HEINEMANN STUDIES – IN BIOLOGY

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Lecturer in Zoology, University of Glasgow

JOHN PAUL
F.R.S.E., M.B., Ch.B., M.R.C.P.Ed., M.C.Path.,
Titular Professor in the Department of Chemistry in the
University of Glasgow

Cell Biology

This is an up-to-date, and extremely readable, introduction to one of the most rapidly developing fields of scientific research. It gives a concise description of the structure and behaviour of cells, mainly from the point of view of molecular biology. Bacteria and other Protista are included, as well as the cells of multicellular animals and plants.

The book is aimed primarily at undergraduates with little knowledge of the subject, but it should also be useful for graduates interested in recent advances. It gives an up-to-date survey of all the important aspects of cell biology, and draws extensively on discoveries hitherto reported only in scientific journals.

M. J. WELLS, B.Sc.
Demonstrator in Zoology and Fellow of Churchill College,
Cambridge

Brain and Behaviour in Cephalopods

The book begins with an introduction to the structure of cephalopods, and an account of what they are able to detect about the world around them and how they can respond. This is followed by sections showing something of the complexity of their behaviour and describing the many experiments which have been made during the last few years.

The second half begins with a description of brain structure and what has been learned about it from electrical stimulation and brain lesions. Finally, there is an addendum discussing the ecological limitations of cephalopods imposed by the peculiarities of their structure.

*A very welcome summary, simply written and well produced, of a field of research in which British biologists have been particularly active.*—N. Tinbergen in The Guardian

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Professor G. Becker, Bundesanstalt für Materialprüfung, Berlin-Dahlem 45. Unter den Eichen 86/87, Germany.

Mr. D.G. Coursey, Department of Biochemistry, Nutrition and Food Science, University of Ghana, Legon, Accra, Ghana.

Dr. J. Garrido, Director, Departamento de Fermentaciones Industriales, Castello 25, Madrid, Spain.

Dr. H.J. Hueck, Central Laboratory TNO, Julianalaan 134, P.O.B.71, Delft, Holland.

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The Bulletin will act as a vehicle for the publication of work on all aspects of biodeterioration generally, i.e. the deterioration of materials of economic importance by microorganisms, insects, rodents etc. Contributions should be in the form of:

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J.G. Savory and R.S. Smith.

**OBSERVACIONES**
STATEMENT ON CO-OPERATIVE RESEARCH.

CO-OPERATIVE RESEARCH IN BIODETERIORATION

Eleanora H. Hueck - van der Plas with a foreword by Francoise Langlais.

FOREWORD

One of the main objectives of the Organisation for Economic Co-operation and Development (O.E.C.D.) is to promote the economic development of its Member countries. Economic growth depends very greatly on industrial development which in turn is very closely linked to research activities and the rate at which new discoveries are applied industrially. Scientific research is therefore an important element in relation to economic development and hence has a significant place in the context of O.E.C.D. activities.

To meet the research needs arising from economic expansion, the Committee for Scientific Research of the O.E.C.D. promotes the development of the scientific potential of its Member countries through international co-operation. This co-operation may be promoted in different ways, such as the confrontation of common problems and exchange of experience between policy makers and research workers, the co-ordination of national science programmes, sector reviews, the construction in common of highly expensive equipment, and finally the implementation of research programmes established in common, in which each laboratory undertakes to do part of the work and the results of which are shared by all. This last mode of work has been developed over the years by the O.E.C.D.'s Central Service for International Co-operation in Scientific Research which provides the administrative support for extensive research work, programmed at meetings organised by the O.E.C.D. and financed by and executed in the laboratories of Member countries interested in a given project.

2. Organisation for Economic Co-operation and Development, 2, Rue Andre - Pascal, Paris 16e, France.
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The topics which are most suited to co-operation of this kind are those which require extensive testing either in time, geographically, or both, and which one laboratory would find hard to undertake alone.

INTRODUCTION

Although the phenomena accompanying the biological deterioration of materials probably have been known as long as man has used materials, this problem has been studied scientifically only relatively recently. Methods for protecting materials against attack, such as tanning of fishing-gear and sails and impregnation of wood, however, have been widely used for quite a long time, but on a purely empirical basis.

The study of the biodeterioration of materials has been greatly stimulated by the world war II. Military materials had to be used under many different, often very deteriorative, climatic conditions, while the transport of materials to the fighting theatres was difficult, especially in the tropics. Accordingly, military laboratories started looking for ways and means to protect materials. At the same time it was realized that basic scientific knowledge of the attack by organisms is necessary for the development of adequate protective measures.

Until now this basic research has developed in many laboratories, mainly industrial and military and some civilian government laboratories. Although contacts among the different research workers have not been infrequent, the development has nevertheless been largely due to individual research workers.

A CO-OPERATIVE APPROACH

In 1962 the Committee for Scientific Research of the Organisation for Economic Co-operation and Development adopted a proposal of the Dutch delegation for initiating a co-operative study on the biodeterioration of materials. As a consequence, a group of experts has been established. This group is undertaking co-operative studies. As the O.E.C.D. is an economical organisation, all such projects are evaluated in this respect as far as possible. From the proposal just mentioned, some data can be quoted on the occurrence of biological deterioration and the damage it causes in money value.
Value in millions of U.S. $ of selected perishable materials.

Data refer to annual production or consumption drawn from statistics of the years 1959 and 1960.

<table>
<thead>
<tr>
<th>Materials</th>
<th>U.S. $ x 10^6</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>cotton</td>
<td>2800</td>
<td>raw material, consumption</td>
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<tr>
<td>jute</td>
<td>140</td>
<td>import of raw material</td>
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<tr>
<td>rayon</td>
<td>1300</td>
<td>production of filament yarn</td>
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<tr>
<td>paper</td>
<td>4200</td>
<td>woodpulp, production 1962</td>
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<tr>
<td>wood</td>
<td>6600</td>
<td>production</td>
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<tr>
<td>wool</td>
<td>2200</td>
<td>industrial consumption</td>
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<tr>
<td>leather</td>
<td>5000</td>
<td>shoes</td>
</tr>
<tr>
<td>mineral oils</td>
<td>10700</td>
<td>crude oil, refinery throughput</td>
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<tr>
<td>plastics</td>
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<td>consumption</td>
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<tr>
<td>synthetic rubbers</td>
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<td>consumption</td>
</tr>
<tr>
<td>natural rubber</td>
<td>800</td>
<td>consumption</td>
</tr>
<tr>
<td>paint</td>
<td>2900</td>
<td>consumption</td>
</tr>
<tr>
<td>electric instruments etc.</td>
<td>13700</td>
<td>sales</td>
</tr>
<tr>
<td>optical instruments</td>
<td>400</td>
<td>production</td>
</tr>
<tr>
<td>Total</td>
<td>53600</td>
<td></td>
</tr>
</tbody>
</table>

As a round figure U.S. $50.000.000.000 will be taken to represent the annual turn-over of materials threatened by biogenic deterioration. This leads to an estimate of annual loss due to this type of deterioration of about U.S. $1.000.000.000.

In this list only direct damage has been accounted for. In some cases indirect damage however may be very high as is the case in the deterioration of joints in pipelines, the occurrence of bacterial slime in paper mills, the formation of deposits in jet fuels, etc.

The above figures may be contested, however it should be realised that:

1. The figure given here is in good agreement with earlier estimates given above.

2. The estimate of the value of the annual turnover of perishable materials is apt to be too low because finished materials have a much higher value than the raw materials generally used as a basis for this list.

The subject of biogenic deterioration of materials appears to be particularly relevant to the economic objectives of the O.E.C.D. since its growth target cannot only be attained by increased industrial development, but also by ensuring that the phenomenal losses through deterioration as shown above are reduced.
The aim of the work of this group is also described in this proposal, viz:

1. To gather information on the occurrence of biological degradation of materials.

2. To gather knowledge on causative organisms.

3. To find and to standardize suitable test methods.

4. To evaluate methods of protection against the biological deterioration of materials.

5. To initiate studies in the prevention of the biological deterioration of materials.

6. To further the spread of knowledge of the phenomenon in question among interested industries.

The subjects studied have to be carefully selected to ensure optimum profit from the work done.

In most fields of research, two types of studies can be distinguished, viz. those unsuitable and suitable for a co-operative approach.

Subjects in the first group are characterized by the fact that basic knowledge is still scarce or lacking and that a considerable creative effort is needed for further progress. This creative work, in general, can best be performed by the individual research worker.

The subjects in the second group do not concern basic principles so much, but rather the comparison of a great mass of data. These data can be gathered more efficiently by a group of research workers than by one individual laboratory. This assertion can be illustrated with some examples, culled from the biodeterioration field.

1. Physiological studies on the way microorganisms attack materials, or on the mode of action of biocides, belong to the first group. Here, generally speaking, fundamental knowledge is still lacking to such an extent that it is not yet possible to formulate a theory that can be checked by planned experiments. Advance is made by a step by step procedure to be decided on at the bench.

2. Studies on the principles of biological tests applied in the field of biodeterioration, however, lend themselves easily to a co-operative approach because it consists essentially of a comparison and evaluation of already existing methods.

By gathering data from a great number of tests and by comparing these, the influence of various factors on the results of the test procedures can be studied. Through the co-operation of a number of laboratories a mass of
data can be acquired far exceeding that which any one laboratory could possibly produce.

THE PROGRAMME OF THE GROUP OF EXPERTS ON BIOLOGICAL DETERIORATION OF MATERIALS

Keeping the above restrictions in mind, the O.E.C.D. group of experts has drawn up a programme of studies to be carried out co-operatively. In order to do the work as efficiently as possible, a few special task groups have been set up; they are to work out different parts of the programme. At the moment there are the following task groups:

1. taxonomy
2. textiles
3. plastics
4. ecology
5. documentation

Taxonomy

The taxonomy group was the first to be established, as it was realized that, in all studies, organisms had to be used and that it is an absolute necessity to know exactly the organism with which the study is carried out and, furthermore, to be sure of the purity of the strains used. It has already undertaken various activities. In the first place this group is establishing a catalogue of microorganisms which play an active part in the biodeterioration of materials. In this catalogue, a number of characteristics of each strain will be summarized, such as: name of the strain, its history, with the numbers by which it had been designated in other collections, its biodegradative potency, etc. Furthermore physiological and any other important data will be provided in additional data sheets.

The organisms described in the catalogue will also be brought together in an O.E.C.D. collection of biodeteriorating microorganisms. Any research worker who wants to use these strains in his study can receive them from the O.E.C.D. collections. Strains from these collections will also be used for co-operative studies. This group is furthermore preparing a "check list" which summarizes the organisms which have been considered to be biodeteriorating. From this check list too, organisms can be chosen for further study.

It may be asked whether only microorganisms are considered as biodeteriogens since it is well known that insects may cause very important damage. This is certainly true, but in order not to overburden the group it has been decided to concentrate the research effort on those organisms which are least known. At the moment, therefore, insects are not being considered.
Textiles and Plastics

As has been stated above, care must be taken not to overburden the experts. For this reason it has been agreed to start with the study of textiles and plastics only, and, to defer the study of other materials. These materials were chosen because they are economically important, while hardly any co-operative work is done on them.

Since principles of testing, as has been discussed in paragraph 2, constitute a good subject for co-operative research, both groups are setting up a programme in this field.

The textile group is going to do an experiment on the activity of mixed cultures as compared with the same organisms in single pure cultures. The study is carried out with cotton treated with different fungicides. In this way, a comparison of strains can be made while the intra- and interlaboratory reproducibility of the test method is studied at the same time.

The plastics group is going to compare the activity of the groups of fungi, as prescribed in a few specifications, on pvc-sheets plasticized with different plasticizers. It is hoped that some insight into the usefulness of these fungal mixtures as test "organisms" will thus be gained. At the same time, the vulnerability of these pvc types will be studied. Finally, some methods for determining the degree of attack, viz. visual observation and determination of the change of the elasticity modulus, will be compared.

