INTERNATIONAL
BIODETERIORATION
BULLETIN

A QUARTERLY JOURNAL OF BIODETERIORATION

BIODETERIORATION CENTRE
THE UNIVERSITY OF ASTON IN BIRMINGHAM
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BULLETIN

BIODETERIORATION CENTRE
UNIVERSITY OF ASTON
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NOTES FOR CONTRIBUTORS

The International Biodeterioration Bulletin is published four times per year (Spring, Summer, Autumn and Winter). Typescript contributions should be sent to the Editor, Professor T.A. Oxley, at the above address. The Bulletin acts as a vehicle for the publication of original works, including reviews, on all aspects of biodeterioration, i.e., deterioration of materials, artefacts or facilities, of economic importance by living organisms, which include microorganisms, insects, rodents, birds, higher plants, etc. Articles on biodegradation, that is conversion of materials to less objectionable, more easily disposable, or higher value products by living organisms, are also published.

Contributions are published only in English. Each article must be accompanied by a summary in 50-150 words which will be translated into French, German and Spanish. Native speakers of these languages are invited to submit their summaries in their own language; in certain circumstances complete articles may be submitted in French, German or Spanish and will be translated into English for publication.

Illustrations must be very clearly drawn, normally larger than the size finally desired. The suggested final size should be clearly indicated but the Editor reserves the right to vary this in the interests of economy and clarity.

As far as possible diagrams will be reduced to single column width (30 mm) or to half page (170 mm). In any event, neither these nor half tone photographs can exceed full page (260 by 170 mm). Authors should bear in mind that it is generally more convenient for readers if legends which accompany diagrams or photographs appear with them on the same page and should proportion their illustrations accordingly. Lettering on diagrams will normally be inserted by the printer; authors are therefore asked to insert lettering or symbols in pencil on the originals or in ink on a copy.

All articles are submitted by the Editor to one or more independent referees for advice on their clarity, originality, and general suitability for publication, but the final decision whether or not to publish an article rests with the Editors. If articles are rejected the substance of the referee's report will usually be communicated to the author and in suitable cases the Editor will be pleased to help authors to improve their pages with a view to possible publication.

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BIODETERIORATION SOCIETY NEWSLETTER

Proceedings of the Berlin Symposium

There have been continuing difficulties over printing of the Proceedings but these are now resolved and it is confidently expected that printing and binding will have been completed some time in November. Official publication should be possible some time after Christmas. The Society greatly regrets this delay but is confident that the volume will be well accepted when it appears.

The Society has entered into an arrangement to publish jointly with Pitmans Publishers Ltd. Pitmans will handle all sales and have given very great assistance in overcoming many of the difficulties.

The Biodeterioration Centre

During August the Centre moved from the building within the main campus of the University (at 80 Coleshill Street) which it had occupied almost since its inception, to St. Peter’s College. St. Peter’s is a former Teachers’ Training College which was recently acquired by the University, about two miles from the main campus. It consists of a series of old and new buildings on three sides of a very pleasant sports field.

The Biodeterioration Centre occupies part of the former Drama and Science departments of the original college. Both office and laboratory accommodation are very much improved and the staff are agreed that the facilities are in every way more satisfactory than those formerly used.

About the same time the word “Information” was dropped from the title of the Centre. This does not imply any reduction in the information activities of the Centre, indeed they are being enhanced, but it does reflect a continuing increase in the amount of contract research and industrial consultancy work being undertaken.

The full address is now:
The Biodeterioration Centre,
University of Aston,
St. Peter’s College,
College Road, Saltley,
BIRMINGHAM B9 3TE.
Telephone: 021-328 5950 (the Centre’s own lines).

The Centre cannot be reached by telephone through the University’s own line but if difficulty is experienced with the number given above the Centre may be contacted through St. Peter’s College (021-327 3734).

Dr. Howard Eggins

The Biodeterioration Society is glad to congratulate its founder member and prime instigator, Dr. H.O.W. Eggins, on his appointment by the University of Aston as Warden of St. Peter’s College.


The 12th Autumn meeting of I.B.R.G. was held on the 25th – 27th September at the new premises of the Biodeterioration Centre, St. Peter’s College, University of Aston in Birmingham. Over twenty members took part, from seven European countries; Denmark, France, West Germany, Holland, Norway, Switzerland and the U.K. Working Groups which held meetings were those on Paints, Constructional Materials, Taxonomy – Ecology and Industrial Problems. The latter group acts as an umbrella organisation for small groups and new groups just forming and at present includes biocides and rodents in its field of interests. Bureau (the steering committee of IBRG) and Plenary meetings were also held.

A new Working Group, previously part of the Industrial Problems Group, came into being as a full Working Group in its own right. The new group, the Cutting Oils Working Group, will pursue a co-operative investigation and discussion programme on the biodeterioration of cutting oils, and until the election of a Chairman and Technical Secretary, will be supervised by Dr. R.N. Smith of Hatfield Polytechnic, U.K.

As the work of IBRG is almost wholly concerned with the biological breakdown of materials of economic importance, it has been agreed that the name should be changed to the International Biodeterioration Research Group, as the word biodegradation is now closely associated with waste treatment processes. The familiar initials of IBRG will of course remain unchanged.

Membership of IBRG is open to all who wish to participate in the co-operative investigations carried out by the Working Groups. At present there is an annual subscription of £30 (Thirty Pounds) sterling, to finance the Secretariat, and meetings to discuss progress and plan work are held twice each year.

Readers who would like further information on IBRG and its current activities, are asked to contact Dr. Dennis Allsopp, Secretary-General IBRG at the Biodeterioration Centre. (See earlier newsletter item for full address and telephone number).

IBRG has members representing Universities, Polytechnics, Government Departments and Industrial Companies, and welcomes enquiries from prospective members.
Abstracts of some papers presented at a Symposium on

TECHNIQUES FOR BIODEGRADATION STUDIES

Held at Cardiff University College on 5–6th July 1979
on the occasion of the Society's Summer Meeting

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**Title:** Biodegradation: Definition, Mechanism and Implications.
**Author:** Professor David E. Hughes.
**Address:** Department of Microbiology, University College, Newport Road, CARDIFF CF2 1TA.

**ABSTRACT**

Biodegradation was seen to be essential to all living organisms and linked closely to their growth and reproduction. Microbes, because of their long evolutionary history, have the greatest potentiality to degrade organic and inorganic materials. Thus, in order to discover whether any material is biodegradable, the search can be concentrated on finding a microbe to degrade it. Various strategies for carrying out this search were discussed, including modifications to such methods as: elective/selective culture, mixed organism cultures, maintenance and catabolic systems. The need to identify pathways, intermediates and end products of breakdown was stressed as well as the evolution of degradative enzymes.

---

**Title:** Physical Strength Testing as a Measure of Microbial Deterioration.
**Author:** K. Hardie
**Address:** Biodeterioration Centre, University of ASTON, St. Peter's College, Saltley, BIRMINGHAM B8 3TE.

**ABSTRACT**

The choice of appropriate criteria for full assessment of microbial effect on a material is obviously important. Measurement of strength loss provides useful information on the extent of physical damage and on the effect this damage may have on the practical use of the material. Different types of attack by microorganisms and the effect that these attacks may have on the inherent strength properties of a material were discussed, particularly in relation to the biodeterioration of wood. Any solid material may possess one or more of the following strength properties:— compressive, tensile, shearing, impact bending, static bending and hardness. Methods of assessing the various strength properties and some of the difficulties encountered were discussed.

Literature on assessment of strength loss was reviewed and strength loss was found to compare favourably with the more usual criterion of weight loss. Immediate reductions in strength properties have frequently been found with the onset of decay, strength losses of 50% having been recorded for weight losses of 10% or less. During the initial stages of biodeterioration strength loss may, therefore, prove a more valuable criterion than weight loss in certain situations.

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**Title:** Biodegradability: Simulated Sewage Treatment.
**Author:** H.A. Painter
**Address:** Water Research Centre, Elder Way, Stevenage, Herts. SG1 1TH.

**ABSTRACT**

A strategy was set out for guidance through the tangled web of the many methods available for the assessment of biodegradability. Several of the more popular preliminary (screening or batch) tests were described in detail and indications given as to their application. A simple method was described for producing an acclimatized inoculum for use in the screening tests. Standard methods for simulating the treatment by activated sludge and percolating filters of sewage and industrial waste waters were given. Finally, some recent results obtained by using all of these methods were reviewed.
ABSTRACT

The spoilage of two-phase systems, such as cutting oils and cosmetics, is well documented and hence we may consider bio-treatment as a disposal method. Conventional test methods for biodegradability are complicated by the complex chemistry of formulations, the migration of substances between the two-phases, adsorption onto surfaces (particularly sludge) and the presence of preservatives.

The surface area of the oil phase can be increased to speed up bio-degradation by adsorption onto filter paper, asbestos fibre or colloidal silica. The latter method is preferred and is sufficiently rapid for respirometric techniques to be used.

There is as yet no general agreement on biodegradability on test methods or even on analytical techniques to assess results.
Summary. The fixation of copper/chromium/arsenic wood preservative in softwoods and hardwoods has been investigated. Three softwoods and six hardwoods were examined using carefully controlled fixation conditions and a leaching procedure. Differences in leaching were found to be slight and unlikely to cause great variation in the performance of treated timbers.

Introduction

Pressure treatment with copper/chrome/arsenic (CCA) preservatives has been widely accepted as a means of protecting vulnerable timbers during service in ground contact. The efficacy of such treatments has been demonstrated in field trials in the UK over a period of more than forty years (Purslow 1975). However, recent tests in other parts of the world using timbers treated with CCA have indicated that early failures in hardwoods may occur.

In the present study a laboratory leaching technique was used on samples having a high surface area: volume ratio. This approach should maximise the degree of leaching likely to take place, thus increasing the possibility of recognising variations in loss patterns dependent on species.

Henningson (1974) reported the results of field tests in Sweden, Denmark, Finland and Norway. After 5 years, satisfactory results were obtained with CCA treated Scots pine (Pinus sylvestris) samples in three of the test sites, but treated beech (Fagus sylvatica), birch (Betula pubescens) and alder (Alnus incana) had not performed well; no comparative tests were made at the fourth site. He also tested a number of other preservatives which showed a similar pattern of poorer performance in the hardwoods.

Tamblyn (1973, 1975) reported the results of field tests in Australia and Papua New Guinea, carried out at four different sites. After 10 years testing, satisfactory results were obtained with CCA treated radiata pine (Pinus radiata) but similarly treated samples of Yorkshire oak (Eucalyptus regnans) had decayed. This pattern was repeated with creosote and pentachlorophenol-type preservatives.

The situation is therefore far from clear. Acceptable performance of CCA-treated hardwoods has been found in the UK but elsewhere hardwoods have failed on the same exposure sites where softwoods have performed well. It appears that some difference, either in the distribution of preservative in softwoods and hardwoods or in the fixation mechanism of the preservative, is playing a significant part in premature failure.

