

Two substances which answer these criteria have been reasonably tried and tested. Both of these partition from fuel to water.

The first of these is ethylene glycol monomethyl ether or 2:methoxyethanol, the anti-icing additive known as FSII or A.1.A. Other anti-icing additives such as diethylene glycol monomethyl ether or ethylene glycol monobutyl ether have similar effects. Formulations of this additive used to contain glycerol until this was discovered to actively encourage the growth of many different micro-fungi beside *Cladosporium resinae*. Without the glycerol, ethylene glycol monomethyl ether is a growth suppressor or a biostat rather than biocide. If added to fuel at around 0·10% to 0·15%, concentrations in the water build by partition to 20% or more. This prevents icing because the osmotic pressure of the water is increased, and the growth of *Cladosporium resinae* is suppressed for the same reason. At high osmotic pressures, micro-organisms cannot obtain water through their cell walls which are semi-permeable membranes. In many instances, dehydration of the cell occurs and biocidal effect is noted with time. Preservation of foods by salting and the use of sugar in jam making are examples of the application of this theory.

The effectiveness of ethylene glycol monomethyl ether in practice is governed by the amount of water present in the tank or system. Concentrations of or above 15% in water are desirable and care is needed to ensure that concentrations in the fuel are sufficient to yield a high enough concentration in the water. This can be difficult, or even impossible, where abnormally high water concentrations are expected, such as those in water-displaced fuel tanks.

If fuel containing additives passes via storage, tanker, or bowsers, etc., over a number of water bottoms, then its content of additive can be drastically reduced by repeated partition.

This is one of the reasons for the apparent failure of this additive to give either adequate anti-icing protection or to be effective against microbial growth. A good-housekeeping programme of water reduction in conjunction with this additive must, therefore, be advised together with quality assurance to ensure adequate concentrations.

The second substance commercially available is Biobor JF, a mixture of organo-boron compounds which, like FSII, partition into water. This also has the effect of increasing osmotic pressure but as its partition product is chiefly boric acid, it will act as a biocide. Lower concentrations than those with FSII are effective, 135 ppm in the fuel controlling growth under usual conditions.

Because boron compounds act as fluxes some concern has been expressed with reference to possible depositions on turbine blades or even corrosion because of the acidity produced in the water. Limited trials have proven favourable and it is generally accepted that it may be used in a 'one shot' disinfection programme, usually at the higher concentration of 270 ppm in the fuel. Disinfection is essentially a time/temperature process and contact for 48–72 hours is desirable.

Biocides all have a capacitance limit. This means that 'x' millilitres of such a substance will kill 'y' organisms but once that reaction is complete it is no longer effective. For this reason it is desirable that tanks and systems be manually cleaned prior to the use of biocides. Cleaning is desirable also because no biocide dissolves growth, and dead organisms can cause as much blockage as live ones. Dead material may well be more readily freed from walls or floors of tanks and disinfection without prior cleaning may increase the chance of such problems.

Conclusions

Microbial contamination should be considered a possibility if slimes and sludges are found in kerosene fuels, hydraulic fluids, or lubricating oils. Black or dark patches on filter elements or on the walls of tanks should raise suspicion. Examination by a microbiologist is essential for proper diagnosis and his advice as to remedial measures may well be tempered by the implications of growths in a specific area or specific fluid.

Research to discover alternative biocides and investigations into the relationship of *Cladosporium resinae* with other bacteria, yeasts, and fungi in fuels continues, but, at present, a combination of 'good housekeeping' and the judicious use of one of the tested biocides must be advised where problems occur.

It is interesting to speculate that *Cladosporium resinae* probably appeared in diesel fuel many years ago but because such fuel was used in less sophisticated reciprocating diesel engines, it probably passed unnoticed until its presence in jet fuels caused concern. Diesel-fuelled gas turbine engines naturally give rise to more concern. Anyone who has been concerned with tank cleaning over a number of years will confirm that similar sludges were seen in diesel tanks many years ago!

The cotton sleeving of coalescer filter elements can, under certain circumstances, raise other microbial problems. If these are left in drained down coalescer vessels where the humidity remains high or stored under damp conditions, other organisms able to decay the cotton have been shown seriously to deteriorate the fabric (FIG. 3).

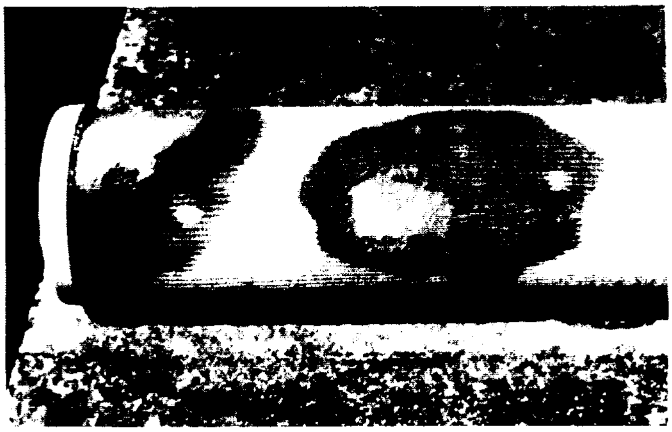

FIG. 3—THE COTTON SLEEVING OF A COALESCER FILTER ELEMENT SHOWING ATTACK BY *STACHYBOTRYS ATRA* WHEN THE ELEMENT HAS BEEN LEFT IN THE HIGH HUMIDITY OF A DRAINED DOWN COALESCER VESSEL

This factor and the possibility of organisms other than *Cladosporium resinae* causing problems in kerosene fraction oils, fuels, and hydraulic fluids, makes accurate microbial analysis important in every case.

Acknowledgement

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NOTE: *Cladosporium resinae* (Lindau) de Vries, the imperfect state of *Amorphotheca resinae* Parbery is now known as *Hormoconis resinae* (Lindau) Arx and de Vries. The former name is retained in the above article to avoid confusion, as many people are already familiar with the name Cladosporium.

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