Both groups have sent out a questionnaire on the various aspects of biodeterioration of these materials and of special problems which should be studied in order to locate problems in these fields of biodeterioration. The answers received are being analyzed.

Ecology

One of the difficulties of the study of biodeterioration is that little or nothing is known about the distribution of biodeteriorating microorganisms, nor about the interactions between them and other microorganisms in their natural environment. The group on ecology intends to study these problems, and, as a start, is setting up a programme for comparing isolation methods, as these have to be the basis for analyzing populations. As a first approach, methods for isolating cellulolytic microorganisms from soil will be studied.

This group, too, has sent out a questionnaire, which is being worked out.
Documentation

In biodeterioration, lastly, as in all modern science, there exists a need for keeping up with the literature. Until recently, the Prevention of Deterioration Centre had met this need. Now that the publication of its Abstracts has been stopped, the group is trying to find a way out by gathering data for a title index which will be published regularly, possibly in this Bulletin.

At the same time, a survey is being made of products for protecting materials produced and sold in the different co-operating countries. This survey will contain data on active compounds, fields of application, trade names, as well as names and addresses of suppliers and producers. It is meant to give an exhaustive survey of compounds; without evaluating them, however.

CONCLUSIONS

In the foregoing it has been shown that not all research is suitable for a co-operative approach, but that specific parts of the research on biodeterioration certainly are. For these studies which, at the moment, mainly concern the principles of test methods, the bringing together of a great mass of data is essential, as, from comparison of these data, the influence of various factors in the testing procedure can be established.

It is certainly not the intention of the group to duplicate the work of other groups, such as I.S.O. and A.S.T.M., which develop test procedures. It only wants to analyze the procedures themselves with the ultimate aim of being able to advise on how to choose a method best fitting any specific requirement.

The activities of the taxonomy, ecology and documentation groups, too, can be considered as data-gathering. Ultimately, the group can be said to aim at being able to predict what type of biodeterioration will occur under certain circumstances, and to indicate measures to prevent this.
During recent years the Commonwealth Mycological Institute has, among its other activities, become increasingly concerned with consultation and advisory work in the field of biodeterioration. A wide experience of the taxonomy and biology of the fungi has enabled assistance to be given to numerous industrial and research establishments faced with problems involving fungal damage to materials or interference with industrial processes.

The C.M.I. was founded in 1920 to provide an information service and a fungus identification service for plant pathologists overseas. Originally named the Imperial Bureau of Mycology, and later the Imperial Mycological Institute (hence the internationally registered prefix "IMI" which is still attached to its herbarium specimens and culture serial numbers), the Institute assumed its present title in 1947. Since 1935 it has been a unit of the Commonwealth Agricultural Bureaux, a group of 13 bureaux and institutes financed jointly by the Commonwealth Governments to provide information services for the various branches of agricultural science.

Today the basic function of the C.M.I. remains unchanged. Among its publications the Review of Applied Mycology, first published in 1920, is still the only abstracting journal devoted to plant pathology. This journal also contains occasional abstracts of papers dealing with fungal damage to stored agricultural products and manufactured materials, and extensively covers the literature on agricultural fungicides. However, it does not attempt any comprehensive coverage of the literature on biodeterioration. In addition, the C.M.I.'s publications now include the Review of Medical and Veterinary Mycology, comprising abstracts from the world literature on fungus diseases of man and animals, the Index of Fungi, listing names of new genera and species published, the Bibliography of Systematic Mycology, Mycological Papers, mainly consisting of taxonomic studies, and Ainsworth & Bisby's Dictionary of the Fungi, an invaluable reference book for all workers concerned with mycological problems. The identification service of the C.M.I. is based on the herbarium which now contains more than 112,000 reference specimens of fungus material. Nearly 10,000 specimens or cultures are received annually for examination, this work being undertaken by 12 mycologists specialising in the taxonomy of various groups. A small bacteriology unit provides a similar service for plant pathogenic bacteria.
The culture collection of the C.M.I. functions both as a living extension of the herbarium, providing a reservoir of named cultures to assist the mycologists with their identification work, and also as a public culture collection. 5,000 strains of fungi of industrial, educational, plant pathological or taxonomic interest are currently maintained in culture, and about 3,000 cultures are supplied annually to laboratories throughout the world. The C.M.I. is also participating in the maintenance of mould cultures for the O.E.C.D. Biological Deterioration of Materials group.

The C.M.I.'s work on the biological deterioration of materials by fungi is based on the culture collection department. For a number of years one important aspect of the culture collection's work has been to act as the main supplier in the U.K. of mould cultures for use in mould resistance tests carried out to certain official specifications. This activity resulted in the Institute's specialised knowledge of fungi becoming more widely known to industry, and there was a corresponding increase in the numbers of requests for assistance in overcoming mould growth problems. This function soon became officially encouraged, and the industrial side of the Institute's work is now supported by a grant from the Ministry of Technology. Unfortunately present space and facilities do not permit very large scale investigations to be carried out and limit the amount of basic research which can be undertaken. However the C.M.I. currently deals with more than 200 enquiries per year relating to the biodeterioration of a wide variety of materials, ranging from requests for information to problems requiring detailed laboratory study. One important problem now under investigation, in conjunction with Liverpool University and the National Coal Board, is the rotting of coal mine conveyor belting. Attempts are being made, with the assistance of laboratory trials, to relate the extent of rotting to various conditions underground, and to determine to what extent strength loss of the nylon component of cotton/nylon mixture belts may be due to microbiological agents. Examples of other current or recent fields of interest are various aspects of the occurrence of Cladosporium resinae in kerosene aircraft fuel, the microbiology of cattle foodstuff during long term storage, an evaluation of fungus resistance tests on cork products, and the testing of the mould resistance of adhesives for plastic coating wall coverings.

The C.M.I.'s taxonomic facilities are available to industry and research establishments to assist with the identification of fungi associated with deterioration, and friendly co-operation has thus been established with government departments and industrial Research Associations, which are frequently engaged with the problems of particular materials. As a specialist taxonomic centre and as an advisory and consultative laboratory dealing with specialised problems, the C.M.I. forms a useful part of the network of laboratories in the U.K. engaged in biodeterioration research.
AN ADHESIVE TAPE TECHNIQUE FOR THE MICROSCOPICAL EXAMINATION OF SURFACES SUPPORTING, OR SUSPECTED OF SUPPORTING MOULD GROWTH.

A.O. Lloyd

The method is based on the use of adhesive cellulose tape, such as "Sellotape". It has been usefully employed in at least two laboratories, over a number of years, although the method was not published until January 1964, when it was shortly described by Hendey as a means of examining laboratory cultures of micro-fungi.

The writer has found the method extremely valuable in examining the surfaces of a great variety of materials, including textiles, timber and plastics, especially in those cases where drying out, or other causes, have rendered orthodox methods difficult. By the examination of "Sellotape" impressions of affected surfaces it is often possible to identify morphological characteristics which would be destroyed by attempted transference of the growth to a microscope slide by means of a needle or knife.

In the case of robust articles a short length of adhesive tape is pressed on to the surface and then carefully peeled off. For more delicate surfaces less pressure is used, and in the case of laboratory cultures on agar, the adhesive side of the tape is gently brought into contact with the fungus. A suitable part of the tape impression is selected and a half inch square cut off. This is mounted, sticky side up, in lactophenol/cotton blue stain mountant. A cover glass is quickly placed on top before the tape has time to curl. A semi-permanent slide can be made by merely sealing the edge of the cover-slip with nail varnish.

The mounted "Sellotape" impression may be examined by low or medium power objectives or even by oil immersion, and the method is useful in the preparation of slides for photography. The accompanying illustration, Figure 1, is a photo-micrograph of a "Sellotape" impression of mould fungi growing on woven paper fabric. Here it is quite evident that we have an Aspergillus species. The dimensions and characteristics of the vesicle, metulae and conidia give an indication of the possible species with which it conforms. There can be little doubt that the dark spores belong to a species of Stemphylium or a close relation of that organism.

Usually these microscopical observations can be supported with other evidence, such as colour or character of the growths, which may assist in giving positive identification. The growths can often be revived on nutrients, and, provided that these show the same morphological features as those observed on the tape impression, the organism may be further studied in culture.

The great advantage of this technique is that the fragile structures are firmly held in the same position which they occupied before transference to the tape. It is often possible to identify organisms which are no longer viable when attempts are made to revive them on nutrients.

Also, observation of the actual organisms responsible for spoilage is much more satisfactory than relying only on recognition based on the transfer of fragments to nutrients, in which quick growing contaminants may readily supplant the original growths. This is especially true when investigating dried-up, (and sometimes much-handled), specimens of textiles, paper and plastics, when the risk of error due to such a cause is exceedingly great.

REFERENCES


2. Lacto-phenol/cotton-blue

Lactic acid 20 g.
Phenol 20 g.
Glycerol 40 g.
Water 20 g.
Cotton Blue 0.05 g.
Fig. 1. Section of "Sellotape" impression of mould fungi on paper. (Magnification X 620 on print)
A RAPID METHOD FOR THE DETERMINATION OF THE ACTIVITY OF BACTERIOSTATICALLY TREATED FABRICS USING THE STANDARD WARBURG TECHNIQUE

J. van der Toorn, Dorothea M.M. Adema and H.J. Hueck

INTRODUCTION

A manometric technique measuring the respiration of fungi on textile materials, using a Barcroft differential manometer is described by Siu (1951). Darby (1958) has compared this method with the Warburg technique for assaying microbiological susceptibility and fungistatic activity of materials. Both methods gave good results. A modification of the method of Siu to study attack on plastic films is given by Burgess and Darby (1964).

For the testing of bacteriostatically finished textiles a similar technique may be envisaged. It would be of advantage if standard laboratory equipment, such as the Warburg apparatus, could be used for the purpose. It was thought that such a technique would allow a rapid evaluation of bacteriostatic properties.

For testing bacteriostatics as such, several manometric techniques with the standard Warburg apparatus have been published. Baker, Harrison and Miller (1941) and Ordal and Borg (1942) used resting cells while Sevag and Ross (1944), Wood, Ono and Bessey (1958) and Bornside, Merritt and Weil (1964) used growing cells.

The resting cell technique has been applied in our laboratory to bacteriostatically finished fabrics; results of this investigation are published in this note. Experiments with growing cells, which have also been carried out, will be published in the near future.

The principle of the test is rather simple. From the amount of oxygen consumed the inhibition of the respiration of resting bacteria on the material is calculated and compared with the respiration on blank material.

DESCRIPTION OF THE METHOD

The bacterial strains used are:

- **Staphylococcus aureus**, Rosenbach 1884; strain ATCC 6538
- **Escherichia coli** (Migula 1895) Castellani and Chalmers 1919; strain ATCC 11229.

The bacteria are grown in nutrient broth (Difco B3) for 24 hours. After this time a new culture is made in fresh nutrient broth. This culture again is incubated for 24 hours. The cells are then centrifuged, washed 3 times with phosphate buffer (0.05 M, pH 6.8) and resuspended in phosphate buffer containing 0.3% glucose and 0.1% tween-80. By centrifuging 1 ml of the resuspended culture in a graduated centrifuge tube, the cell concentration can be estimated. Care must be taken that the concentration of cells is not too high in order to allow for a good aeration. A practical concentration in some instances appeared to be such that in the Warburg vessel, the final suspension, which may range from 0.1 - 1.5 ml contains a total of 10^{13} bacteria. As a check on the activity of the bacteria used, it is taken that with the untreated samples the amount of oxygen consumed, must be at least 50-100 μl/hr.