Differences in the fixation mechanism would lead to variation in the amount of water-soluble preservative present, and thus leaching is an ideal technique for studying variations in fixation pattern. Nicholson and Levi (1971) compared the leachability of CCA in radiata pine and spotted gum (Eucalyptus maculata). They found greater losses of preservative from the hardwood species but total salt losses were only about 10 per cent of uptake. Although this difference was small it appeared to indicate that some difference in fixation of CCA in softwoods and hardwoods may occur, and that study of fixation in more species would be worthwhile.

Experimental

Timbers

Three softwoods and six hardwoods were examined.

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Dr. Henshaw's present address: c/o John & E. Sturge Ltd., Denison Road, Selby, North Yorkshire Y08 8EF.

(Received February 1979).
Fixation of copper, chromium and arsenic in softwoods and hardwoods, B. Henshaw.

Softwoods: Scots pine (*Pinus sylvestris*)
Whitewood (*Picea abies*)
Western red cedar (*Thuja plicata*)

Hardwoods: Beech (*Fagus sylvatica*)
Birch (*Betula pubescens*)
Elm (*Ulmus procera*)
Lime (*Tilia spp*)
Oak (*Quercus robur*)
Sycamore (*Acer pseudoplatanus*)

Sample preparation

Blocks 50 mm [longitudinally] x 25 mm [radially] x 25 mm [tangentially] free from knots and wane were cut from air-dried planks of each timber. Where possible the blocks were cut from sapwood areas but in cases where the sapwood/heartwood boundary could not be defined, they were cut from that portion of the plank furthest from the centre of the tree.

300 μm thick sections were then cut from each block parallel to a radial face, using a microtome. This procedure ensured that a representative amount of earlywood and late wood was present in each section.

Impregnation

The sections were numbered and weighed then sandwiched in “matched sets” of five (one from each block) between two pieces of Scots pine (50 x 25 x 3 mm). Each sandwich was secured with a rubber band and impregnated with a 3 per cent copper/chromium/arsenic solution formulated to Type 2 BS 4072: 1974 using the following treatment schedule:
- vacuum (-0.97 bar/15 minutes), introduction of solution, final pressure (6 bar/30 minutes).

After treatment, excess solution was wiped from the surface of the sections and the solution uptake of each section was determined by weighing.

Equilibration of samples

One matched set from each timber was examined for CCA content immediately after treatment. A number of matched sets of Scots pine were equilibrated under three sets of conditions before leaching; (a) by air drying in the laboratory at 20°C; (b) 20°C/83 per cent rh; (c) 10°C/81 per cent rh. In the main series of investigation equilibration was carried out at 20°C/83 per cent rh for appropriate periods.

Leaching

The equilibrated sections from matched sets were leached individually using 70 ml of deionised water in a 250 ml beaker, at 20°C and with continuous magnetic stirring. The stirring bars were separated from the samples by nylon mesh to avoid mechanical breakdown of the sample and the beakers were sealed with wax-impregnated film. At the end of the leaching period (100 hours) the sections were removed and the leaching liquor was analysed for copper, chromium and arsenic content.

Analyses

All analyses were carried out by atomic absorption spectrophotometry using the method of Williams (1972). The CCA content of each individual section or leaching liquor was measured and average figures for the five samples in each matched set calculated.

Results and Discussion

Analysis of impregnated timbers immediately after treatment indicated that in every case the amount of preservative taken up exceeded that predicted by uptake weight of solution, sometimes by a considerable margin. While this effect may be exaggerated by the relatively large surface area of the samples, other workers have noted that CCA salts can be adsorbed from solution during treatment (McMahon et al., 1942). The detailed comparison is shown in Table 1, from which it can be seen that variation occurs in both the total amounts of preservative absorbed and in the relative proportions of copper, chromium and arsenic. The case of elm is particularly noteworthy. Several mechanisms can be postulated to rationalise the figures obtained. All the values are in excess of unity and this can be partially explained in terms of dissolution of soluble material from the sections leading to a lowering of the initial weight (and hence an under-estimate of the uptake of CCA). However, this mechanism does not explain the variation in proportions of copper, chromium and arsenic found by analysis to amounts expected from uptake weight.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Ratio of copper, chromium and arsenic found by analysis to amounts expected from uptake weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Softwoods</td>
<td>Hardwoods</td>
</tr>
<tr>
<td>Pine</td>
<td>Spruce</td>
</tr>
<tr>
<td>Copper</td>
<td>1.24</td>
</tr>
<tr>
<td>Chromium</td>
<td>1.45</td>
</tr>
<tr>
<td>Arsenic</td>
<td>1.29</td>
</tr>
</tbody>
</table>
arsenic and this may be ascribed to adsorption, precipitation, chemical reactions between the preservative and specific wood components, or a combination of the three. The factors in Table I were used to adjust the observed uptake weights of copper, chromium and arsenic in each section. The adjusted figures were used in calculating the amounts lost during leaching. It was hoped that any errors caused by the effect described would be minimised by this procedure.

A leaching period of 100 hours was chosen as this was found to be necessary to obtain complete removal of soluble copper compounds.

Figures 1 to 3 show the leaching profiles produced with the equilibration conditions described previously. Fixation in air-dried samples proceeded less rapidly than in "wet" samples, and lower levels of fixation were achieved. Copper was of particular interest as the amount leached appeared to increase with fixation time, during dry fixation (see Figure 1). The rapid formation of a copper/cell wall complex has been postulated by Wilson (1971) and the breakdown of this complex in relatively dry conditions could account for the increasing amount of copper leached.

The difference in fixation profiles of the "wet" and "dry" samples is considerable, but the large surface area/volume ratio of the thin sections used led to very rapid drying and hence magnified any effect caused by lack of available water. However, it is apparent that water is necessary for effective fixation to take place and that samples must, therefore, not be allowed to dry too rapidly. The effect of water on fixation has been noted by several authors, notably Dahlgren (1975).

The effect of decreasing the fixation temperature was less marked with only a marginal decrease in the fixation rate for copper and arsenic. Chromium demonstrated the most noticeable change in fixation rate but similar levels of fixation of all three elements were eventually obtained. A slower fixation rate at lower temperatures has also been observed by Wilson (1971) and Dahlgren (1975). Dahlgren postulated that more chromium remained in the hexavalent state at lower temperatures and the more marked effect of temperature on chromium fixation found in this work may lend support to this view.

The fixation profiles of five timbers were determined under controlled fixation conditions (20°C/83 per cent R.H.). The timbers examined were Scots pine, lime, spruce, oak and western red cedar, and the profiles obtained are shown in Figures 4 to 8. No systematic difference was found between the fixation patterns of softwoods and hardwoods. Copper and chromium produced characteristic asymptotic curves in virtually all the timbers examined but a noticeable 'bump' was
Fixation of copper, chromium and arsenic in softwoods and hardwoods, B. Henshaw.

Fig. 2. Chromium fixation under various conditions in Scots pine sapwood

- a) Air drying at 20°C
- b) 20°C, 83% r.h.
- c) 10°C, 81% r.h.

Fig. 4. Pine fixation profile 20°C / 83% r.h.
Fixation of copper, chromium and arsenic in softwoods and hardwoods, B. Henshaw.

Fig. 3. Arsenic fixation under various conditions in Scots pine sapwood

Fig. 5. Oak fixation profile 20°C/83% r.h.
Fixation of copper, chromium and arsenic in softwoods and hardwoods, B. Henshaw.

Fig. 6. Lime fixation profile 20°C / 83% r.h.

Fig. 7. Spruce fixation profile 20°C / 83% r.h.
observed in the arsenic fixation profile. This is presumably due to chemical reactions taking place within the timber causing variation in the proportion of water-soluble arsenic. However, in the complex interaction between the preservative and timber structure, it is difficult to define the particular mechanism which causes this variation in the proportion of soluble arsenic salts. Wet fixation under the conditions described was largely complete within two weeks.

A further four suspect hardwoods were subjected to leaching after two weeks fixation under controlled conditions (20°C/83% r.h.). The timbers studied were beech, birch, elm and sycamore, and the quantities of salts leached from these and the five timbers mentioned previously under the above conditions are shown in Table 2. The preservative components appeared to fix to about the same degree in softwoods and hardwoods. The total amount of preservative salts leached after two weeks varied from 4--8 per cent of the uptake (see Table 2). Losses of this magnitude in the severe leaching system used are unlikely to affect preservative performance. Losses of arsenic accounted for 57--84 per cent of the total preservative lost despite the fact that the arsenic salt accounts for only 20 per cent of the dry weight of preservative.

Conclusions

Water is necessary for fixation reactions to proceed to completion in pine sapwood. Fixation is virtually complete after two weeks at 20°C in moist conditions (83 per cent relative humidity). It proceeds less rapidly with decreasing temperature although similar levels are eventually reached. Hence samples should not be allowed to dry too quickly.

The course of fixation appears to be similar in both hardwoods and softwoods. No systematic difference in the levels of fixation achieved was apparent and it seems unlikely that variation in the fixation mechanism, leading to preferential leaching of CCA, is responsible for the reported premature failures in hardwoods. In all cases the component which leached most was the arsenic salt.
Fixation of copper, chromium and arsenic in softwoods and hardwoods, B. Henshaw.

Considerable variation has been observed in the distribution of CCA between softwoods and hardwoods (Dickinson *et al.* 1976, Greaves, 1972, 1973, Aston and Watson, 1974). Further study of the effect of distribution on the decay characteristics may provide the answer to the problem of early failure of treated hardwoods.

### Table 2
Copper, chromium and arsenic leached from different timber species after two weeks wet fixation at 20°C

<table>
<thead>
<tr>
<th>Timber</th>
<th>CuSO₄·5H₂O</th>
<th>K₂Cr₂O₇</th>
<th>As₂O₅·2H₂O</th>
<th>Total Salts</th>
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<td>0.66</td>
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<td>1.50</td>
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<td>6.29</td>
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<td>0.53</td>
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<td>6.22</td>
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<td>Pine</td>
<td>0.69</td>
<td>0.30</td>
<td>4.48</td>
<td>5.47</td>
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<tr>
<td>Birch</td>
<td>0.61</td>
<td>0.44</td>
<td>4.33</td>
<td>5.38</td>
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<tr>
<td>Lime</td>
<td>1.20</td>
<td>0.26</td>
<td>3.82</td>
<td>5.28</td>
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<tr>
<td>Spruce</td>
<td>0.42</td>
<td>0.34</td>
<td>4.04</td>
<td>4.80</td>
</tr>
<tr>
<td>Oak</td>
<td>0.36</td>
<td>0.51</td>
<td>3.69</td>
<td>4.56</td>
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<tr>
<td>Beech</td>
<td>0.34</td>
<td>0.50</td>
<td>2.98</td>
<td>3.82</td>
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THE DETERIORATION OF COMMERCIAL MAIZE (Zea mays) BY INSECTS AND FUNGI

Matt F. Ivbijaro1, E.O. Osisanya2 and E.E. Akinlade2

Summary. Attack by insects and fungi on maize in open markets in South Western Nigeria results in losses in quantity and quality of the grain. Sitophilus oryzae (L.) and S. zeamais (Motsch.) accounted for about 92% of the insect population while Tribolium castaneum (Herbst.) and Rhizopertha domincus (F.) accounted for 6% and 2% respectively. The grains were also attacked by fungi belonging to the genera Aspergillus, Penicillium, Rhiizopus and Fusarium.