For determining the antibacterial action of bacteriostatically treated fabrics small circular patterns of the fabric with two crossed central slits (as given in the figure) both treated and untreated are placed on the bottom of the standard Warburg vessels around the center-well. The center-well contains 0.2 ml 20% KOH solution. The amount of bacterial suspension for inoculating the samples is so small as to wet the fabric without leaving any free liquid. Depending on the material this will range from 0.1 - 1.5 ml. The amount of oxygen consumed by the bacteria is determined according to the normal Warburg constant volume procedure for 1.5 hours at 27°C with a shaking rate of 90 oscillations per minute (Umbreit, Burris and Stauffer, 1957). Readings have been carried out with intervals of 10 minutes. The experiments are carried out in duplicate. Each experiment is repeated once with another batch of bacteria.

The amount of oxygen consumed versus time gives a straight line for the untreated and the treated fabrics. The inhibition of respiration is calculated from the amount (x₄μl) consumed by the bacteria on the treated sample after 60 minutes in comparison with the amount (x₀μl) consumed by the bacteria on the untreated sample according to the formula:
Inhibition of respiration: \( \frac{x_2 - x_1}{x_0} \times 100\% \)

Because of the linear relationship found, the inhibition within the duration of the experiment may be taken to be independent of time. The results are calculated as multiples of ten. As in repeat-experiments rather large variations appeared to occur, the end results were evaluated in our laboratory arbitrarily as follows:

Inhibition: 80 - 100% - good - ++
40 - 70% - trace - +
0 - 30% - absent - -

AN EXAMPLE OF RESULTS

Cotton was bacteriostatically treated with an Ag-containing finish at concentrations ranging from 0.001 to 0.03% of the active substance.

The results of the test are summarized in table 1, including an investigation of the washfastness of the treatment.

DISCUSSION

The method described above apparently yields rather crude results, though consistent in repeat-experiments if the large variation in results is taken into account; its main advantage is the rapidity of the procedure. It appears, therefore, that the method is suitable for purposes of rapid screening. More detailed observations and a comparison with other methods will be published elsewhere.

LITERATURE


<table>
<thead>
<tr>
<th>Micro-organism</th>
<th>Treatment concentration (% Ag)</th>
<th>First series</th>
<th>Second Series</th>
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<tr>
<td></td>
<td></td>
<td>O₂ consumption after 60 min.</td>
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<tr>
<td></td>
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Microbial deterioration of a raw material before it has entered into the stream of commerce results in a direct loss to the producer, and in the jungle of commerce such deterioration results in claims between parties. These claims are frequently covered by insurance but the cost is ultimately borne by the final consumer as insurance and legal costs are taken into account when arriving at commodity prices. Frequently materials pass through many hands before reaching the final consumer and deterioration may occur at any stage of storage, transport and distribution, and manufacture. When deterioration has occurred claims for damages are made against one or more of the earlier parties involved in the commercial progress of the materials.

The microbiologist may be called in to examine materials showing deterioration with the view of establishing at which stage in the commercial history of the material the deterioration occurred, and so help to establish which party is responsible and thus fix the economic liability.

With perishable commodities it is necessary to have an understanding of all the factors involved in the normal commercial life of the particular product. It is necessary to be able to distinguish the particular micro-organisms associated with each stage in the processing and storage of these materials, the effects of changes in physical conditions on the sequence of organisms, and the changes that these organisms may produce in the product. Then by establishing the type of deterioration and the types of associated organisms it is possible to draw some conclusions as to the most probable stage in the life history that the deterioration commenced and what were the contributory factors.

With non-perishable commodities the situation is quite different. Although in some instances there may be specific organisms associated with a particular material, e.g. cellulose or wood decaying organisms, the general position is that the organisms developing are not governed by the product but rather by the physical environment, the two most important factors being the available moisture and temperature.

In this situation "indicator organisms" may give the clue as to the kind of conditions responsible for the onset of deterioration. It is often only possible to indicate that at some time the material was subjected to a certain humidity-temperature environment and it is necessary to look for other indications to establish at which stages these conditions prevailed. Examples of such indications are contamination with salt which might indicate contamination with sea water and hence onset of deterioration during ship transport or if a material is coated or dyed it might be possible to establish whether deterioration occurred before or after this stage. Also by establishing the degree of humidity, i.e. low or high, it may be possible to correlate this with climatic conditions in the particular places the material had been.

The vital part of any examination of deteriorated materials is to obtain a true picture of the spectrum of organisms present and to establish as far as possible which of the organisms produced the deterioration. The next stage is to reproduce the deterioration with these particular organisms under defined physical conditions. Unfortunately techniques for isolating the full spectrum of organisms are far from perfect and it is desirable to use as many approaches as possible. A sound appreciation of the principles of microbial ecology is a necessary prerequisite to any such investigation. There are still many gaps in our knowledge of the "physical constants" of different organisms and of the significance of particular successions of organisms. Because of this the microbiologist often can only express an opinion on the probable course of events leading to deterioration.

The microbiologist (or deterioration expert) will report his findings to one of the parties in a dispute and he may then be required to appear as an expert witness for this party in the course of legal proceedings. Only those aspects of his report considered by counsel to favour his case will be presented as evidence. It is then up to the other counsel by means of cross-examination to extract the "other half" of the truth. For the scientist this is not very satisfactory and many a claim has been lost or won simply because the right questions were not asked. There is much to be said for the system operating in some countries where expert advisors are appointed by the courts.

The other situation which is much more satisfying is when the two parties to a dispute request the scientist to act as an arbitrator. Then it is possible for him to examine all the evidence in relation to the technological background of the material and the normal commercial procedures and come to some conclusion as to the possible cause of deterioration and hence establish the economic liability.

There is usually a very different attitude between manufacturers and merchants. The manufacturer wishes to know the cause so that in addition to obtaining compensation where appropriate he can take measures to prevent the recurrence of such deterioration. The merchant on the other hand is
usually only interested in obtaining compensation. This attitude usually results in a very long time lag before suit is brought.

A very surprising feature is the number of claims that are filed without material ever having been examined by an expert and often the time lag may be one or more years and material is no longer available for examination. Even when such material is available it is not possible to be certain what it was like one or more years previously. Often there are only vague documents to study, which may bring joy to the legal parties but only frustration to the scientist.

An unknown factor in many legal disputes is "inherent vice". When deterioration has been shown to have occurred many a defendant hopes to establish that this was the result of inherent vice and so be relieved of any financial responsibility. Inherent vice defies definition but in the context of this discussion it could mean deterioration which is inherent to a particular product and is likely to occur during what is at present regarded as being satisfactory storage and processing. Obviously inherent vice is put to use by commodity speculators.

BITUMEN DEGRADATION UNDER NATURAL CONDITIONS:
PRELIMINARY STUDIES

Richard W. Traxler

For the past several years our laboratory has been concerned with the degradation of asphalts by bacteria (Phillips and Traxler, 1963; Traxler 1962). This work has been theoretical in nature with little emphasis on those aspects dealing with the applied or economic importances of bacteria on bituminous materials under natural conditions. The literature on microbial oxidation of pure aliphatic and aromatic hydrocarbons is extensive, but little information is available on the action of bacteria on the more complex materials of petroleum origin.

Stone, Fenske and White (1942) demonstrated that bacteria were capable of oxidizing all fractions of petroleum including asphalts, waxes and oils. They observed that light to medium molecular weight fractions from crude petroleum were more easily oxidized than higher molecular weight constituents. Harris, Kline, and Crumpton (1956) described the action of microorganisms at the soil - asphalt interface of roadmats, and Harris (1959) described the growth of bacteria on pipeline coatings of various composition. Burgess (1956) confirmed bacterial action on asphalts in his studies and suggested undersealing the oil mat to inhibit the oxidation of road oils.

1. Biodegradation Center, Microbiology Department, University of Southwestern Louisiana, Lafayette, Louisiana, U.S.A.
Kulman (1958) and Martin (1961) further confirmed the action of microorganisms on asphalt and bituminous products under natural conditions and, to a limited extent, under laboratory conditions. They used the soil burial technique or a modification thereof and assessed microbial action by visual inspection and various standard tests on the bituminous materials. Since most of their tests were performed on mixtures of bitumen with other materials it is most difficult to determine the microbial effect on the bitumen.

Our present investigations are aimed at determining the effect of natural conditions on the degradation of different bitumens. Laboratory experiments (Phillips and Traxler, 1963) have demonstrated a rapid degradation of asphalts under optimum conditions, and a difference in the specificity of attack. We want to determine if these same effects occur under natural conditions.

MATERIALS AND METHODS

Soil Burial Technique:

Eight bitumens from different sources have been used in the soil burial test. They are labeled A, C, E, F, FT, 1A, 3A, and 6A. Some physical characteristics of these materials are summarized in Table 1. Birch tongue blades were weighed to 1 mg then coated with melted bitumen and the excess allowed to drain off. Each bitumen coated blade was reweighed to determine the bitumen coat weight. The tongue blades with each bitumen were divided into three groups. Group I was buried in finely cultivated garden soil which was subject to normal rain-fall and temperature changes. Group II was buried in soil subject to the same temperature changes but protected from rain-fall. Group III consisted of the control group which was not given soil exposure. Three blades were removed from each plot at weekly intervals, gently rinsed in water to remove loose soil, air dried and reweighed to obtain the average weight change for the specimen. The temperature range during the course of this experiment was 14°F to 99°F with a daily average of 57°F.

Soil Percolation:

Two bitumens (1A and D) were chosen for percolation experiments. Two percolators were set up as shown in Figure 1. A volume of 1500 ml of sterile washed sand was placed in the column over a glass wool support. This was covered with 20 gms of fresh garden soil and the column charged with approximately 350 ml of mineral salts medium (Table 2). The test column was charged with 15 gms of bitumen dissolved in a minimum amount of benzene to allow pouring and air dried to remove the benzene prior to adding the soil inoculum. The control column did not receive the asphalt sample. Both columns were maintained at room temperature (20°C).

Dilution plate counts were made in triplicate from both columns at 0, 1, 2, 3 and 4 days in Trypticase Soy agar (BBL), incubated for two days at room temperature then counted. Counts were also made for each colony...
RESULTS AND DISCUSSION

The data on bitumen weight loss (Figures 2 and 3) is an indication of the differences in susceptibility of various bitumens to microbial action. This agrees with the laboratory experiments performed using pure cultures of a Bacillus isolated by enrichment culture methods (Traxler, 1962). It is apparent in the series shown in Figure 2 that Bitumen A is the most susceptible to weight loss in moist soil followed in order by Bitumens C, F, and E. All the bitumens except Bitumen A appear to be resistant to weight loss in dry soil, at least for the length of this experiment. Bitumen FT which is reported to be treated with a bactericidal material is apparently protected from weight loss even in moist soil. The three materials shown in Figure 3 are also subject to weight loss but none of them to the extent of Bitumen A in Figure 2. It is to be noted that Bitumens 1A and 3A even show some weight loss in the dry soil.

The data from the soil percolation experiments (Table 3) is not definitive but does indicate a trend. For example, the control column counts are fairly constant on all four determinations indicating a fairly stable total bacterial population in the absence of bitumen. The counts in the test columns gradually increase in the case of Bitumen 1A to a maximum population in four days. The total population in the test column with Bitumen D reaches a maximum at 2 days then declines. Also, it is noticed that Bitumen 1A supports a higher bacterial population than Bitumen D. This may be more apparent than real and must be verified by additional determination.

It is concluded from this information that in the natural environment as in the laboratory there is considerable variation in susceptibility of different bitumens to degradation. Also, it is evident that a moist environment is essential for rapid microbial action. Further tests must be run using longer exposure times before the dry soil condition can be adequately determined.

Since the action of the soil flora on bitumen is likely to be due to more than one type of bacterium it is believed that the soil perfusion experiments may be of great value in the elucidation of this interrelationship. This technique will provide information not only on total population effects but also on population sequences. By further testing we hope to be able to determine those bacterial types which exert the greatest effect on bitumens. Chemical analyses for byproducts may also be made from these columns.
REFERENCES


Table 1. Bitumen Characteristics

<table>
<thead>
<tr>
<th>Bitumen</th>
<th>Softening Point °F</th>
<th>Specific Gravity at 77°F</th>
<th>Penetration at 77°F/100g/5 sec</th>
</tr>
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<tbody>
<tr>
<td>A</td>
<td>195</td>
<td>1.015</td>
<td>48</td>
</tr>
<tr>
<td>C</td>
<td>214</td>
<td>1.06</td>
<td>3</td>
</tr>
<tr>
<td>E</td>
<td>167</td>
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<td>0</td>
</tr>
<tr>
<td>F</td>
<td>170</td>
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</tr>
<tr>
<td>FT</td>
<td>173</td>
<td>1.226</td>
<td>0</td>
</tr>
<tr>
<td>1A</td>
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<td>0.999</td>
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<tr>
<td>3A</td>
<td>111</td>
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<td>142</td>
</tr>
<tr>
<td>6A</td>
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Table 2. **Mineral Salts Medium**

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<tr>
<td>Na₂HPO₄</td>
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</tr>
<tr>
<td>KH₂PO₄</td>
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</tr>
<tr>
<td>NH₄NO₃</td>
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<td>FeCl₃</td>
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<tr>
<td>MnSO₄</td>
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</tr>
<tr>
<td>KI</td>
<td>0.001</td>
</tr>
<tr>
<td>CuSO₄</td>
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</table>

pH 6.8 - 7.0

Table 3. **Dilution plate counts from percolation columns**

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<th>Time of Sample (Days)</th>
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<tr>
<td>0</td>
<td>220,000</td>
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<td>1</td>
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<td>3</td>
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<td>Experiment 2</td>
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<td>0</td>
<td>28,900</td>
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<tr>
<td>2</td>
<td>706,000</td>
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</tr>
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<td>3</td>
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<td>4</td>
<td>878,000</td>
<td>17,300</td>
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</table>
Fig. 1. Percolation Column.
Figs. 2 & 3. Effect of moist and dry soil on bitumen weight loss. Birch tongue blades coated with bitumen of known weight.
AN INTERLABORATORY EXPERIMENT WITH THE SOIL BURIAL TEST

H.J. Hueck and J. van der Toorn

INTRODUCTION

In 1959 representatives of nine laboratories met under the aegis of an ad hoc committee, set up at the initiative of the Central Laboratory TNO, in order to discuss possibilities of standardization of the soil burial test. An interlaboratory test was agreed, aiming at evaluation of a suggestion made by Hueck (1960) in that mathematical means could be used that express the aggressivity of the experimental soil. Thus, this aggressivity could be determined by establishing a breakdown curve of untreated standardized cotton fabric.

The test calls for the determination of residual strength in successive periods of time. The residual strengths thus obtained are transformed according to the formula of the logistic curve:

\[ \log \frac{A - S}{S} = b \log \frac{t}{\tau} \]  

where

- \( A \) = initial strength of the test fabric (100%)
- \( S \) = residual strength of the test fabric at time \( t \)
- \( b = \tan \alpha \) = a constant determining slope of the curve
- \( \tau \) = half-life period = time at which residual strength = 50%,

in that case \( \log \frac{A - S}{S} = \log \frac{100 - 50}{50} = \log 1 = 0 \).

If \( \log \frac{A - S}{S} \) is plotted against \( \log t \), a straight line results (cf Hueck loc cit).

1. Central Laboratory TNO, Delft, The Netherlands
2. Sub Committee on Interlaboratory Experiments of the European Committee on Standardization of Biological Test methods for Rotproofness. This main committee was instituted by the 1954 Paris Botanical Congress, but has never been convened. The ad hoc committee only met once.
The results of the experiments have already been reported informally to the participants (v.d. Toorn and Hueck, 1963). Since the experiments will apparently not be resumed on the same basis, a summary of the main results is submitted below in order to prevent a possible loss of data, and duplication of effort. At the same time, a few clerical errors in the informal report are now corrected. Finally, suggestions for future research are given.

The co-operating laboratories were:

- Farbenfabriken Bayer A.G., Krefeld-Uerdingen, Germany.
- CIBA A.G., Basel, Switzerland.
- Eidgenössische Materialprüfungs- und Versuchs-Anstalt, St. Gallen, Switzerland.
- J.R. Geigy A.G., Basel, Switzerland.
- Farbwerke Hoechst A.G., Hoechst, Frankfurt, Germany.
- Centre d'Etudes du Bouchet, Le Bouchet, France.
- Central Laboratory TNO, Delft, The Netherlands.

EXPERIMENTAL SET UP.

The experiment was carried out with a standard cotton fabric of 250g/m² partly untreated and partly impregnated with 0.0%, 0.2%, 0.4%, and 0.8% Cu-naphthenate calculated as % Cu/dry weight of fabric. The treatment with 0.0% Cu was carried out in the same way as that for the other concentrations, but the solvent used did not contain copper. The strips of fabric were buried by each laboratory in its own soil at 28±1°C, the water content of the soil being 25±2% of the dry weight. For each treatment, five burial periods were observed with six strips each. Strength was determined with the laboratories' own dynamometers. Copper content of unburied strips was determined, after the test by the Central Laboratory TNO.

The aim of the experiments was to relate the results for the impregnated fabric to those for the untreated fabric in order to find out whether this relationship would lead to a better agreement among laboratories than may be expected when laboratories carry out absolute determinations using the soil burial test.

RESULTS.

In Table 1, the original strength of the test fabric as determined by each laboratory, and the copper content of the fabric used in each laboratory, are recorded. The copper content was determined by the Central Laboratory TNO. The order in which the laboratories are mentioned is arbitrary, and the same in all tables. The accuracy of the chemical determination was, on the average, 0.03%.

From Table 1 it will be seen that considerable variations exist in the individual determinations of tensile strength. Laboratory 1 was
consistently too low, and Laboratory 6 too high. This result was obtained notwithstanding a request to calibrate the dynamometers beforehand. The difference can be straightened out by using residual strength for the sake of comparison. It may serve, however, as a warning that not all dynamometers are what they should be.

The variation in Cu-content is less disturbing. Though some extremes exist, it is felt that they will not contribute substantially to the error in the present soil burial test, since this test is rather insensitive to increases in concentration over a certain level of Cu.

As will be shown, most laboratories could hardly distinguish between 0.5 and 1.0% Cu. It will be noted that actual concentrations of Cu were higher than those initially aimed at. These latter notations will be retained for indication purposes, however.

In Table 2, the results of strength determinations after burial are recorded. The essential constants, as far as they could be determined, are recorded in Table 3. It must be pointed out that the constants have been recalculated from curves constructed by applying the method of least squares, whereas in the informal report they had been drawn arbitrarily and, therefore, they may differ from those in the informal report. It will be noted that the experiment was not continued long enough to enable sufficient data to be obtained with Cu-impregnated fabric.

In general, constants were calculated when three data between 10 and 90% residual strength were available. Data obtained by extrapolation are bracketed.

DISCUSSION

It was found that formula (I) could be easily fitted to the data of the untreated and 0.0% series. Usually sufficient data in the more reliable range between 10 and 90% residual strength were available for the purpose. It is also clear that practically all the laboratories reported basically the same results for cotton untreated and containing 0.0% Cu. This means not only that these fabrics do not differ in this test, but also that the results obtained by each laboratory are consistent as such. Furthermore, the difference in constants between laboratories in these series, are not such that a "disturbing disagreement" results. For the impregnated series, however, results are rather erratic. Generally too few points in the desirable range were available for construction of a curve fitting the data tolerably well. In a number of cases, the data fit the formula well to excellently. Many times, however, the points were scattered so much that fitting of quite a different mathematical expression (as advanced by other authors) must be deemed possible too.

The evidence, therefore, was not conclusive. This being the case it was not possible to satisfy the original aim of the interlaboratory effort, viz. to find a correlation between results in the untreated and
impregnated series. Nevertheless, the conclusion may be drawn that future experiments in this field should aim at obtaining more accurate information in the range between 10% and 90% residual strength, both by using more than six replicates and a more accurate timing of harvesting.

The latter point may be achieved by all laboratories first carrying out a preliminary experiment from which the ideal harvesting periods can be agreed. Thereafter the whole experiment should be repeated with a convenient spacing of the incubation periods. Since the data from each laboratory were already found to be consistent as such this procedure should be possible. If a suitable mathematical expression is used for the breakdown (deterioration) curve all the laboratories need not harvest at the same time. It is only necessary to establish the essential constants, viz. slope and half-life period.

It will be clear from the above that future experiments must deal with either lower concentrations of Cu or with considerably extended periods of burial. As to the constants of the breakdown curve in the test under report, it may be intimated, though not with certainty because of the inconclusive evidence, that the half-life period appears to be more variable than the slope: this was found earlier (Hueck, 1960) whilst Kempton et al (1963), though not using a mathematical transformation, came to the same conclusion. They explain this as being due to biological breakdown beginning only after a certain low level of fungicide is reached, by other, presumably non-biological processes, which makes the slope of the biological breakdown process of untreated and impregnated fabrics intercomparable to a certain extent. This plausible explanation can be checked, if accurate breakdown studies are carried out, combining strength determinations with chemical analysis during the process. For a good understanding of the process it is rather important to know whether this hypothesis is exactly true, or only approximately so. In the latter case, other factors, e.g. diminished growth rates, be it that they occur only in a restricted range of concentrations of the toxic substance, must be considered, which complicates the description of the process. The mathematical implications of the occurrence of such factors will be discussed elsewhere.

In the experiment reported here it was not possible to calculate accurate mean values of the breakdown constants. On the basis of the maximum and minimum values found in Table 3, the following constants were arbitrarily chosen as representative. A slope tan $= 3.0$ was combined with half-life periods of $3.0$ days; $26$ days; $45$ days and $60$ days for, respectively, untreated; $0.2\%$; $0.4\%$ and $0.8\%$ fabric. The hypothetical residual strength values obtained from these curves are represented in the bottom line of Table 2 to show the usefulness of such a mathematical procedure for comparative purposes.

LITERATURE


Table 1. Initial strength and actual Cu-content of test fabrics in several laboratories.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Initial strength in kg/cm of test fabric</th>
<th>Cu-content in % (dry-weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>untreated 0.0% 0.2% 0.4% 0.8%</td>
<td>0.2% 0.4% 0.8%</td>
</tr>
<tr>
<td>1</td>
<td>43.7 39.8 39.7 42.9 47.1</td>
<td>0.28 0.56 1.06</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.33 0.48 1.01</td>
</tr>
<tr>
<td>2</td>
<td>53.1 54.3 58.6 58.3 60.0</td>
<td>0.29 0.51 0.91</td>
</tr>
<tr>
<td>3</td>
<td>57.3 57.6 56.9 58.5 60.9</td>
<td>0.33 0.53 0.97</td>
</tr>
<tr>
<td>4</td>
<td>54.8 56.0 53.7 57.7 58.7</td>
<td>0.31 0.51 0.91</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.32 0.53 0.99</td>
</tr>
<tr>
<td>5</td>
<td>53.6 51.9 56.7 56.3 58.5</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>69.1 68.3 68.6 72.3 74.8</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>54.5 55.1 52.5 55.1 60.8</td>
<td>0.26 0.49 1.04</td>
</tr>
<tr>
<td>8a (own dynamometer)</td>
<td>56.3 53.9 56.1 61.0 63.1</td>
<td>0.37 0.60 1.26</td>
</tr>
<tr>
<td>8b (dynamometer Lab 9)</td>
<td>52.9 50.7 56.1 56.5 60.9</td>
<td></td>
</tr>
<tr>
<td>9a (first series)</td>
<td>53.0 49.2 54.8 55.0 61.0</td>
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<td>9b (second series)</td>
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</tr>
<tr>
<td>Mean</td>
<td>54.6 53.3 55.4 57.6 60.9</td>
<td>0.31 0.53 1.01</td>
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Table 2: Residual strength of cotton fabric, impregnated with different concentrations of Cu-naphthenate, after different incubation periods in a number of laboratories.