Viability of the infested maize was 37% compared to 97% in clean samples. Fat acidity value was three times higher in the infested sample while the uric acid content of infested maize was almost double that of clean samples. The need for improved drying of maize, better handling and distribution methods to obviate possible danger to human life is discussed.

Introduction

Maize, (Zea mays L.) is a staple food among millions of Nigerians. When fresh, maize is boiled or roasted. When dry it is made into porridge (akamu, ogi) or into a white solid (agidi, etc). Because the moisture content of fresh maize at the time of harvest in southern Nigeria ranges from 22 to 32% between May and July, maize thus stored becomes mouldy and grain damage is further aggravated by rodents during storage and distribution. In addition to biochemical changes which insect infestation causes to stored grain, discoloration and mycotoxicosis often result from fungal attack.

Most earlier data on losses in stored grains were from laboratory studies. This paper, however, examines the changes caused by insects and moulds to maize during storage and distribution in open markets in southern Nigeria.

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The deterioration of commercial maize (Zea mays) by insects and fungi, Matt F. Irvijaro, E.O. Osisanya and E.E. Akinlade.

(iii) Viability. The viability of the maize was determined in apparently clean, and in infested grains, from each market by observing the emergence of the hypocotyl in 5 subsamples each of 20 grains, of clean and of infested maize, placed on moistened Whatman No. 1 filter paper in a petri dish at 27° ± 1°C.

(iv) Mould types. Five samples of clean, and of infested maize, each of 20 grains, were put on moistened Whatman No. 1 filter paper in petri dishes and covered and incubated under near ultraviolet light to hasten sporulation. Mycelial growth was observed and spores identified.

2. Biochemical measurements

(i) Free fatty acids. Infested and apparently clean maize grains were ground separately. From each portion, 6 samples of 5g each were analysed for free fatty acids according to the method of the American Association of Cereal Chemists (1962).

(ii) Reducing sugars. Soluble carbohydrates were determined by the method of the Association of Official Agricultural Chemists (1950) while sucrose was extracted using the modified anthrone method of Firby et al. (1973).

(iii) Uric acid was determined according to the method of Eichhorn et al. (1961).

Results

In the commercial maize samples examined, Sitophilus sp. (probably either or both of S. oryzae L. and S. zeamais Motsch., but not distinguished as to species) accounted for 92% of the total insect population while Tribolium castaneum (Herbst.) and Rhyzopertha dominica (F.) accounted for 6% and 2% respectively. Table 1 shows that with increase in infestation, percentage kernel damage increased, while viability decreased. Free fatty acid value in infested maize was 4.5 mg KOH per 100 g grain compared with 1.3mg KOH per 100g in the apparently clean samples. True uric acid content (mg/100 ml) was 2.05 in infested samples. Insect-infested maize grains were also found to contain moulds belonging to the genera Aspergillus, Penicillium, Rhizopus and Fusarium in descending order of abundance.

Discussion and Conclusion

This study shows that maize sold on open markets in Ibadan had physical damage and reduced viability. Increase in fat acidity value in the commercial samples showed that the oily material in the maize must have suffered oxidative degeneration leading to rancidity during storage and distribution.

Uric acid, a major component of insect excreta, has been used as an index of insect infestation in grains (Coombs, 1963; Subramanyan et al., 1955) although creatinine, guanine and allantoin may also be present.

Table 1

<table>
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<tr>
<th>Market</th>
<th>Insect population (mean no. per 5 cob sample)</th>
<th>Kernel damage (mean no. per 100)</th>
<th>Viability %</th>
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<tr>
<td>Sango</td>
<td>48.2</td>
<td>41.0</td>
<td>100</td>
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<td></td>
<td></td>
<td></td>
<td>26</td>
</tr>
<tr>
<td>Dugbe</td>
<td>36.4</td>
<td>35.5</td>
<td>94</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>36</td>
</tr>
<tr>
<td>Oja Oba</td>
<td>15.4</td>
<td>23.5</td>
<td>98</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>50</td>
</tr>
</tbody>
</table>

It has, however, not been reported whether storage fungi produce uric acid in fungal-infected stored produce. It can thus be reasonably assumed that the increase in uric acid in the commercial maize sold in Ibadan is probably due only to insect attack. The presence of small quantities of uric acid in the apparently clean samples could have been due to undetected insect infestation. Purine and adenine also presumably come only from insect infestation. Therefore the low value of 1.3mg KOH/100g can be explained by undetected infestation in the 'apparently clean' samples. On open markets in southern Nigeria, maize cobs with apparent insect infestation are usually sorted from the bulck before sale, hence the overall mean percentage insect population, kernel damage and biochemical changes will be higher than we have observed.

In addition to the already observed infestation, the lipolytic properties of the infecting moulds may have contributed to the increase in fat acidity value in the infested maize. Increase in fat acidity value is a useful measure of deterioration. For example, Pingale et al. (1954) recorded a rise of 44, 46 and 49 mg KOH per 100g of wheat infested by three different insects compared to 19–37 mg KOH per 100g in uninfested wheat.

Although insect activity in stored grains which results in heating and increased grain moisture encourages the growth of fungi, stored products insects can also be responsible for the introduction and dissemination of storage fungi in produce (Agrawal et al., 1957, 1958). Mycotoxocosis especially by the Aspergillus group has been a major cause for concern (Forgass et al., 1959, 1966) hence fungal attack of maize becomes very important if we consider that porridge, a popular weaning diet all over Nigeria and a major light meal for adults, comes from maize.

It is in the light of this that farmers and distributors of maize must consider seriously, methods of improving maize drying, better handling and
The deterioration of commercial maize (Zea mays) by insects and fungi, Matt F. Ivbijaro, E.O. Osisanya and E.E. Akinlade.

distribution systems to obviate a possible threat to health.

Acknowledgement

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THE EFFECT OF TEMPERATURE AND CULTURE MEDIUM ON THE GROWTH AND REPRODUCTION OF CHAETOMIUM GLOBOSUM

P.M.T. Curran and B. Hegarty

Summary. The effect of six temperatures and four agar media made up with seawater and distilled water on the radial growth and perithecial production of Chaetomium globosum has been studied. Generally, optimum growth occurred between 20 and 30°C. Sporulation depended mainly upon the nature of the medium. Seawater did not inhibit radial growth but did affect perithecial production.

Introduction

Although terrestrial Ascomycetes are rarely found in the sea, C. globosum was found by Byrne (1971) to develop on Scots pine test blocks submerged in 100% seawater when subsequently incubated for several weeks. Jones (1962) reported the occurrence of C. globosum on beech test blocks submerged in the sea. Byrne (1971) reported that C. globosum, grown in still yeast extract liquid medium made up with seawater, produces optimum growth in seawater than in the same medium made up with distilled water. Banerjee and Jones (1975) examined reproduction of C. globosum extensively in wood rotting studies (Savory and Pinion, 1958; Corbett, 1965; Eaton & Jones, 1971). Very little work has been carried out on the effect of culture medium on its growth and reproduction. Eanes and Lowy (1970) studied the effect of culture medium on the pattern of wood decay by C. globosum and Byrne & Jones (1975) examined reproduction of C. globosum in response to salinity. The present work was undertaken to study the effect of temperature and culture medium (using seawater and distilled water for comparison) on the radial growth and production of perithecia of C. globosum.

Materials and Methods

Four media were used in the temperature experiment: (a) 2% Malt Extract Sea Water Agar (2% ME SW) containing malt extract, 20 g; agar, 20 g; sea water, 1 litre. (b) Johnson and Sparrow's (1961) Sea Water medium (J.S.) containing peptone, 0.1 g; glucose, 1 g; dibasic potassium phosphate, 0.05 g; ferric citrate, 0.01 g; agar, 18 g; sea water 1 litre. (c) Glucose Yeast Extract Sea Water Medium (GYE SW) containing glucose, 10 g; yeast extract, 3 g; agar, 20 g; sea water 1 litre. (d) Sea Water Agar (SWA) containing agar, 18 g; sea water 1 litre. The same media, prepared with glass distilled water, were also used. All media were autoclaved at 15 lb/in² for 15 min and cooled before pouring into 8.5 cm sterile disposable petri dishes.

To prepare the spore suspension inoculum, perithecia of Chaetomium globosum Kunze IMI 16203 were scraped gently and aseptically from the surface of mature, two week cultures (grown on GYE SW and 2% ME DW) and placed in 1 ml of sterile 0.02% Tween 80 with glass beads in a McCartney bottle. The perithecia were broken by vigorous shaking; sterile glass rods with flattened tips were also used to crush the perithecia. Perithecial wall fragments were also allowed to settle and the ascospore supernatent was decanted into an empty McCartney bottle. Spore density was estimated using a Neubauer counting chamber and adjusted with sterile distilled water to approximately 10⁵ spores/ml.

The fungus was grown in triplicate on the seawater and distilled water media at 5°, 12°, 15°, 20° and 30°C. The dried surface of each agar plate was inoculated centrally with one drop of a spore suspension of C. globosum from a sterile pasteur pipette, calibrated to deliver 0.02 ml per drop. The pasteur pipette was held at right angles to the agar surface and approximately...
The effect of temperature and culture medium on the growth and reproduction of Chaetomium globosum, P.M.T. Curran and B. Hegarty.

one inch from it. Then, one drop was allowed to fall onto the agar surface, carefully to avoid "splashing". The inoculated plates were kept flat on the laboratory bench until the drops had dried, so as to achieve circular colonies on incubation. The culture plates were then incubated at the appropriate temperatures for two weeks.

For growth estimation, two measures (in cm) of each colony at right angles to each other were taken. A mean diameter was calculated from each triplicate set of plates (Figure 1). Standard errors of the mean were either zero or very low, the highest being 0.54 cm (for an almost 'full' 8.5 cm plate at 30°C). For sporulation estimation, two series of triplicate sets of the seawater and distilled water media were inoculated (a) one series using spore suspension as for growth estimation and (b) the second series using a mycelial plug (diameter 6 mm) taken from a two week old culture on SWA or DWA. The plates were incubated at various temperatures (Figure 2) for two weeks.