<table>
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<tr>
<th>Laboratory</th>
<th>Impregnation (%Cu)</th>
<th>Incubation period in days.</th>
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<tr>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>untreated</td>
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</tr>
<tr>
<td></td>
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<td>100</td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>0.4</td>
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</tr>
<tr>
<td></td>
<td>0.8</td>
<td>100</td>
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<tr>
<td>2</td>
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<tr>
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Table 2 continued

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<th>Incubation period in days</th>
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</tr>
<tr>
<td>0.2</td>
<td>100 98 105 105 100 84</td>
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</tr>
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<td>0.4</td>
<td>100 97 105 105 100 83</td>
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<tr>
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<tr>
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<td>100 59 16 8 0 0</td>
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<tr>
<td>0.2</td>
<td>100 93 91 96 72 43</td>
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</tr>
<tr>
<td>0.4</td>
<td>100 105 95 58 25 8</td>
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</tr>
<tr>
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<td>100 101 101 85 39 34</td>
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<td>6</td>
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Table 2 continued
Table 2 continued

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<tr>
<td>deterioration</td>
<td>0.2</td>
<td>100</td>
</tr>
<tr>
<td>curve</td>
<td>0.4</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>0.8</td>
<td>100</td>
</tr>
</tbody>
</table>
Table 3. Half-life period and slope of deterioration curves from Table 2.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>( \tau = \text{half-life period (days)} )</th>
<th>( \tan \alpha ) = slope</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>untreated 0.0 0.2 0.4 0.8</td>
<td>untreated 0.0 0.2 0.4 0.8</td>
</tr>
<tr>
<td>1</td>
<td>2.5 2.8 - - -</td>
<td>3.4 3.8 - - -</td>
</tr>
<tr>
<td>2</td>
<td>2.8 3.1 - - -</td>
<td>4.0 3.8 - - -</td>
</tr>
<tr>
<td>3</td>
<td>2.2 2.1 (35) 35 25</td>
<td>2.2 2.1 (3.0) 2.0 1.9</td>
</tr>
<tr>
<td>4</td>
<td>3.1 3.0 - - -</td>
<td>3.8 3.4 - - -</td>
</tr>
<tr>
<td>5</td>
<td>2.3 2.3 19 26 33</td>
<td>3.4 2.8 5.3 5.4 4.9</td>
</tr>
<tr>
<td>6</td>
<td>2.2 2.0 (80) -</td>
<td>1.8 1.5 (1.2) -</td>
</tr>
<tr>
<td>7</td>
<td>2.7 3.5 12 (15) 15</td>
<td>2.7 3.9 3.9 (5.7) 2.6</td>
</tr>
<tr>
<td>8a first series</td>
<td>3.8 3.0 (26) - -</td>
<td>2.2 1.6 (3.4) -</td>
</tr>
<tr>
<td>8b second series</td>
<td>4.6 2.3 (25) (76) (62)</td>
<td>3.5 3.8 (1.8) (1.2) (1.2)</td>
</tr>
<tr>
<td>9a old soil</td>
<td>5.0 5.6 - 44 51</td>
<td>3.0 6.2 - 2.6 2.6</td>
</tr>
<tr>
<td>9b new soil</td>
<td>2.3 2.3 (34) (81) (93)</td>
<td>2.9 2.7 (2.4) (0.9) (1.1)</td>
</tr>
<tr>
<td>Mean</td>
<td>3.1 3.0</td>
<td>3.0 3.2</td>
</tr>
<tr>
<td>Minimum value found</td>
<td>2.2 2.0 - 12 (15) 15</td>
<td>1.8 1.5 (1.8) (0.9) (1.2)</td>
</tr>
<tr>
<td>Maximum value found</td>
<td>5.0 5.6 (35) (81) (93)</td>
<td>4.0 6.2 5.3 (5.7) 4.9</td>
</tr>
</tbody>
</table>
EXTRACTION OF AFLATOXIN FROM GROUNDNUT MEAL WITH ACETONE-HEXANE-WATER AZEOTROPE

L.A. Goldblatt and J.A. Robertson Jr.¹

Groundnuts may become infected with strains of the common mold Aspergillus flavus that produce a group of highly toxic substances known collectively as aflatoxin, (Spensley, 1963). Contaminated groundnut meal has in the past caused the death of large numbers of farm animals. This is of concern to the animal feed industry but it is also important in connection with international protein-food efforts as groundnut meal serves as the protein concentrate for many proposed diets in protein deficient areas. It is essential that aflatoxin, if it is present, be removed if the meal is to be used for feed or food; but the customary methods of processing groundnuts, mechanical expression or extraction with hydrocarbon solvents, leave the aflatoxin in the residual meal or cake.

Aflatoxin is soluble in polar solvents such as methanol. Indeed this solubility in methanol forms the basis for a quantitative method of assay for aflatoxin, (Armbrecht et al. 1963). However, methanol is a relatively poor solvent for lipids and extracts a large proportion of nonlipid material in addition to aflatoxin. In this laboratory an azeotrope of acetone, hexane and water has been under investigation as a solvent for removal of lipids and gossypol from cottonseed for several years, (King et al. 1961). There is evidence that this is a practical solvent which can be recovered and reused. We have found that this azeotrope is an excellent extractant for aflatoxin from groundnut meal with the added advantage that it does not extract as much of the nonlipid constituents as does methanol. The azotrope has been reported to be composed of 42.1% acetone, 56.5% hexane and 1.4% water (by volume), (King and Frampton, 1961). It is readily prepared by distilling a mixture of acetone, commercial hexane (Skellysolve B), and water, 44:55:4 (water in excess), and collecting the heart cut which distills at 47.5-48°C. A small (2 to 3%) lower layer relatively richer in water and acetone settles out of the azetropete on standing at room temperature, so it should be thoroughly mixed before use for extraction.

A commercial groundnut meal extracted with methanol and assayed for aflatoxin according to the procedure of Armbrecht, et al., was estimated to contain 3 ppm of aflatoxin. The estimate was based on comparison of intensity of fluorescence, after development on a chromatoplate coated with Silica Gel G, with the fluorescence produced

¹ Oilseed Crops Laboratory
Southern Utilization Research and Development Division, Agricultural Research Service, U.S. Department of Agriculture, New Orleans, Louisiana, U.S.A.
by a standard reference sample of partially purified aflatoxin estimated to contain 25% aflatoxin B and 25% aflatoxin G. A parallel assay performed in the same way but with the substitution of the acetone-hexane-water azeotrope for methanol also afforded a value of 3 ppm. However, the crude primary extract obtained on extraction of the groundnut meal with the azeotrope was much less contaminated with extraneous material than was the primary extract obtained with methanol. The residue obtained on evaporation of the methanolic extract of 100 grams of the commercial groundnut meal weighed 11.5 g. whereas that obtained after evaporation of the extract obtained with azeotrope weighed only 3.1 g. Accordingly, the subsequent purification of the extract required for assay for aflatoxin by thin layer chromatography is much simpler if the azeotrope is used as the extractant.

The azeotrope would seem to be the solvent of choice for the extraction of aflatoxin from contaminated groundnut meals. The aflatoxin which is removed quite readily is accompanied by only about 3% of other extractables from the groundnut meal whereas methanol removes about 11% in addition to the aflatoxins. The azeotrope has been proposed for commercial use in the extraction of cottonseed, (King and Frampton, 1961), and it may be that the azeotrope would be the solvent of choice in producing aflatoxin-free products.

REFERENCES


INTRODUCTION

One of the problems to be solved by the worker in the field of the biodeterioration of materials, is the evaluation of the microbial degradation.

As a criterion for attack of cellulosic materials the determination of tensile strength, weight loss, absorption of alkali, fluidity, microscopic appearance etc. are valuable. In general in the biodeterioration of these materials, processes of degradation of the polymer are involved. The attack of plastics is fundamentally different, however, as the basic polymer remains unattacked. In this case the degradation is mainly limited, as far as is known, to the added compounds like plasticizers, stabilizers, fillers etc., or to impurities in the raw materials.

Properties of plastics, which might be used as a measure for microbial attack are tensile strength, strain at break, elasticity modulus, stiffness, electrical conductivity, etc.

For plasticized PVC, the most interesting type of plastic in this respect, the change of stiffness or elasticity seems to be an appropriate criterion for attack.

The properties mentioned above can be determined by a number of methods, namely:

a. the self weighted cantilever method as described in Federal Standard Stock Catalog CCC-T-1916 method 5204;

b. the cantilever bending method as described in Federal Standard Stock Catalog CCC-T-1916 method 5206;

c. the torsional test according to ASTM Designation D 1043-51;

d. the 1% or 100% modulus from the stress-strain diagram as recorded by the dynamometer;

e. the vibrating-reed method as described in this paper.

In the following special attention shall be given to the last mentioned method. After a short review and discussion of the principle of the method, an apparatus for measurement of the resonance frequency is described. Finally this technique is compared with tensile tests, as illustrated by some experimental results.


2. Northern Regional Technical Institute TNO, Groningen, The Netherlands
Nolle (1948) was among the first to employ the vibrating-reed method in the present form for the study of the elastic properties of rubber-like materials. More recently the method has been used by Weyland (1961), especially for the determination of the dynamic elasticity modulus of fibres.

A modification of this method is given by Joshi (1964). Karas and Warburton (1962) applied the principle to rubber-reinforced polystyrenes and Gibbs and Theberge (1963) to ceramic materials.

A vibrating-reed apparatus was developed by one of the authors (H.A. Waterman) provided with a stroboscope and a measuring microscope for the measurement of the dynamic elasticity modulus and internal friction of plastic films. The apparatus to be described here is a simplified version of the original one, its capability being limited to comparative measurements of elasticity moduli.

PRINCIPLE OF THE METHOD

The principle of the method consists of the determination of the frequency corresponding with the fundamental or one of the overtones of the sample. The dynamic elasticity modulus of the sample can be calculated from the formula:

$$E = \frac{12v^2 D l^4}{cd^2} \quad \text{......... (I)}$$

$E$ = elasticity modulus in dyne/cm$^2$
$v$ = resonance frequency in Hz
$d$ = thickness of strip in cm
$l$ = free length of strip in cm
$D$ = density in g/cm$^3$
$c$ = constant, depending on the tone

The value of $c$ is 0.3136 for the fundamental, 12.32 for the first overtone and 96.43 for the second overtone.

DESCRIPTION OF THE APPARATUS

The apparatus consisted of two main parts viz. the variable frequency oscillator and the electro-magnetic driver.

The variable frequency oscillator

The variable frequency oscillator was a commercial apparatus$^3$, modified in some details upon our request. The oscillator works on the resistance-capacity principle. The maximum output was 25 volts, the total output frequency covered a range from 3 - 10,000 Hz.

3. RC-Oscillator Type 31A delivered by Laboratory for Electronics Pekel N.V. 1, Alblasstraat, ROTTERDAM, The Netherlands.
The frequency-range engraved in the dial consisted of range A from 3 - 10 Hz and range B from 10 - 35 Hz. By means of the frequency-range switch these ranges can be multiplied by 1, 10, 100 or 1000. The amplitude of the output signal could be varied continuously. The frequency calibration accuracy was given to be ±1.5%. In our experience already the frequency-range of 0-500 Hz sufficed for the materials tested in our laboratory.

The electro-magnetic driver

For the electro-magnetic driver a professional gramophone cutting head can be used. At the time we built this apparatus these cutting heads were not readily available, so we decided to build one ourselves.

The electro-magnetic driver is fed with the output signal of the oscillator. A schematic drawing (front view) of the driving mechanism is given in Figure 1. It consists of a permanent magnet (a) with soft iron polar pieces as shown in the figure. A coil (c) of thin copper windings is attached between the polar pieces. The ends of the coil are connected with the output of the oscillator.

A small pointed anchor (d) in the centre of the coil, supported by a film of rubber or plastic, is tightened at the top between two pieces of soft rubber (b). The anchor, when magnetized by the alternating current in the coil, tends to turn itself into the direction of the permanent magnetic field. This movement is suppressed, however, by the rubber buffers causing the anchor to vibrate with a small amplitude. The anchor can rotate at a knife-edge, fitted into an equal shaped groove in the plate supporting the coil.

A small aluminium clamp (e), which holds the end of the sample (f), is terminated in a shank which fits into a hole of the anchor. The clamp and its shank must have very small mass, in order to place the mechanical resonance of the loaded cutter at the highest possible frequency, as it is impossible to make satisfactory experimental observations at frequencies very far above the cutter resonance.

The whole is mounted on a metal frame, provided with an on-off switch for easy changing of the sample (see figure 2).

PROCEDURE

The measurements of the resonance frequency have been carried out in a conditioned room at constant temperature and relative humidity. Especially variations in temperature appeared to have a pronounced influence on the measurements.

Strips of the material to be tested of 10 x 100 mm were cut to the desired length after the microbiological tests, because - as will be shown later - the dimensions of plasticized PVC decrease when the plasticizer is consumed by micro-organisms. The strips are conditioned in the above mentioned room for at least 24 hours.
The best suited length of the strips to be measured depends on the type of material under investigation. In our case it was taken as 23 mm. The strips were attached to the clamp of the electro-magnetic driver with a small screw, 3 mm being needed for the attachment. The driver is connected with the output plugs of the oscillator (see figure 2). The frequency of the oscillator is gradually increased till the strip begins to resonate. The maximum amplitude of the resonance is established visually.

Because the output signal of the oscillator is not exactly sinusoidal the strip may resonate at the keynote \( xH \) when the oscillator dial reads \( \frac{1}{2} xH \), though with a much smaller amplitude. This difficulty can be overcome by first determining the frequency of the supposed fundamental \( xH \), and then the frequency of the first overtone, which has to be about 6.3 times the frequency of the fundamental. In practice these complications are rather easily recognized, however.

The dynamic elasticity modulus can be calculated from the resonance frequency, the specific weight and the dimensions of the sample according to formula (I). It must be stressed, however, that the apparatus described in this paper is not meant in the first place for the absolute measurement of the elasticity modulus, but is very useful for comparative measurements of changes in elasticity. When only moderate changes are involved the relative value of the elasticity modulus can easily be calculated with the formula:

\[
E_{rr} = \frac{E_{1}}{E_{0}} = \frac{\overline{v}^2}{v_{0}^2} \quad \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots (II)
\]

in which \( \overline{v}^2 \) can be approximated by \( \overline{v}^2 = S^2 \) (\( S = \) standard deviation).

In this case the changes of specific weight and dimensions are neglected. For a more severe attack, a correction for the dimensions and the density of the sample should be introduced. This can be achieved by measuring the weight of the sample before and after the biological test, and by calculating the mean density, assuming that the weight loss is caused only by a loss of plasticizer, and that the density of polymer and plasticizer are known.

The same applies for the thickness of the sample. From these observations the relative elasticity modulus can be calculated according to the formula:

\[
E_{rr} = \frac{E_{1}}{E_{0}} = \frac{\overline{2} \quad D_{1} \quad d_{1}^2}{\overline{2} \quad D_{0} \quad d_{0}^2} \quad \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots (III)
\]
It must be pointed out that several corrections of the quantities thus found are possible. The method is, however, still in a developmental phase and has not yet reached its ultimate form. Our purpose with this preliminary publication is only to show the feasibility of the approach and to enable other workers in the field to gain experience with it.

RESULTS OF SOME EXPERIMENTS WITH PVC PLASTICIZED WITH DI-HEXYLADIPATE

An experiment was carried out to compare the tensile tests, and the vibrating-reed method as a means of evaluating microbial attack of plastics. Strips of 1 x 10 cm were prepared from plasticized PVC consisting of 30% di-hexyladipate and 70% PVC (Solvic 122). These samples were randomly distributed into 3 groups. The first group was kept in the laboratory as a control, the second and third group were buried in soil during respectively 2 and 4 weeks according to method VITNO BIO Al. The moisture content of the soil was 29.3% at the beginning and 17.3% at the end of the experiment.

After the soil burial test, the samples were rinsed with tap water, dried and divided into two sub groups, one of which was used for the determination of the tensile strength, the other for the determination of the resonance frequency.

The tensile tests were carried out with 10 replicates, the results of which are given in Table 1, the resonance frequency measurements with 30 replicates, the results of which are given in Table 2.

As can be seen from Table 1, the tensile strength and the 1% modulus increase, and the strain at break decreases during the soil burial test.

The relative elasticity modulus \( \varepsilon_{rt} \), calculated as the ratio of the 1% modulus after and before the soil burial test, increased 21.4 and 27.4 times after respectively 2 & 4 weeks soil burial. The difference between the 2nd and 4th week are only small, but according to the method of Wilcoxon statistically significant with a level of significance of \( \alpha = 0.025 \) for the tensile strength, and \( \alpha < 0.005 \) for the strain at break and the 1% modulus.

Table 2 shows an increase of the resonance frequency during the soil burial test, with a corresponding uncorrected \( \varepsilon_{rr} \) value of 16.5 and 17.8 after respectively 2 & 4 weeks incubation. In this case the difference in the resonance frequency between the two periods are not statistically significant. The values of \( \varepsilon_{rr} \) corrected for changes in density and thickness are 21.0 and 22.4 respectively.
Table 1. Tensile strength, strain at break and modulus at 1% strain of plasticized PVC (30% di-hexyl adipate + 70% PVC) before and after the soil burial test.

<table>
<thead>
<tr>
<th>Incubation</th>
<th>Tensile strength</th>
<th>Strain at break</th>
<th>1% modulus</th>
<th>Standard deviation</th>
<th>E&lt;sub&gt;Tr&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>kg/cm&lt;sup&gt;2&lt;/sup&gt;</td>
<td>%</td>
<td>kg/cm&lt;sup&gt;2&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>83.7</td>
<td>61.6</td>
<td>4.0</td>
<td>0.5</td>
<td>1.0</td>
</tr>
<tr>
<td>2 weeks</td>
<td>241.0</td>
<td>10.4</td>
<td>85.6</td>
<td>17.8</td>
<td>21.4</td>
</tr>
<tr>
<td>4 weeks</td>
<td>294.0</td>
<td>5.0</td>
<td>109.4</td>
<td>11.7</td>
<td>27.4</td>
</tr>
</tbody>
</table>

Speed of moving clamp: 5 cm/min. Distance between the jaws 5 cm (carried out with Instron Dynamometer). Temperature 21°C.

Table 2. Resonance frequency, density, thickness and relative elasticity modulus of plasticized PVC, temperature 23°C.

<table>
<thead>
<tr>
<th>Incubation</th>
<th>Resonance frequency</th>
<th>Standard E&lt;sub&gt;Tr&lt;/sub&gt;</th>
<th>Un- corrected loss</th>
<th>Weight density</th>
<th>Thickness</th>
<th>Corrected E&lt;sub&gt;Tr&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hz</td>
<td>deviation</td>
<td>%</td>
<td>g/cm&lt;sup&gt;3&lt;/sup&gt;</td>
<td>cm</td>
<td>corrected</td>
</tr>
<tr>
<td>control</td>
<td>30</td>
<td>3</td>
<td>1.0</td>
<td>1.26</td>
<td>0.022</td>
<td>1.0</td>
</tr>
<tr>
<td>2 weeks</td>
<td>122</td>
<td>12</td>
<td>16.5</td>
<td>14.8</td>
<td>0.020</td>
<td>21.0</td>
</tr>
<tr>
<td>4 weeks</td>
<td>126</td>
<td>18</td>
<td>17.8</td>
<td>17.4</td>
<td>0.020</td>
<td>22.4</td>
</tr>
</tbody>
</table>

So the E<sub>Tr</sub>-values obtained according to both methods appear to agree very well, especially when the use of different principles is taken into account. For the purpose of evaluating the attack of plasticized PVC both methods seem to have their own merits.

One of the advantages of the vibrating-reed method is that it can also be applied to composite materials like coated fabrics (e.g. nylon fabric coated with plasticized PVC). The tensile properties of such fabrics are principally determined by the fabric, and not by the coating.

(4), density of PVC = 1.40. Density of di-hexyl adipate = 0.933
Changes in stiffness of the coating will bring about changes in the resonance frequency, but no change in tensile strength.

Other advantages are the dimensions of the samples to be used, the relatively low cost of the apparatus and its modest dimensions allowing it to be worked with on the laboratory bench.

REFERENCES


Fig. 1. Schematic drawing of the electromagnetic driver.

Fig. 2. Apparatus for the determination of the resonance frequency.
DESTRUCTIVE TERMITES

W.V. Harris

Insects of the order Isoptera, commonly known as termites, are active agents of biodeterioration throughout the warmer regions of the world. Well known for the damage they do to structural timbers, termites also cause considerable losses through their attacks on furniture, fabrics, books, merchandise in storage, and to a variety of plastics used for coating electric cables, in packaging and for insulation of buildings. Two characteristics of termite behaviour are (1) intensity of attack which can result from the social organisation of these insects allowing thousands of workers from a single community to be concentrated upon one food source; and (2) an innate "curiosity" in foraging worker termites which drives them to bore exploratory tunnels through materials of no nutritional value.

Termite damage to structural timbers is not, as a rule, noticed for a considerable time, usually when something collapses. Damage to merchandise etc. is observed much earlier because inspection is easier, injury tends to be more superficial and at the same time out of proportion to the actual amount of material removed. "Tropicalising" of furniture, television sets, apparatus and the like, and the provision of termite-proof packaging for goods in transit within the Tropics are matters worthy of the attention of exporters.

Two aspects of the work of the Termite Research Unit (Ministry of Overseas Development) directly concerned with biodeterioration are the identification of termite damage, and the preparation of reference specimens of damage for the information of manufacturers and exporters in the United Kingdom. Laboratory colonies of termites have been maintained for the past eleven years, and the two species found most useful for the rapid demonstration of attack on various materials are Reticulitermes lucifugus from southern Europe and R. hesperus from North America.

Goods imported into the United Kingdom from tropical countries exhibit damage that falls into two distinct groups according to whether the cause has been a dry-wood or a subterranean termite. Timber from South America, Parana pine and balsa, has arrived with recently established breeding pairs of the dry-wood termite Cryptotermes brevis in galleries just below the surface. Such infestation no doubt took place while the parcels of wood were stored at coastal ports awaiting shipment during the swarming season. Well developed colonies of this termite have been found in furniture from the West Indies, while related species of the genus have been found similarly from West Africa and south-east Asia. Dry-wood termite infestation is

indicated by the presence of seed-like frass, and galleries excavated broadly within the wood, avoiding the denser medullary rays. Under suitable conditions of temperature and humidity dry-wood termites continue to live within woodwork once it has become infested.

Damage by subterranean termites ceases when goods are moved and contact with the nest is broken. Live termites are not, as a rule, to be found after shipment though careful search may produce fragments of dead termites sufficient for identification purposes. Earth or carton material is usually associated with subterranean termite damage as it is the usual practice for these insects to use such material to maintain stability within their excavations or to provide covered ways through which they can approach the food supply in safety. In other cases the paths of the worker termites are indicated by a spattering of brown faecal spots, probably a trail laying technique (Fig.3). Bales of unbleached cotton cloth from India have been found with subterranean termite damage, the earth lined excavations being typical of the genus Odontotermes (Fig.2). A diagnostic character of subterranean termite feeding on wood and hardboard is the minutely furry appearance of the damage. The worker termite gets a grip on one or more fibres with the points of its mandibles and levers upwards until the fibres snap. The piece of fibre is then detached with a side-to-side sawing movement leaving the rough ends behind.