To count the perithecia, a paper disc (Curran, 1971) with 12 holes (four on each of 3 concentric circles, central, median and peripheral) was used to mark 12 points on each culture plate base. Mature perithecia were counted in a microscopic field (x100) by focusing, initially on each marked point, and then onto the perithecial level, having the base of the plate on the stage of the microscope. The total number of perithecia in the 12 microscopic fields was obtained for each culture plate; each point on the graph (Figure 2) is the mean of 36 counts (triplicate plates).

Results

Radial growth of C. globosum was enhanced from 5° to 25°C on all seawater and distilled water media except GYE SW on which radial growth was enhanced from 5° to 15°C (Figures 1 and 2). However, colony morphology was visually different on the four media using either seawater or distilled water (Figures 5.3 and 5.4). Even though Figures 1 and 2 show similar radial growth rates particularly on 2% ME, J.S. and the water agars, Figures 3 and 4 clearly show that the number of mature perithecia produced on the media differed, using either seawater or distilled water. While mature perithecial production occurred on 2% ME, GYE and J.S., 2% ME supported greatest sporulation using either seawater or distilled water. No perithecia were observed on SWA at any temperature; perithecial production was negligible on DWA. No perithecia were produced at 12° or 15°C on any of the seawater media. Using distilled water, perithecia were produced at 12°C on 2% ME only, and at 15°C on 2% ME, GYE and J.S.; mature perithecial production was greater on GYE DW than on GYE SW. The optimum temperatures for mature perithecial production were 20° - 30°C on 2% ME, 30°C on GYE and 30°C on J.S. using either seawater or distilled water. Figures 5.1 and 5.2 show visually the production of mature perithecia (on 2% ME, SW and 2% ME DW respectively) at six temperatures.

Discussion

The results of the work presented here shows that although it is evident that C. globosum produces similar radial growth rates on different culture media, it is obvious that colony morphology differs on each of the four media employed. This difference appears to be mainly due to sporulation response of the fungus to the culture media, and, although easily observed on agar media, was not detected when liquid cultures were employed (Curran, 1977).

Contrary to what might be expected, seawater had no inhibitory affect on the radial growth of C. globosum. However, it did have an inhibitory effect on perithecial production at 12° and 15°C, mainly on 2% ME; perithecial production was inhibited on GYE SW at all temperatures tested in comparison to production on GYE DW. Byrne and Jones (1975), employing corn meal agar at 20°C, found that C. globosum Kunze ex Fr. produced 45 perithecia per cm² on the media prepared with 40% seawater and 29 perithecia per cm² on the media made up with distilled water. However, the number of perithecia significantly decreased from 40% seawater medium to 70% seawater medium and they found no perithecia on 80, 90 and 100% seawater media. Gray, Pinto and Pathak (1963) studied the effect of seawater on protein synthesis and growth of twenty three terrestrial fungi (not including C. globosum) and found that the dry weight of mycelia was greater from seawater media than from distilled water media in all except two fungi employed.

Malt extract was found to be the optimum medium for the radial growth and perithecial production of C. globosum. This result complements those of Kaune (1970) and Banerjee and Levy (1970) when they employed malt extract in wood decay studies using C. globosum.

It is evident from the results presented here and those of Richie (1959), Gray et al., (1963) and Byrne (1971) that some terrestrial fungi can grow well in seawater and in distilled water media. It is also evident from the results presented here and those of Byrne and Jones (1975) that sexual reproduction of some terrestrial fungi, e.g., C. globosum is more sensitive to salinity stresses than vegetative growth.

References


The effect of temperature and culture medium on the growth and reproduction of *Chaetomium globosum*, P.M.T. Curran and B. Hegarty.

Fig. 1. Effect of temperature and seawater culture media on the radial growth of *C. globosum*.
- ○ 2% ME SW; □ GYE SW; ○ J.S. SW; □ SWA.
  Incubation period: 14 days.

Fig. 2. Effect of temperature and distilled water culture media on the radial growth of *C. globosum*.
- ○ 2% ME DW; □ GYE DW; ○ J.S. DW; □ DWA.
  Incubation period: 14 days.

Fig. 3. Effect of temperature and seawater culture media on mature perithecial production of *C. globosum*
using spore suspension inoculum --- and mycelial plug inoculum

Symbols for media as in Figs. 1 and 2.
Incubation period: 14 days.

Fig. 4. Effect of temperature and distilled water culture media on mature perithecial production of *C. globosum*
using spore suspension inoculum --- and mycelial plug inoculum

Symbols for media as in Figs. 1 and 2.
Incubation period: 14 days.
The effect of temperature and culture medium on the growth and reproduction of *Chaetomium globosum*, P.M.T. Curran and B. Hegarty.

1. *C. globosum* on 2% ME SW at (a) 5°, (b) 12°, (c) 15°, (d) 20°, (e) 25° and (f) 30°C after two weeks.

3. *C. globosum* on (a) J.S.SW, (b) 2% ME SW, (c) SWA and (d) GYE SW. Two weeks at 25°C.

2. *C. globosum* on 2% ME DW at (a) 5°, (b) 12°, (c) 15°, (d) 20°, (e) 25° and (f) 30°C after two weeks.

4. *C. globosum* on (a) J.S. DW, (b) 2% ME DW, (c) DWA and (d) GYE DW. Two weeks at 25°C.

**Figure 5**
The effect of temperature and culture medium on the growth and reproduction of *Chaetomium globosum*, P.M.T. Curran and B. Hegarty.


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LABORATORY TEST METHODS FOR ASSESSING THE ALGICIDAL PROPERTIES OF BIOCIDAL WASH PREPARATIONS

L.H.G. Morton

Summary. A method of obtaining bacteria-free algal suspensions from fouled stonework is presented together with a test method for assessing the algicidal properties of biocidal wash preparations. Two methods for determining the toxic level of algae are described. Persistence or otherwise of green pigmentation is the criterion used for assessing the viability of algae.

Methods d'essais de laboratoire pour estimer les propriétés algicides de préparations bloclées de lavage. On présente une méthode pour obtenir des suspensions d'algues sans bactéries à partir d'ouvrages en pierre infectés ainsi qu'une méthodes d'essais pour mesurer les propriétés algicides des préparations bloclées de lavage. Deux méthodes pour estimer le seuil toxique du biocide sont décrites. La persistance ou, par ailleurs, une pigmentation verte sont les critères utilisés pour mesurer la viabilité des algues.

Introduction

Epiphytic algal growth occurs on porous stonework and on painted surfaces where conditions of dampness, warmth and light are available (Hueck-van der Plas, 1968; Whiteley, 1973; Richardson, 1973; and Springle, 1975). Smooth surfaces are less affected than rough cement based surfaces with high porosity. Fouled stone surfaces are in the main considered to be simply dirty, neglected and unsightly. However, algae, when dead, may constitute the major source of dirt and are considered to be the for-runners of lichens and mosses capable of extensive corrosive activity.

There are in existence numerous toxic washes which may be used for the elimination and prevention of algal growths on stonework and other surfaces. The Building Research Digest 139 (Anon. 1972) lists some of these compounds whilst others are included in Hueck-van der Plas (1968) and in Richardson (1973).

The choice of a toxic wash preparation may involve extensive preliminary trials in order to assess its suitability and often time does not permit this. There is need, therefore, for a rapid laboratory sorting test.

A laboratory assay procedure of this kind requires suitable algal cultures obtained preferably from the fouled surface to be tested. The collection and maintenance of such cultures is often a difficult procedure since the autotrophic bacteria collected with the algae thrive well in media containing mineral salts. Goryunova et al. (1965) and Wieringa (1968) review methods which have been used to obtain pure cultures of blue green algae. Whilst many workers have been successful, the techniques employed often require considerable skill and extensive subculturing onto suitable media to assess the levels of bacterial persistence.

This paper presents a relatively simple experimental procedure for obtaining bacteria-free mixed algal cultures and also outlines two suitable test procedures.

Preparation of Mixed Algal Culture

Epiphytic algal growth on stonework is removed by scraping the surface with a flame'd scalpel. The algal 'dust' obtained in this manner is suspended in 10 ml of sterile distilled water contained in a universal bottle. This crude algal suspension is inoculated directly into 150 ml of Medium C (Kratz and Myers, 1955) containing 12 units/ml of penicillin, held in a conical flask. The flask is placed in an illuminated orbital shaker and incubated at 25°C for 7-10 days by which time a mixed culture of algae should be available for assay purposes.

A shaker which has been found suitable for this test is made by Gallenkamp; it has seven fluorescent tubes of 30 w each, mounted 350 mm above the flasks. Alternatively, the test will work quite well if flasks are placed in direct sunlight on a window ledge. However, this is only successful in summer. The flasks must be shaken daily.

Culture Medium

Medium C of Kratz and Myers (1955) is as follows:

Medium C of Kratz and Myers (1955) is as follows:

1 Preston Polytechnic, Corporation Street, Preston PR1 2TQ.

(Received, July 1979).

MgSO₄ · 7H₂O 
K₂HPO₄ 
Ca(NO₃)₂ · 4H₂O 
KNO₃ 
Na₃ citrate 2H₂O 
NaHCO₃ 
Fe₂(SO₄)₃ · 6H₂O 
Microelements solution
Water to one litre.

Microelements solution:
H₃BO₃ 
MnCl₂ · 4H₂O 
ZnSO₄ · 7H₂O 
MoO₃ 
CuSO₄ · 5H₂O 
Water to one litre.

The medium can conveniently be made up by using the following concentrated stock solution to make up one litre:

MgSO₄ · 7H₂O 50 g in 200 ml. Add 1 ml.
KH₂PO₄ 125 g in 500 ml. Add 4 ml.
Ca(NO₃)₂ · 4H₂O 5 g in 200 ml. Add 1 ml.
Na₃ citrate 2H₂O 33 g mixed in 200 ml. Add 1 ml.
Fe₂(SO₄)₃ · 6H₂O 0.08 g
KNO₃ and NaHCO₃ are added as solids.

In order to suppress non-algal growths in the above Kratz and Myers medium, penicillin is added at the rate of 12 units per millilitre. A suitable stock solution of penicillin is Penicillin - G (Benzylpenicillin), SIGMA Stock No. PEN - NA. The medium is first sterilized by autoclaving at 121°C for 20 minutes. When cool the penicillin is added. The penicillin solution may be sterilized by filtration before it is added to the stock but this has not been found to be essential.

Determination of toxic level (lethal dose) of biocide

Method 1 By the addition of increasing quantities of biocide to a fixed volume of algal suspension.

In this method quantities of the biocidal preparation being assayed ranging from 0.05 to 5.0 ml are introduced into sterile tubes containing 5.0 ml of algal suspension. The volume in each tube is made up to 10 ml with sterile distilled water where necessary so that each viability assessment can be made in the same volume of liquid. The tubes are illuminated as above and held at room temperature for 10 days. The criterion for assessing the viability of the algae is the persistence or otherwise of green pigmentation.