Merchandise exported to the Tropics may be exposed to termite attack before reaching its final destination, particularly in warehouses at ports. The foraging termites are particularly interested in wood, cardboard, paper and fabrics of rayon and vegetable fibres, and remove them en masse. Exploratory tunnels are made into non-edible materials such as plastics and the softer metals, the amount removed by the termites being governed by the softness of the material. A small amount of termite damage may lead to disproportionate losses in value, as for example when packing materials and labels are eaten from bottles and jars. A subterranean termite, Microcerotermes diversus is active at Aden and ports in the Persian Gulf, where, among other things, damage has been done to boxes of pharmaceuticals, cigarettes and mattresses in storage. (Fig.1 & 3). A mobile crane which had been stored for some time at an East African port was found on reaching its destination to have had the insulation removed from most of its electric wiring by subterranean termites.

Cable manufacturers have long been aware of the possibilities of termite damage to coatings of synthetic rubber, plastics and lead in many parts of the world (Colwill, 1964). If wrappings of jute and cotton, unprotected by insecticides, are encountered the damage is intensified, as seen in an example of Macrotermes attack on neoprene coated signals cable in Rhodesia some years ago (Fig.6). The development of plastic water-pipes and the use of plastic sheeting for lining temporary water tanks; protecting concrete work etc. offer further opportunities for termite damage. Expanded plastics used for insulation of buildings offer little barrier to termite movement. (Fig.5).
In different parts of the world different genera of termites are responsible for damage to materials. Apart from the genera already mentioned in Africa, India and the Middle East, there is a particularly destructive genus, Coptotermes, which is the main agent in south-east Asia. Coptotermes damage has a number of distinctive characters, one of which is the mottled colouring of the carton material left around the damage. Two examples of Coptotermes damage are shown (Fig.4) from Singapore. Further examples of damage to merchandise are given by Becker (1962) and Weidner (1962).

Small laboratory colonies of the two species of Reticulitermes already mentioned have been used to demonstrate the varying susceptibilities of a wide variety of plastics, in the form of film, sheet and expanded foams. Some of this has been carried out at the request of manufacturers, and the remainder in order to provide reference specimens. Testing for resistance in materials treated with repellents has not been carried out here, use being made of the work of Gay and Wetherly (1962). The ability of termites to penetrate aluminium foil was demonstrated in a series of trials. It had been proposed to employ the foil in multiwall sacks for tropical produce, but it was found that in order to exclude termites the foil had to be of a thickness such that the necessary pliability had been lost.

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Colwill, D.J. (1964) Damage to transmission lines and cables by pests: *Pestic. Abstr. A.* 10; 393-487


COLONIZATION OF ORGANIC TRAPS BY MICROORGANISMS
IN KWAJALEIN ISLAND SOIL

Oscar H. Calderon\(^1\) and E.E. Staffeldt\(^2\)

INTRODUCTION.

Organic residues are continually being added to the soil where they are colonized and decomposed by the soil flora and fauna. This decomposition or chemical breakdown in the soil of plants, animals and their wastes is accomplished by the natural processes of microorganisms. Sadasivan (1939), Walker (1941), Sörgel (1941), Harder (1948), Semeniluk (1948), Remy (1949) and Staffeldt (1951), buried organic materials in soils to trap the microorganisms capable of digesting the buried materials. These organic traps included sterilized and non-sterilized stems of wheat, oats and clover, fragments of grass, pollen grains, hemp seeds, houseflies and ant larvae. Procedures used in handling the organic traps, e.g. surface sterilization following colonization and aerobic versus anaerobic conditions, would alter the complex of microorganisms growing from the traps. Staffeldt (1951), using the straw colonization technique under aerobic conditions and without surface sterilization, recognized 38 genera of fungi and representatives of the Actinomycetes, Myxomycetes and Basidiomycetes. The predominant genera of fungi observed included species of Pythium, Trichoderma, Myrothecium, Rhinotrichum, Stachybotrys, Fusarium, Verticillium, Alternaria, Chaetomium, Phoma, Rhizopus, Aspergillus and Penicillium.

The use of organic segments to trap the organisms responsible for the digestion of the buried materials reveals the qualitative microbial inhabitants common to a specific soil.

MATERIALS AND METHODS.

Kwajalein atoll, is part of the west central Marshall Islands located in the Central Pacific Ocean. Kwajalein island is three miles long and one-half mile wide. Six soil samples, coral in composition, were collected, in 1962, near and away from the beach on Kwajalein Island. Soil sample number one was obtained near the beach where there was some vegetation present. Soil samples number two and six, were obtained away from the beach and with some vegetation present; while samples number three and five, were taken inland where heavy vegetation was found. Soil sample number four was obtained from the coral beach with little or no vegetation present. These soils were placed aseptically into two and one-half inch, sterile, cylindrical, collecting jars, sealed and sent to the Microbiological Laboratory at White Sands Missile Range, New Mexico.

1. Microbiological Laboratory, E & I Branch; MSD; White Sands; Missile Range, New Mexico, U.S.A.
2. Biology Department, New Mexico State University, New Mexico, U.S.A.
The microorganisms inhabiting these soil samples were trapped by allowing them to grow into sterile organic plant parts. Healthy, unbroken straws were selected from baled alfalfa, (Medicago sativa), hay obtained from the New Mexico State University, Agronomy Farm. The straws were cut into pieces two inches in length, moistened under evacuation, and steam sterilized for one hour at 15 pounds pressure.

These sterile straws were placed aseptically into each collecting jar, containing the soils, by tilting the jar to cause a soil surface incline and by placing the straws on the soil surface. Twelve straws were added to each jar in this manner. Four straws were removed aseptically after 7, 21 and 56 days. These straws were washed free of adhering soil particles with a sterilized brush and running tap water. In addition, they were rinsed six times in sterile, distilled water and placed in sterile paper towels to absorb the free moisture. Each straw was then aseptically cut into three equal parts and planted at three points on the surface of carrot-decoction agar and Rose Bengal agar, which was contained in 9 cm. petri plates. This made a total of four plates for each of the six soils or 24 plates per examination date. Three straws placed on each plate would allow a microbe to appear 72 times per examination.

The straws removed from the Kwajalein soil samples were examined within 24 to 48 hours for growth of fungi, bacteria and Actinomycetes. Representatives of unidentified microbes were transferred to slanted agar in test tubes for further study and identification. The petri plates were also examined at frequent intervals, thereafter, to note the presence of additional microbes that had a slower growing rate. Organisms that had not been apparent earlier were transferred to carrot agar slants for further study. All microorganisms isolated from the Kwajalein soil were lyophilized and placed in the WSMR microbial collection.

RESULTS.

Fungi and bacteria colonized all of the sterile organic traps within one week. Fusarium spp., represented by F. bulbigenum, F. scirpi and F. solani, colonized 100 per cent of the traps. This genus was followed by forms representing Bacteria No. 1, Bacteria No. 2 and Myrothecium verrucaria which were found in 63, 39 and 30 per cent of the traps, respectively. The remaining organisms colonized a smaller percentage of the traps and were represented by Stachybotrys atra (19%), Chaetosporium cladosporioides (17%), an unidentified species (17%), F. moniliforme (17%), Rhizoctonia solani (15%), Cunninghamella echinulata (11%), Pythium sp. (11%), Curvularia spp. (11%). Fewer than 10 per cent of the straws were colonized by Trichoderma lignorum (9%), Bacteria No. 2 (9%), Sclerotium sp. (7%), Alternaria spp. (4%), Micromonilla echinata (2%), Mucor sp. (2%), Sphaeroma sp. (2%), Aspergillus sp. (2%) and Thielavia terricola (2%).

Examination of the stems after burial for 21 days revealed that most of the same types of microorganisms were present but the numbers of colonies of certain organisms had changed. Again Fusarium spp. was the
The most prevalent genus of the microorganisms noted and was found on 99 percent of the traps. The most common microorganisms found after 21 days burial included Fusarium spp., Bacteria No.1 (51%), Fusarium moniliforme (38%), Bacteria No.3 (32%), Bacteria No.2 (26%), Rhizoctonia solani (22%), Curvularia spp. (17%), an unidentified fungus (13%) and Myrothecium verrucaria (10%). Less prevalent microorganisms included Pythium sp. (8%), Stachybotrys atra (7%), Sclerotium sp. (7%), Gliocladium roseum (7%), Cunninghamamella echinulata (6%), Chaetostroma cladosporioides (4%), Sphaeronema sp. (4%), Trichoderma lignorum (4%), Alternaria humicola (3%), Mucor sp. (1%), and Penicillium sp. (1%).

Information on the numbers and types of microorganisms found colonized stem segments after 56 days burial was not obtained. The stems had decomposed too far, could not be handled and were considered humus.

Percentages of occurrence of microorganisms found in the six soil samples are summarized in Table 1. Soil sample No.4 which was removed from the beach and had little or no organic matter, also exhibited a restricted microbial population. The soil sample removed near the beach (No.2) and containing some organic material revealed a more diverse group of microorganisms. As the soil samples were taken further from the beach (No.1 and No.6) and with increases in vegetation (No.5 & No.3) the soil microflora became more diverse. When the microorganisms were common, they were usually found in almost equal abundance in all the soil samples, but when they were not too common their presence was usually associated with distance from the beach and quantity of organic matter. The unidentified fungus, a coremia producer, requires additional investigation due to its unusually high occurrence in one soil. Some of the Phycomycetes, Pythium sp. and Cunninghamamella echinulata, although not too common were found in most soil samples.

DISCUSSION

Sterilized plant parts were buried in Kwajalein Island soil samples. These organic traps were removed from the soil, washed free of adhering soil particles and plated onto the surface of agar contained in petri dishes. Nineteen genera of fungi and three bacteria were observed and isolated as they grew from these organic traps. Fourteen of the nineteen genera of fungi observed were the same as the genera of organisms isolated from Iowa soils (Staffeldt, 1951). The determination of the cosmopolitan nature of these microorganisms will be more meaningful when species identifications are completed. The use of sterilized plant parts for trapping these organisms appears to be an efficient tool for microbial removal. In addition to revealing the qualitative microbial distribution, it also gave indications of ecological changes that take place during normal decomposition of organic material.

It was reported that common microorganisms found in Kwajalein soil were found in almost equal abundance, and the presence of others that were not too prevalent was usually associated with distance from the beach and quantity of organic matter. The use of other organic traps and anaerobic conditions might add a great deal of information to the interpretation of
findings reported herein.

The length of time that plant segments were buried and removed will have to be changed, because decomposition had progressed too far by 56 days. Additional studies are being contemplated in the near future.

ACKNOWLEDGMENT:

The authors wish to express their gratitude to Dr. Preston Kampfmeier for collecting the soil samples.

REFERENCES:


Table 1: The percentage of times 19 genera of fungi and 3 bacteria were found in six Kwajalein Island soil samples.
FRACTIONATION OF MYROTHECIUM VERRUCARIA CELLULASE
BY GEL-FILTRATION


Culture filtrates from M. verrucaria have been fractionated on Sephadex G-75 to give three major cellulolytic components with molecular weights of about: I, 55,000; II, 30,000; III, 5,300. II has 90% of the total carboxymethylcellulase activity and is little affected by exposure to cotton. I and III, which are both active towards cotton, are removed or deactivated by exposure to it. These observations accord with the previously recorded behaviour of the whole culture filtrate (Biochem. J. (1963), 88, 288). Factors affecting the sharpness and reproducibility of separations by gel-filtration are discussed.

SOME OBSERVATIONS ON CLADOSPORIUM RESINAE AS A FUEL CONTAMINANT AND ITS POSSIBLE ROLE IN THE CORROSION OF ALUMINIUM ALLOY FUEL TANKS.


Considerable interest has been evoked recently by reports of biological growths in storage tanks and aircraft fuel tanks containing kerosene-type fuels. Most of these reports are somewhat difficult of access as they are unpublished accounts of work undertaken in Service establishments or in laboratories of industrial concerns, but the problem is dealt with in some detail in the above paper.

It is now well known that the problem of microbial attack exists wherever large quantities of kerosene-type fuels are stored. Several organisms have been reported as being present in fuels obtained from different sources, but it is likely that one organism only, a fungus, is common to all the samples.