The volume of biocide to be added will, of course, depend on its concentration and effectiveness, but two suitable working ranges of biocide/algal suspension have been found to be:

Millilitres of biocide solution added to 5.0 ml aliquots of algal suspension:
Range 1 0.05 0.1 0.15 0.2 0.25 0.3 0.35 0.4 0.45 0.5
Range 2 1.0 1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0

Method 2 By the introduction of a standard volume of algal suspension into a solution containing varying amounts of growth medium and biocide solution.

In this method 10 ml aliquots of algal suspension are introduced into flasks containing a standard volume of solution made up of varying amounts of growth medium and biocide solution. The flasks are incubated in the orbital shaker with illumination for one week at 25°C. Viability of the algal test organism is assessed by the pigmentation criterion. A suitable initial working range of dilutions is as follows:

<table>
<thead>
<tr>
<th>Flask</th>
<th>Volume of medium C ml</th>
<th>Volume of biocide ml</th>
<th>Volume of algal suspension ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>90</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>B</td>
<td>80</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td>C</td>
<td>60</td>
<td>40</td>
<td>10</td>
</tr>
<tr>
<td>D</td>
<td>40</td>
<td>60</td>
<td>10</td>
</tr>
<tr>
<td>E</td>
<td>20</td>
<td>80</td>
<td>10</td>
</tr>
<tr>
<td>F</td>
<td>100</td>
<td>0</td>
<td>10</td>
</tr>
</tbody>
</table>

Confirmation of Algal Death

Algal death can be confirmed by collecting algal debris resulting from method 1 or 2. The debris should be washed by decanting and centrifuging techniques and then introduced into flasks containing sterile 100 ml volumes of medium C. The flasks should be incubated at 25°C in the illuminated orbital shaker or incubated at room temperature, for 2 weeks. Death of the algae is confirmed when there is no re-occurrence of pigmentation.

References

Anon. (1972). Control of lichens, moulds, and similar growths. Building Research Station Digest No. 139 (March).


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THE EFFECTS OF FILTERING AGENTS UPON THE ACTIVITY OF PRESERVATIVES IN CUTTING FLUIDS

I.U. Onyekwelu and E.O. Bennett

Summary. The effects of kaolin, bentonite, diatomaceous earth and cellulose filtering agents upon preservatives in petroleum base, semisynthetic and synthetic cutting fluids were studied. These generally had an adverse effect upon inhibitory properties; however, the specific effect was related not only to the filtering agent/preservative combination but also to the particular type of cutting fluid as well. Cellulose was found to have the least deleterious effect upon the inhibitors in all three types of coolants.

The inhibitory properties of Dowcide A, Milidin TI-10 and Dowcide 75 were increased by the filtering agents in petroleum base products. Dowcide A exhibited improved activity in the presence of the filtering agents in semisynthetic coolants and bentonite, diatomaceous earth and cellulose increased the inhibitory activity of Proxel CRL in these same coolants. The inhibitory properties of Sodium Omadine were improved by the agents in synthetic fluids.

The inhibitory properties of Tris Nitro were markedly reduced by the filtering agents in all three classes of cutting fluids.

The test units consisted of wide mouth glass contain­ers of sufficient size to hold approximately 1L of liquid. Each unit was aerated so that unifonn rolling of the coolant was obtained in each unit.

Experimental Procedure

The test units consisted of wide mouth glass contain­ers of sufficient size to hold approximately 1L of liquid. Each unit was aerated so that uniform rolling of the coolant was obtained in each unit.

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The effects of filtering agents upon the activity of preservatives in cutting fluids, I.U. Onyekwelu and E.O. Bennett

Five hundred ml of tap water (approximately 120 ppm hardness) and then the additives were added to each container. The kaolin powder was acid washed American standard quality obtained from Fisher Scientific Co.; the bentonite powder was laboratory grade obtained from Fisher Scientific Co.; the diatomaceous earth powder was analytical grade obtained from J.T. Baker Chemical Co.; and the cellulose powder consisted of medium length fibres with a maximum ash of 0.015% containing no more than 5 ppm Fe and 2 ppm Cu obtained from Whatman, Ltd., England. Five gms of each filtering agent were added to the test units and mixed thoroughly. Bentonite and diatomaceous earth settled to the bottoms of the test units while kaolin and cellulose powders remained suspended in the lubricants.

The preservatives and concentrations employed in this investigation were as follows:

**DOWICIDE A**  
- 1000 ppm - A 97% active powder of sodium o-phenyl-phenate produced by The Dow Chemical Company, Midland, Mich., U.S.A.

**TRIS NITRO**  
- 1000 ppm - A 50% solution of tris(hydroxymethyl)nitromethane produced by the IMC Chemical Group, Des Plaines, Ill., U.S.A.

**GROTAN**  
- 1000 ppm - A 78.5% solution of hexahydro-1,3,5-tris(2-hydroxyethyl)1,2-triazine produced by The Lehn & Fink Industrial Products Div. of Sterling Drug, Inc., Montvale, N.J., U.S.A.

**MILIDIN Ti-10**  
- 1000 ppm - A solution (concentration not given) of hexahydro-1,3,5-tris(2-hydroxyethyl)1,2-triazine-iodine complex produced by The DeMille Chemical Corp., Jersey City, N.J., U.S.A.

**VANCIDE TH**  
- 1000 ppm - A 95% solution of hexahydro-1,3,5-triethyl1,2-triazine produced by The R.T. Vanderbilt Company, Inc., New York, N.Y., U.S.A.

**DOWICIL 75**  
- 1000 ppm - A 67.5% active powder of 1-(3-chloroallyl)-3,5,7-triaza-azolialdiamantane chloride produced by The Dow Chemical Company, Midland, Mich., U.S.A.

**BIOBAN P-1487**  
- 1000 ppm - A solution containing 70% 4-(2-nitrobutyl)morpholine and 20% 4,4-(2-ethyl-2-nitromethylene)dimorpholine produced by IMC Chemical Group, Des Plaines, Ill., U.S.A.

**SODIUM OMADINE**  
- 1000 ppm - A 40% solution of sodium pyridine thiol-4-oxide produced by Olin Corp., Stamford, Conn., U.S.A.

**PROXEL CRL**  
- 500 ppm - A 30% solution of 1,2-benzisothiazolin-3-one produced by ICI United States, Inc., Wilmington, Del., U.S.A.

**KATHON 886**  
- 100 ppm - A solution containing 8.6% of 5-chloro-2-methyl-4-isothiazolin-3-one and 2.8% 2-methyl-4-isothiazolin-3-one and 2.6% 2-methyl-4-isothiazolin-3-one produced by Rohm & Haas Company, Philadelphia, Pa. U.S.A.

In order to avoid confusion to those who use these products, they were used as received in the concentrations given.

The coolant concentrate was added (15.0 ml) and thoroughly mixed until a uniform emulsion was formed. The petroleum base products consisted of Shell Dromus B and Texaco 591, the synthetic products consisted of Shamrock and Monroe coolants and the semi-synthetic products were DoAll and Quaker formulations.

The coolant was diluted to 600.0 ml by adding tap water and the liquid level was carefully marked upon the container. Once each week, distilled water was added to bring the liquid level back to this mark. Distilled water was used in order to avoid a buildup of organic salts.

Each unit was inoculated with 1.0 ml of each of a bacterial and a mould inoculum and reinoculated once each week thereafter with 1.0 ml of a 50–50 mixture of the two inocula. The organisms found in the inocula originally came from spoiled samples of industrial cutting fluids. The bacterial inoculum, while subject to some variation, consisted predominantly of different species of pseudomonads with lesser numbers of *Paracolobacterium*, *Proteus* and *Klebsiella* species. The mould culture consisted mostly of *Fusarium* and *Cephalosporium* species with minor numbers of *Candida* and *Monilia* organisms. The bacterial inoculum was maintained under constant aeration in a petroleum base cutting fluid known to be highly susceptible to bacterial attack. The mould inoculum was maintained under constant aeration in a synthetic coolant which was highly susceptible to slime formation. Each week the containers were shaken vigorously and approximately three-fourths of the coolant removed and fresh fluid added in order to maintain vigorous growth. Neither of the two inocula had been grown on any substrate other than cutting fluids for several years. The bacterial inoculum contained between 25 to 100 million organisms while the mould culture contained between 100,000 to 250,000 units/ml.
Once each week, each unit was examined for its microbial content using standard microbiological techniques designed to elucidate numbers of bacteria and molds. Subculture media were incubated at 35°C and read after 48 hours. Tests were continued until two consecutive counts in excess of 100,000 organisms/ml were obtained or until visible slime formed in the units. All units which contained less than 100,000 organisms/ml or did not develop slime were studied for 105 days.

Every effort was made to provide maximum challenge to the chemicals employed to treat the coolants. All units were open to aerial contamination; no effort was made to prevent introduction of organisms via the compressed air or the water employed to dilute the coolants.

All counts, inoculations, subculturing make-up and any other practice was done at the same time each week in order to minimize any variation in results from this source.

All experiments were done at room temperature (25°C). The coolants employed exhibited a pH range from 7.6 to 9.6. The products consisted of two synthetic, two semisynthetic and two petroleum base lubricants.

Since the investigation constitutes a continuation of previous work pertaining to antimicrobial agents in cutting fluids, a number of controls were included. One set of controls consisted of four units containing a common petroleum base product treated with 1000 ppm of Milidin TI-10. These units have failed in 21 to 28 days over the past several years and performed normally during this investigation. Duplicate controls of each cutting fluid without preservative were included and showed no inhibitory activity. Duplicate controls containing each filtering agent showed no inhibitory activity. All experiments were done in duplicate and where the date was of significant importance, experiments were repeated a number of times in order to assure reproducibility.

The data exhibited in Table 1 were developed by averaging the results of each experiment and calculating the percentage differences between the controls of preserved coolant and those consisting of preserved coolant plus the filtering agent.

Results

Table 1 exhibits the results of the study with filtering agents. Due to the way the results are presented it was concluded that the inclusion of control data might confuse the reader so this information was not included. It is sufficient to state that none of the filtering agents exhibited antimicrobial properties in the cutting fluids alone, nor did any of the cutting fluids exhibit antimicrobial properties in the absence of treatment with a preservative.

At the beginning of the work it was expected that the results would be much more clearcut than those obtained. It was thought that a particular filtering agent would have an uniform effect upon a preservative. It was expected that groups of cutting fluid preservatives such as the triazines would exhibit a similar pattern. It may be noted that this is not the case. The specific effect of a filtering agent is not only related to the particular preservative involved but is also related to the type of cutting fluid used.

All of the filtering agents improved the inhibitory properties of Dowicide A in petroleum base and semisynthetic fluids. Kaolin improved the inhibitory properties of Dowicide A in synthetic fluids; bentonite produced a 50% reduction in activity and diatomaceous earth and cellulose had no significant effect upon the inhibitor.

The antimicrobial properties of Tris Nitro were adversely affected by the filtration agents in all cutting fluids. Tris Nitro owes a major portion of its antimicrobial properties to the liberation of formaldehyde and was expected to show similar results to those obtained with the triazine compounds.

It might be expected that the effects of the filtration agents upon the triazine compounds would be similar since these chemicals also release formaldehyde. The results are quite different indicating that the effects of the filtering agents are probably related to some type of reaction with the original compound rather than some interaction with formaldehyde after it is produced. Milidin TI-10 was least adversely affected in the petroleum base products; Milidin TI-10 and Vancide TH were least affected in the semisynthetic products and Grotan and Vancide TH were the least affected in the synthetic products.

Dowicil 75 exhibited improved antimicrobial properties in the presence of the filtering agents in the petroleum based products; however, the preservative was adversely affected by the filtering agents in the semi- and synthetic products.

Bioban P-1487 was adversely affected by kaolin, bentonite and diatomaceous earth while cellulose improved inhibitory activity in the petroleum products.

Kaolin had an adverse effect upon Proxel CRL in all cutting fluids while bentonite, diatomaceous earth and cellulose either had no effect or improved inhibitory properties of the preservative in petroleum and semisynthetic fluids. Unfortunately, due to the unusual effectiveness of Kathon 886 all experiments ran the entire 105 day test period in the petroleum and semisynthetic fluids so no differences could be detected. Proxel CRL and Kathon 886 both were adversely affected by the filtering agents in the synthetic products.

Discussion

There are several areas of concern when filtration materials come in contact with cutting fluids. Filtering agents may be a source of contamination of the
Table 1
Effect of filtration materials on the inhibitory activities of preservatives in petroleum base, semi-synthetic and synthetic cutting fluids.
The figures in the body of the table indicate the percent change from coolant preservative control.

<table>
<thead>
<tr>
<th>Preservative</th>
<th>Petroleum base</th>
<th>Semi-synthetic</th>
<th>Synthetic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Dowcide A</td>
<td>+88</td>
<td>+84</td>
<td>+102</td>
</tr>
<tr>
<td>Tris Nitro</td>
<td>-76</td>
<td>-76</td>
<td>-76</td>
</tr>
<tr>
<td>Grotan</td>
<td>-20</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Milidin TI-10</td>
<td>*</td>
<td>+83</td>
<td>+85</td>
</tr>
<tr>
<td>Vancide TH</td>
<td>-20</td>
<td>-43</td>
<td>*</td>
</tr>
<tr>
<td>Dowcrl 75</td>
<td>+81</td>
<td>+144</td>
<td>+85</td>
</tr>
<tr>
<td>Bioban P-1487</td>
<td>-33</td>
<td>-17</td>
<td>-17</td>
</tr>
<tr>
<td>Sodium Omadine</td>
<td>-12 *</td>
<td>-12</td>
<td>-71</td>
</tr>
<tr>
<td>Proxel CRL</td>
<td>-19</td>
<td>*</td>
<td>+26</td>
</tr>
<tr>
<td>Kathon 886</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
</tbody>
</table>

1-40 oil to water ratio

* Difference less than 10%.

Each number represents an average of duplicate determination on two different cutting fluids.

Filtering agents — 5 gms/test unit
1. Kaolin
2. Bentonite
3. Diatomaceous earth
4. Cellulose
lubricant; they may serve as an environment for microbial growth; they may remove components of the cutting fluid; and they may interfere with preservative action.

**Filtering agents as a source of contamination**

Filteration materials can contain organisms that contaminate products being filtered (Woodward & McNamara, 1970). Twenty-three samples of kaolin and sixteen samples of chalk were found to contain from 100 to over 10,000 organisms/gm (Westwood & Pin-Lim, 1971). Clay employed in pharmaceutical formulations has been found to contain up to 40,000 mould segments/10.9 gms. In one instance, the clay employed in the pharmaceutical product was the source of organisms creating a biodeterioration problem (Sokolski et al., 1962). In a similar instance, it was found that sulfate-reducers contaminated kaolin during industrial processing and later produced a deterioration problem when the material was used as a filtering medium (Gay, 1971).

**Filtering agents as an environment for microbial growth**

Filter materials can serve as a site of attachment of microorganisms where they feed upon the liquid passing through the equipment. Kaolin can adsorb staphylococci (Barr, 1957), streptococci (Oksentyan, 1940), Saccharomyces species (Oksentyan, 1940), and oil bacteria (Mueller & Hcikisch, 1972) but not the gram-negative organisms (Barr, 1957). On the other hand, one of the major problems associated with filtration of coolants has been slime formation which binds the filter and reduces the rate of flow (Shuhan, 1970; Carter, 1970).

Filtering agents have different effects upon metabolic activities and microorganisms. They can reduce metabolism (Oksentyan, 1940), respiratory rates (Puelleaux & Maigman, 1976) and hinder nitrate oxidation (Tandon & Mishra, 1968).

Kaolin and bentonite stimulate the growth of a number of organisms including pseudomonads (Lahar & Keynan, 1962; Stotzky & Rem, 1966; Vagner & Stroubikova, 1970). Increasing concentration of bentonite improves the rate of ammonization of microorganisms (Filip, 1969), increases the production of ethyl alcohol and the biomass of yeast (Velikanov & Zvyagintsev, 1967). The degree of stimulation increases as the pH is adjusted upward.

It has been suggested that these clay products constitute a source of minerals required for growth; however, all of the stimulation cannot be accounted for on this basis alone. It has been demonstrated that sulfate-reducing organisms cannot grow in cutting fluids in the absence of aerobic organisms (Guymes & Bennett, 1959). Bentonite and diatomaceous earth absorb the toxic components and allow growth of the organisms (Isenberg & Bennett, 1959). Other workers (Pollock, 1947; Teitaro, 1953; Hirsch, 1954; Gorelick et al., 1951) have noted that absorbant materials remove toxic materials and promote growth of a number of organisms in microbiological media. The rate of oxidation of a saturated oil fraction component of a cutting fluid increases when the material is absorbed upon celite (Pierce & Clesceri, 1975).

**Filtering agents removing components from cutting fluids**

Coolant filters may remove components from lubricants and modify their composition. Cellulose filters generally absorb water while synthetic fibres take up oil which may disturb the oil-water ratio. Kaolin absorbs fatty acids such as sodium stearate, sodium caprylate, sodium oleate (Miskarli & Bairamov, 1964), heptadecanoic acid (Meyers & Quinn, 1973) and sorbic acid (McCarthy, 1969). Bentonite can be used to separate fatty acids from mixtures because its absorbtive capacity is greater for saturated than for unsaturated fats (Uyeno et al., 1943). This material also reduces the peroxide value of fatty products (Saka et al., 1957; Khair et al., 1958); it absorbs stearic acid (Tsitsishvile & Barnabishvili, 1965) and heptadecanoic acid (Myers & Quinn, 1973). It can be used to emulsify fats and oils. Bentonite may remove many cationic compounds from solutions (Nakashima & Miller, 1955). Talc and kieselguhr have been noted to absorb sorbic acid (McCarthy, 1969).

Kaolin absorbs cationic, anionic and nonionic surfactants (Barbaro and Hunter, 1967; Malik et al., 1972; Demyanova, 1965). Grinding fluids are often filtered to remove metal particulates and they commonly contain phosphate chemicals which may be absorbed by kaolin (Ginzburg, 1953). Bentonite absorbs cationic surfactants, boron compounds, electrolytes (Miskarli et al., 1971), glycols (Heller, 1965), triethanolamines (Batt, 1972) and amines (Slabaugh & Colbertson, 1951; Kurilenko & Mikhailuk, 1959; Uspenskaya & Shevchenko, 1972). It softens water (Bai, 1981); however, in the presence of triethanolamine, it releases cations such as sodium into solution.

There has been an interesting trend towards employing dyes in cutting fluid to color the products. Kaolin absorbs and removes crystal violet (Armstrong & Clarke, 1971), brilliant green (Thomas et al., 1966) and methylene blue (Caspke, 1930; Friedman & Kuydendale, 1934; Hofmann et al., 1967) from solution. The rate of removal of crystal violet increases as the pH is elevated. Bentonite removes acriflavine, gentian violet and methylene blue from solutions (Nakashima & Miller, 1955; Hofmann et al., 1967); however, talc does not absorb brilliant green (Thoma et al., 1966).

**Filtering agents interfering with antimicrobial agents**

There are many reports concerning the interference of filtering agents with antimicrobial agents. Bentonite, diatomaceous earth (kieselguhr, Celites), talc and kaolin neutralize the inhibitory properties of quaternary ammonium compounds (Nakashima & Miller, 1955; Batyjios & Brecht, 1957; Fitzgerald, 1960; Thoma et al., 1966; McCarthy, 1969; Bean & Dempsey, 1971; Rosen & Berke, 1973); however, they antagonize anionic and nonionic agents as well (Harris, 1961).

Kaolin does not absorb phenol or formaldehyde.
The effects of filtering agents upon the activity of preservatives in cutting fluids, I.U. Onyekwelu and E.O. Bennett

(Csipke, 1930) nor does it interfere with the activities of 8-hydroxyquinoline, p-chloro-m-cresol (Thoma et al., 1966; Yousef & El-Nakeeb, 1973), m-cresol (Bean & Dempsey, 1971) or phenol (Horn et al., 1971). Diatomaceous earth, kaolin and talc absorb pentachlorophenol and the rate of uptake increases as the pH is adjusted upwards (Nose et al., 1963). Bentonite neutralizes the inhibitory properties of hexachlorophene and reduces the activity of 2,2-methylene bis (3,4,6-trichlorophenol).

The results of this study indicate that filtering agents can interfere with the antimicrobial activity of formaldehyde releasing compounds. Since the results are quite different for different compounds of this type (for example those produced by tris nitro and the triazine compounds) there is an implication that the filtering agents interfere directly with the chemical rather than with formaldehyde produced as a result of their breakdown. The results also show that the filtering agents generally do not have an adverse effect upon onophenylphenate and in a number of instances there is an improvement in inhibitory activities.

The capacity of bentonite to take up and release antimicrobial agents is dependent upon the origin of the bentonite. The material has been noted to reduce the antimicrobial properties of chloramphenicol (Ask et al., 1973), tetracycline (Ask et al., 1973), sulfathiazole (Kubis, 1972), potassium iodide (Kubis, 1972) and penicillin (Doll, 1960).