The presence of living organisms in kerosene fuels is always associated with the presence of water, quite small traces of which are sufficient to initiate germination. The presence of water in bulk stowage fuel tanks may be due to several causes, but the chief is probably condensation, which is likely to be severe in tropical countries and it is not unusual to find a layer of several centimetres on the floor of a fuel tank. In exceptional circumstances, water has been reported up to a depth of 0.5 m.
The presence of organisms in fuel tanks presents a number of possible types of trouble, namely, deterioration of the fuel, attack of the fuel tank lining, blockage of pipes, filters and gauges and shorting of electrical equipment. From the evidence available, destruction or deterioration of fuel stocks by these organisms has not occurred, but blockages and interference with gauges have been reported.

Some evidence exists that biological metabolites produced by the mould may attack aluminium alloy fuel tanks unless adequate protection is provided. This observation may have some significance to the aircraft industry, particularly where the fuel comes in contact with the aluminium through imperfect application of the tank sealant.

Samples of the water bottom from tanks in which kerosene type fuel for jet aircraft has been stored, both in ships and in land-based fuel stations in this country and in the Far East, have yielded mycelial fragments, which when cultured, produced a brown fungus which has been identified as Cladosporium resinae (Lindau) de Vries.

Microscopical examination of fungus recovered from fuel tanks shows that two forms of the species are equally at home in kerosene. The first agrees in all respects with C. resinae f. avellaneum. This taxon has been recovered from oil-water mixtures from ships, as well as from fuel tanks in the Far East. A second form also develops; whether the two are genetically distinct is not known. The second form differs from the first in microscopical appearance only and corresponds very closely to C. resinae f. resinae, but differs from it in colour. It is unlikely that these forms have any real taxonomic significance as other strains also arose in cultures which appeared to be intermediate and produced spores of several different shapes.

It seems that the "kerosene fungus" may best be regarded as a highly labile species capable of producing a wide variety of morphological forms, several of which manage to survive, and indeed thrive, in hydrocarbon fuels. It is identical with the "creosote fungus" which manages to survive on tar and creosote and similar products containing phenolic compounds that are usually considered to be fungicidal.

Immersion in kerosene at all temperatures from - 25° C to 40° C had no deleterious effect on the subsequent growth of the spores of the fungus. Immersion of the spores in water at 50° C for up to 3 days retarded spore development; immersion at 50° C for 6 days and at 60° C for 1 day inhibited growth when the spores were removed to a culture medium at 22° C.

Dry kerosene does not contain sufficient dissolved moisture to initiate germination, but immersion for 3 months in kerosene did not impair the ability of the spores to germinate when adequate moisture became available. Experiments also indicated that kerosene vapour provides sufficient nutriment to support the development of the spores, providing humidity is high.
A series of cultures set up in which the spores of the kerosene fungus were seeded on to the surface of a carbon-free medium, covered with a column of kerosene and incubated at 30°C, showed evidence of growth after 12 days and heavy growth at 28 days, indicating that the fungus utilised the kerosene as a source of carbon.

In view of the importance of the corrosion problem in aircraft fuel tanks, preliminary tests were carried out to determine whether this fungus could cause corrosion of aluminium.

The tests, in vitro, on aluminium foil of 0.005 cm. thickness of the "commercially pure" grade showed that all samples that had been in contact with the fungus were attacked to the extent of perforation and had lost considerable weight. By contrast, the controls appeared unaffected and had lost no weight.

The corrosion of aluminium in the presence of the mould, requires further research. Little is known at present of the mechanisms of this phenomenon. It is possible that the metal is attacked by a metabolite produced by the fungus.

EVALUATION OF ROT- AND WEATHER-RESISTANCE OF COTTON FABRICS TREATED WITH METHYLOLMELEAMINE RESINS BY WET FIXATION METHODS


Cotton fabric is extremely resistant to both biological and actinic deterioration when treated with methylolmelamine by the formic acid colloid process. This is the so-called "dry cure" process. A detailed report of extensive environmental testing of this fabric was the subject of a previous publication from this laboratory. This report contains the results of numerous comparative tests of the rot- and weather-resistance of fabrics treated with methylolmelamine with hydrogen peroxide as the acid-forming catalyst or methylated methylolmelamine with acid forming metal salts as the catalyst. These are the so-called "wet cure" processes. These results are compared with previous findings of similar trials with the formic acid colloid process.
FURTHER DEVELOPMENTS IN THE MICROBIOLOGICAL TESTING OF PLASTICS MATERIALS BY RESPIROMETRIC AND LOSS OF WEIGHT TECHNIQUES


Evidence is presented to show that the use of these methods may be extended to an examination of damage by *Pseudomonas aeruginosa* and to the evaluation of antimicrobial treatments against attack by this organism and by mixed species of fungi.

*Ps. aeruginosa* appears to attack only those plasticisers used by fungi but its degrading action is more rapid.

Results with fungi in the respirometric test method show that confident distinction can be made between biocide treatments with different degrees of effectiveness, while only one of the treatments examined was found to check the growth of *Ps. aeruginosa*. Copper 8-hydroxyquinolinolate at 1% was fungistatic but not bacteriostatic. It is concluded that the respirometric test, in addition to tracing the daily course of microbial growth, can allow quick rejection of ineffective treatments and within seven days cast doubt on the effectiveness of others which give only temporary protection.

The weight loss test gives results which reflect those of the respirometric test and the two methods appear to be complementary. Weight loss figures for thin films expressed as a percentage of the plasticiser component may conceivably indicate the amount of physical degradation while absolute values may be of more practical interest for thicker materials.
STUDIES ON THEOCOLAX FORMICIFORMIS WESTW.
(HYM. PTEROMALIDAE),
A PARASITE OF ANOBIUM PUNCTATUM (DEG.) (COL. ANOBIIDAE)


A description is given of the life-cycle in Britain of Theocolax formiciformis Westw. (Pteromalidae), a parasite of Anobium punctatum (Deg.) (Anobiidae). Adults emerge from infested wood in large numbers from April to June. Eggs are laid through the wood surface and use was made of this habit as a means of rearing this parasite under observation in the laboratory. Development from egg to adult at 22° and 25°C and 75 per cent relative humidity, averaged about five and six weeks, respectively, compared with 12 weeks out-of-doors in summer. The number of progeny per female was highest (approximately five) at 22°C, at which the ratio of males to females was 1:3. Only about one per cent of the adults reared were winged. The use of Theocolax as a biological control agent is not considered practical.

FOREST PRODUCTS ENTOMOLOGY IN THE UNITED KINGDOM


The current work on wood-boring insects at the Forest Products Research Laboratory is described. The investigations are broadly divided into basic biological studies, measures for preventing and controlling attack and methods of testing insecticidal material.

THE EFFECT OF SPECIMEN SIZE ON THE LIFE OF TIMBER IN CONTACT WITH THE GROUND.


In field service tests the life of standard-size specimens is used to assess the natural durability of timber. Little is known, however, about the relationship between other sizes and service life. The results of this investigation indicate that the life of timber in contact with the ground is directly proportional to its thickness and not its cross-sectional area. The relationship holds for both the perishable and durable timbers tested.
INVESTIGATIONS ON THE SUSCEPTIBILITY OF HOME-GROWN SITKA SPRUCE (PICEA SITCHENSIS) TO THE ATTACK BY THE COMMON FURNITURE BEETLE (ANOBIUM PUNCTATUM DEG.)


The influence of compression wood and chemical composition (alpha-cellulose, Cross and Bevan cellulose, lignin, nitrogen content, pentosans, resin and material soluble in one per cent caustic soda) in both heartwood and sapwood on the development of the common furniture beetle (Anobium punctatum Deg.) has been studied at the three heights in three trees of Sitka spruce (Picea sitchensis). Two methods of test were compared - (1) egg-laying and subsequent larval development and (2) the growth rate of larvae inserted into test blocks; of these the former proved the more sensitive. Both sapwood and heartwood are suitable for larval development but the outer five-ring zone of sapwood (nearest the bark) is markedly superior in this respect - a quality apparently chiefly related to a greater nitrogen content. Quantitative variations in the other components studied appeared to have little effect on the nutritional suitability of the wood, possibly because with the exception of the resin the amounts present were so large that minor variations were unimportant. The compression wood present in these trees did not appear to affect the rate of development of the larvae. Variations in the nutritional suitability of specified zones at the heights studied showed marked differences between trees especially in the most suitable outer zone. It is, therefore, concluded that studies on the susceptibility of timber species to Anobium attack should be undertaken on specimens from several trees. Where durability is important both sapwood and heartwood of Sitka spruce should be treated with a preservative.

DRY ROT - A RE-APPRAISAL


Dry rot is the term often used to describe all forms of timber decay in buildings but this term can lead to confusion. The differences between dry rot and wet rot are described showing that these two types of fungi behave differently in buildings in this country. The principles governing dry rot eradication are given and emphasis is placed upon the fact that fungicide treatment is an adjunct, not an alternative, to restoration of dry conditions within the affected building.
CONTROL OF BLUE-STAIN IN HOME-GROWN PINE LOGS


Loss in value through the development of blue-stain in home-grown pine logs during storage is an increasingly important problem. Experiments in East Anglia have shown that during the summer months log stain is closely related to attack by bark-boring beetles, but treatment with suitable mixtures of fungicides and insecticides provides a good measure of control.

A FIELD TEST OF A LINDANE/DIELDREN SMOKE FOR CONTROL OF THE DEATH-WATCH BEETLE, XESTOBIIUM RUFOVILLOSUM (DEG.) (COLEOPTERA, ANOBIIDAE).


In view of the promising results obtained with insecticidal smokes against the common furniture beetle, Anobium punctatum (Deg.), a field trial was conducted to study the effect of deposits from Lindane/dieldrin smoke on adults of the death-watch beetle, Xestobium rufovillosum (Deg.), emerging from infested roof timbers of a college chapel in Cambridge, England, in 1963. Quantitative determination of the insecticidal deposits was made and methods devised for studying the effect of the treatment on the number of beetles emerging subsequently in order to assess the long-term value of annual treatments for reducing the population. Beetles collected from an untreated building were used for comparison.

From the results obtained it was inferred that the deposits from the treatment with Lindane/dieldrin smoke prevented emerging adults from causing re-infestation of roofing timbers and it is argued that, provided this can be achieved annually by a succession of treatments, the beetle population should ultimately be eliminated or, at least, greatly reduced. Other possible effects would be the accumulation of the insecticide deposits with a consequent increase in toxicity of the timber surfaces and sorption by the timber to a point where they might exert a toxic effect on larvae or pupae beneath the surface.
THE TOXICITY OF AGED CREOSOTE TO LENTINUS LEPIDEUS


Aged creosotes, extracted after 30 years service in posts, have been examined for their toxicity to Lentinus lepideus and Fomes annosus using agar plate and wood block tests. In agar tests aged creosote failed to control the growth of the test fungi whereas the modern creosote was toxic at low levels. When tested by the wood block method, aged creosotes extracted from above and below the ground level of treated posts were about as toxic to L. lepideus as modern B.S.144 creosote.
A one-day symposium on 'The Mechanism of Cellulase Action' is being organised by the Shirley Institute. This meeting will be held on Friday, 28th May 1965, under the auspices of the Molecular Enzymology Group of the Biochemical Society. There will be two sessions, morning and afternoon, followed by informal discussion in the evening.

The chairman will be Dr. J. Honeyman, Shirley Institute, and speakers will include:

- N.J. King. Forest Products Research Laboratory, Princes Risborough.
- C.C. Maitland. Shirley Institute.
- E.T. Reese. Q.M. Research and Engineering Center, Natick, U.S.A.
- B.A. Stone. University of Melbourne, Australia.

The meeting will be held at the Shirley Institute and will start at 9.45 a.m. Coffee, luncheon, and a buffet supper will be available to ticket-holders only. Tickets (Price 1 gn.) can be obtained by application to K. Selby, Shirley Institute, Didsbury, Manchester 20. (Tel. DIDSbury 8811. Ext. 40) by 14th May.
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