Kaolin has an adverse effect upon the inhibitory properties of mercury compounds such as mercuric chloride (Csipke, 1930), penyl mercuric acetate (Thoma et al., 1966) and phenyl mercuric nitrate (Horn et al., 1971), as well as phenothiazine derivatives (Sorby et al., 1966), benzoic acid (Horn et al., 1971), p-chlorobenzoic acid (Horn et al., 1971), dehydroacetic acid (Horn et al., 1971) and chlorhexidine diacetate (McCarthy, 1969). It does not adversely affect the inhibitory properties of sodium ethylmercuric thiosalicylate (Thoma et al., 1966), sodium-6-sulfanilamido-2,4-dimethylpyrimidine (Thoma et al., 1966), copper salts (Malvezin et al., 1943), methyl paraben (Horn et al., 1971), benzy alcohol (Yousef & El-Nakeeb, 1973) and chlorophenoxytol (McCarthy, 1969).

Talc interferes with the activities of phenylmercuric nitrate, benzoic acid, p-chlorobenzoic acid, dehydroacetic acid (Horn et al., 1971) and chlorhexidine diacetate (McCarthy, 1969). It does not affect antimicrobial properties of phenyl mercuric acetate, sodium ethylmercuric thiosalicylate or 6-sulfanilamido-2,4-dimethylpyrimidine (Thoma et al., 1966), methyl paraben (Horn et al., 1971), benzyl alcohol, phenoxytol propylene or chlorophenoxytol (McCarthy, 1969).

Calite (diatomaceous earth) interferes with the inhibitory action of amines, pyridimine, quinone and copper compounds (Fitzgerald, 1960) while cellulose reduces the activity of chlorhexidine diacetate (McCarthy 1969) but not hexadecyl trimethylammonium bromide (Christenson and Shelton, 1948).

It should be noted that the results of this investigation were produced from only two coolants of each representative type. Cutting fluid formulations are very diverse; therefore, the results cannot be directly applied to all cutting fluid products which are available throughout the world. The data does show that agents employed to filter cutting fluids can have a marked effect upon preservative efficacy and therefore, this factor should be carefully considered in the control of biodeterioration of these products.

Acknowledgements

The following companies are acknowledged for their cooperation and financial support which has made this paper possible.


References


The effects of filtering agents upon the activity of preservatives in cutting fluids, I.U. Onyekwelu and E.O. Bennett


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THE ROLE OF INSECTS IN THE BIODETERIORATION OF INDIAN RED PEPPERS BY FUNGI

M. Seenappa, L.W. Stobbs and A.G. Kempston

Summary. Indian red peppers are known to contain Aspergillus flavus and aflatoxin. In an attempt to improve quality at the source, the microbiological quality of red pepper as it entered storage in several locations in India was determined, and factors contributing to deterioration during storage were studied. Before storage began, the total mould count at all locations exceeded permissible limits for finished products in importing countries, and insect infestation was common. Evidence is provided that insects cause a small percentage of pods to become completely filled with aspergilli prior to storage. These pods show no surface growth of mould, but can be readily identified by their yellow discoloration. They act as reservoirs of severe infection which were not represented in the stringent sampling plan used to determine "total" mould growth in a consignment. During storage, the incidence of aspergilli-loaded pods increased, depending on insect infestation and relative humidity. Although the long term solution to storage deterioration would involve humidity control and the elimination of insects, it is recommended that a program be implemented immediately to remove all discoloured, aspergilli-loaded pods by hand, at every stage of production.

Introduction

Aflatoxin-producing strains of Aspergillus have been shown to be a prominent mould contaminant in Indian red pepper (Capsicum annuum L.) in both India itself (Pal and Kundu, 1972) and in importing countries (Christensen et al., 1967; Flannigan and Hui, 1976). The ICMSF (International Commission on Microbiological Specifications for Foods) (1974) has suggested that the "total" mould count in dried spices should not exceed 10,000 per g; but Christensen et al., (1967) reported that red peppers of Indian origin had levels of A. flavus contamination as high as 200,000 per g. Although the degree of A. flavus contamination may not be directly correlated with the amount of aflatoxin in food, Schindler and Eisenberg (1968) found that red pepper was a good substrate for aflatoxin production and all 6 samples of Indian red pepper included in a Canadian study of spices by Scott and Kennedy (1973) did contain aflatoxin.

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Where dried foods may present problems resulting from the presence of mycotoxins, the ICMSF (1974) advocates quality control at the source in preference to testing the final product for the causative organism and direct examination for the mycotoxin. For spices, Pal and Kundu (1972) have explained why control at the source must be preceded by a detailed study of the drying, storage and handling procedures. This report is the result of a study performed in India during the 1977–1978 crop year to determine the factors governing the contamination and proliferation of Aspergillus flavus in red pepper, and to suggest remedial measures for the implied aflatoxin hazard. The culture methods used would exclude members of the Aspergillus glaucus group.

Materials and Methods

During the 1977–1978 crop year, samples were collected from storage godowns and market places throughout the red pepper growing areas of Karnataka State in South India (Bangalore, Byadgi, Chickballapur, Dharwar and Hubli). The relative humidity (RH) at each site was monitored with a hair hygrometer (Fisher).

Red peppers are stored in gunny bags measuring 44 inches by 26½ inches. Using the terminology of the ICMSF (1974), 100 gunny bags at a site were designated as a 'lot'. From each of the 7 lots, 20 or 25 'field samples' of 500g were withdrawn by grab sample using the gloved hand, as randomly as possible. One 10g 'sample unit' from each field sample was analyzed for total mould count using Potato Dextrose Agar (PDA, Difco) supplemented with 30 mg of tetracycline hydrochloride per and incubated for 5 days at 25°C. This technique would select against isolation of members of the A. glaucus group and understandably these were not found among the isolates. However, the intention of the present study was primarily to test for Aspergillus flavus. When the predominant moulds were aspergilli, isolates were transferred to the Aspergillus Differential Medium (ADM) of Bothast and Fennell (1974) to determine the incidence of members of the A. flavus group. Identification of Aspergillus isolates was based on colony colour and gross morphology of conidial heads (Raper and Fennell, 1965). Each lot was also sampled at 5 day intervals for 30 days to determine the incidence of discoloured pods which were pale-coloured or yellow instead of red. In this sampling procedure, the field sample was 500 pods and a decision was made on each pod (n=500).

ADM was also used to determine the presence of A. flavus on insect parts and insect excreta which were associated with the red peppers. To determine the internal flora of pellets of insect excreta, the surface of the pellets was disinfected in a 0.2% solution of sodium hypochlorite prior to plating. Insect parts, and larvae mounted in Lactophenol Cotton Blue, were examined under a light microscope to demonstrate surface contamination of the insects and the internal microflora of their larvae. To confirm the results of light microscopy, insects were mounted directly on aluminum stubs using conductive cement, coated with 300 to 400 Å gold in an International Scientific Instruments (ISI) sputter coater, and examined in an ISI Super III A Stereoscan electron microscope at 25 kV.

Results and Discussion

Red pepper is grown on numerous small plots throughout Karnataka State in South India. The pods are picked by hand and spread on the ground to dry for 8 to 10 days, after which they are transported to the godowns (warehouses) or markets of a few central cities. A 'lot' of 100 bags of pepper pods would include the harvest of several farmers handled by numerous workers and exposed to various soil and weather conditions. By the time the peppers reached the godowns or markets it was expected that the microbiological quality within lots would vary greatly when analyzed by a sampling plan in which the number of 10-g sample units (n) was 20 or 25. It was also expected that this plan would expose differences among lots which would reflect prevailing variables in the godowns and markets, such as RH and insect infestation. The mould counts presented in Table 1 are displayed in accordance with the ICMSF values for a 3-class plan in which \( m=10^2 \) and \( M=10^4 \). There were differences among lots. All 20 samples of lot 5, for example, had counts less than 10,000 per g while 17 samples of lot 3 had more than 10,000 moulds per g. However, these differences have no practical significance since all lots would require sterilization to be accepted by ICMSF specifications. Contrary to expectations, the distribution of mould counts within lots was narrow; rarely exceeding 2 log cycles. Further evidence of consistency where some diversity was anticipated. A. flavus was the predominant organism, or one of the predominant organisms, in every sample of every lot. Even before storage, Indian red peppers contain too many moulds to be acceptable in international trade without sterilization; and the predominant mould has the potential to produce aflatoxin.

The major difference among lots at the beginning of storage was that 5 were infested with insects and 2 were not. It was not possible to express the degree of infestation numerically; but qualitatively, grain moths (Lepidoptera) and beetles (Coleoptera) were visible to the eye. The most predominant were the red flour beetle Tribolium castaneum (Herbst), the confused beetle T. confusum (du Val) and the Angoumois grain moth Sitotroga cerealella (Oliver). The incidence of discoloured pods determined by an \( n=500 \) sampling of each lot is recorded in Table 1; and it was observed that there were some discoloured pods in every lot infested with insects, but none in either of the non-infested lots.

Sectioning revealed that there was a relationship between the discoloration of pepper pods, insect infestation and fungi. Pl. 1, Fig. 1 is a longitudinal section of a normal red pod in which the seeds are
shown as white and there is no evidence of mould or insect invasion. Pl. 1, Fig. 2 is a yellowish discoloured pod in which the contents were completely overgrown with A. niger. Pl. 1, Fig. 3 is a section of another yellow-coloured pod which was olive green inside due to the presence of A. flavus (as confirmed by ADM). Other discoloured pods contained mixed cultures of A. flavus and A. niger or A. flavus and Mucor spp., in which the contents were cinnamon brown, green or black depending on the ratio of the various moulds present. These pods were called "aspergillus-loaded". Although not visible in the photographs, these aspergillus-loaded pods contained pellets of insect excreta which can be seen in Pl. 1, Fig. 4. This section of a mottled-yellow pod containing insect excreta but no visible mould growth may indicate that insects attack first and mould develops subsequently. It is important to note that no mould growth was evident on the outer surface of any aspergillus-loaded discoloured pods. All red peppers exported from India must be graded under the Agriculture Produce Grading and Marking Rules, commonly known as the 'Agmark' specifications (Spices Export Promotion Council, 1968). Red peppers with visible mould are not allowed to be graded under Agmark, but a small percentage of discoloured pods is permitted. Hence, aspergillus-loaded pods could be found in international trade.

Aspergillus-loaded pods were present in red peppers at the beginning of storage at the rate of 8 or less per 500 pods. "Hot spots" present a problem in sampling which is recognized in other products such as peanuts (Dickens, 1977) and pistachios (Thomson and Mehdy, 1978). The ICMSF (1974) suggests a sampling plan for dried spices in which \( n = 5 \), with the general recommendation that \( n \) be increased for non-uniform foods. In this study \( n = 20 \) or 25. Although it was not possible to obtain a mould count for an aspergillus-loaded pod because of the problems associated with fragmenting mycelium and varying rates of sporulation, the deliberate inclusion of 1 loaded pod with 19 normal pods to make up a 10-g sample produced counts between 20–35 million per g. Since the highest total count recorded in this study was 2 million per g, it was concluded that none of the 150 samples included a loaded pod. The mould counts in Table 1 represent the background count only.

To have increased \( n \) beyond 25 to count fungi would have been overly expensive and time consuming for the study of microbiological changes during the storage of red peppers, without a guarantee that the reservoirs of infection called aspergillus-loaded pods would be adequately sampled.

### Table 1

Indices of red pepper quality at the beginning of storage

<table>
<thead>
<tr>
<th>Lot No.</th>
<th>Location</th>
<th>Relative Humidity (%)</th>
<th>Number of samples (n)</th>
<th>Number of samples having mould counts of $(10^2$ $10^3$ $10^4$ $10^4$)</th>
<th>Predominant Moulds</th>
<th>Associated Insects</th>
<th>Discoloured Pods (per 500)</th>
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<tr>
<td>1</td>
<td>Hubli (Market)</td>
<td>75–80</td>
<td>20</td>
<td>0</td>
<td>12</td>
<td>8</td>
<td>A. flavus</td>
</tr>
<tr>
<td>2</td>
<td>Dharwar (Market)</td>
<td>80</td>
<td>20</td>
<td>0</td>
<td>6</td>
<td>14</td>
<td>A. flavus</td>
</tr>
<tr>
<td>3</td>
<td>Dharwar (Godown)</td>
<td>85</td>
<td>20</td>
<td>0</td>
<td>3</td>
<td>17*</td>
<td>A. flavus</td>
</tr>
<tr>
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<td>Bangalore</td>
<td>80–82</td>
<td>25</td>
<td>0</td>
<td>24</td>
<td>1</td>
<td>A. flavus</td>
</tr>
<tr>
<td>5</td>
<td>Chickballapur</td>
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<td>20</td>
<td>1</td>
<td>19</td>
<td>0</td>
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</tr>
<tr>
<td>6</td>
<td>Byadgi</td>
<td>68</td>
<td>25</td>
<td>0</td>
<td>16</td>
<td>9</td>
<td>A. flavus</td>
</tr>
<tr>
<td>7</td>
<td>Hubli (Godown)</td>
<td>80–85</td>
<td>20</td>
<td>0</td>
<td>2</td>
<td>18*</td>
<td>A. nidulans</td>
</tr>
</tbody>
</table>

* none above 2 million per g
The Role of Insects in the Biodeterioration of Indian Red Peppers by Fungi, M. Seenappa, L.W. Stobbs and A.G. Kempton.

Table 2
Incidence of “aspergillus-loaded” pepper pods during storage

<table>
<thead>
<tr>
<th>Lot No.</th>
<th>RH (%)</th>
<th>Associated</th>
<th>Number of “aspergillus-loaded” pods per 500 pod sample</th>
</tr>
</thead>
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<td></td>
<td></td>
<td></td>
<td>Days in storage</td>
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<tr>
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<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>75–80</td>
<td><em>Tribolium</em></td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td>80</td>
<td><em>Tribolium</em></td>
<td>4</td>
</tr>
<tr>
<td>3</td>
<td>85</td>
<td><em>Sitotroga</em></td>
<td>6</td>
</tr>
<tr>
<td>7</td>
<td>80–85</td>
<td><em>Tribolium</em> + <em>Sitotroga</em></td>
<td>8</td>
</tr>
<tr>
<td>5</td>
<td>65</td>
<td><em>Tribolium</em></td>
<td>4</td>
</tr>
<tr>
<td>4</td>
<td>80–82</td>
<td>None</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>68</td>
<td>None</td>
<td>0</td>
</tr>
</tbody>
</table>

**PLATE 1**

Fig. 1 Section of normal pod of red pepper.

Fig. 2 Contents of pepper pod overgrown by *A. niger*. Pod contents black.

Fig. 3 Contents of pepper pod overgrown by *A. flavus*. Pod contents olive green.

Fig. 4 Pod mottled yellow. Pellets of insect excreta indicated by arrow.
Determining only the number of aspergillus-loaded pods at intervals during storage was simple, rapid, and incurred no laboratory expense. In addition, this index of biodeterioration of spores measured the inter-relationships between insects, fungal growth and RH during storage in terms that could be compared to the accumulated knowledge of stored grain recently reviewed by Bulla et al. (1978). As shown in Table 2, the rate of deterioration was greatest in lots 1, 2, 3 and 7; which were insect infested and stored under RH values in excess of 75%. After 30 days, between 3.5 and 6.0% of the pods were classified as aspergillus-loaded. Lot 5 was also infested with Tribolium but the RH was only 65%. According to Bulla et al. (1978) storage fungi such as Aspergillus usually do not grow at an RH below 70% which would explain why the number of aspergillus-loaded pods in lot 5 increased only from 0.5% to 1.6% during the 30 day observation period. Lots 4 and 6 had no aspergillus-loaded pods in a 500 pod sample at any time during storage, even though the RH under which lot 4 was stored exceeded 80%. Neither of these lots was insect infested, which confirms the observations from Plate 1, Figures 1 to 4 that insect excreta is a precursor of discoloured, aspergillus-loaded pods. The only weakness in using aspergillus-loaded pods as an index of deterioration during storage is that it did not distinguish between lots 4 and 6. Direct plate counts may be necessary to determine the effect of RH on fungal growth when there is no insect involvement.

Adults of both Tribolium and Sitotroga were found to be carrying conidia of Aspergillus and other fungi on their body surface. On Tribolium the fungal conidia were aggregated at the intersection of the thorax and legs (Plate 2, Figs. 1, 2), on the compactly arranged trichomes of the legs (Plate 2, Figs. 3, 4) and at the intersection of abdominal sternal plates which are moistened by excretions (Plate 2, Fig. 5). On adults of Sitotroga, conidia of Aspergillus were attached to the rugose scales which cover both body wings (Plate 2, Fig. 9); favoured by the echinulated or warty nature of the conidia (Plate 2, Fig. 10). Both adults and larvae of Tribolium are known to feed on moulds in stored foods (Singh, 1971). Conidia of Aspergillus are too small (5u) to be physically damaged by the mouth parts of mould-feeding larvae; hence intact conidia stained with Lactophenol Cotton Blue can be seen in the digestive tract of larvae (Plate 2, Figs. 6, 7). Viable conidia in excretal pellets taken from discoloured pods were demonstrated by plating surface-sterilized excreta and observing the growth of conidiofores (Plate 2, Fig. 8). Thus, the adults are capable of acting as mechanical vectors in dispersing fungi within a consignment of red pepper; but the function of the reservoirs of infection called aspergillus-loaded pods is the result of a trophic mode of dispersion. Similar conclusions have been drawn regarding the relationship between fungi and insects in stored grains and corn by workers such as Van Wyk et al., (1959) and Lillehoj et al., (1976); but, as far as can be determined, this is the first time that the precise nature of mechanical vectoring of fungi by insects has been confirmed by scanning electron microscopy.

Conclusions

The ultimate methods of preventing fungal deterioration of red peppers in storage in India will probably involve fumigation to eliminate insect vectors and humidity control to restrict fungal growth and the concomitant risk of aflatoxin production. However, as an interim measure it is recommended that all discoloured pods be removed by hand before and after storage. Dickens (1977) has proposed that a small magnifying lens be used to cull individual peanuts showing visible growth of A. flavus which is no less tedious.

Storage fungi, predominantly A. flavus reach levels which are not acceptable by the ICMSF (1974) during drying before storage. Regardless of the control of further deterioration during storage, Indian red pepper will continue to require sterilization prior to retailing in importing countries.

Acknowledgements

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References


The Role of Insects in the Biodeterioration of Indian Red Peppers by Fungi, M. Seenappa, L.W. Stobbs and A.G. Kempton.
The Role of Insects in the Biodeterioration of Indian Red Peppers by Fungi, M. Seenappa, L.W. Stobbs and A.G. Kempton.

PLATE 2

Fig. 1 The surface of Tribolium near the intersection of the prosternellum and the first pair of legs (350x).

Fig. 2 Enlargement of part of Fig. 1 showing aggregation of fungal conidia on the post coxal bridge of Tribolium (1700x).

Fig. 3 Portion of tibia and tarsus of leg of Tribolium showing fungal conidia dispersed on surface (450x).

Fig. 4 Enlargement of boxed portion of Fig. 3 (3400x).

Fig. 5 Conidia adhering at the intersection of two abdominal sternites of Tribolium (1000x).

Fig. 6 Distal end of Tribolium larva stained with Lactophenol Cotton Blue, showing deeply stained fungal conidia in intestines (250x).

Fig. 7 Enlargement of part of Fig. 6 (1200x).

Fig. 8 Excretal pellet of Tribolium taken from discoloured pepper pod, surface disinfected, and plated on PDA. Aspergillus growth indicated by arrow (350x).

Fig. 9 Portion of Sitotroga wing showing Aspergillus conidia on surface (1600x).

Fig. 10 Attachment of warty conidium of Aspergillus to rugose ridge of a scale on the wing of Sitotroga. (12,000x).


BOOK REVIEW

"MICROBIOLOGY" by M.V. Gueev and L.A. Mineeva
Published by Moscow University, 1978 (in Russian).

As explained in the preface, this volume is specially written for students majoring in the biological faculties of universities and polytechnics, but not intending to become microbiologists and is based on a course of lectures given at the M.V. Lomonosov University, Moscow, to biophysicists, biochemists and physiologists. The authors believe that microbiology is important in the training of biologists and that the shaping of the evolutionary view of life, its variety and unity is impossible without such knowledge. To some extent the living procaryotes can be envisaged as "living fossils", as "finger prints" in the steps of evolution and their study would seem to be fruitful, because it allows consideration of many questions concerning evolutionary attitudes within the framework of hypothetical schemes, which seek to regulate the diverse types of living procaryotes.

After three short introductory chapters giving a historical outline of the origin and development of the ideas on the nature of fermentation, putrefaction and on illness and infection; the status of microorganisms in the living world, and on their size; there follows five chapters dealing with the shape, morphological differentiation and chemical composition of procaryotes; the general characteristics of their metabolism; the genetic mechanism of evolution; the problems of microbial systematics and a long but interesting one on the problem of the evolution of life and the emergence of procaryotic cells in the primaeval world, influenced a great deal by the evolutionary ideas of the Russian biologist A.I. Oparin, whose book, in its several editions, on the "Origin of Life", is now well known outside Russia.

The final section discusses the evolution of the energetic processes in Procaryotes — with chapters on substrate phosphorylation (alcoholic, lactic acid, propionic and butyric acid fermentations); photophosphorylation and the problems of photosynthesis; molecular oxygen as a factor in evolution; and on oxidative phosphorylation with particular reference to chemolithothrophs and chemoheterotrophs.

A short final chapter deals with viruses, their structure, multiplication and biological nature.

All the material given seems to be up to date and the evolutionary slant given throughout is an intriguing one. It may be supposed, as the authors say, that in accord with Oparin's view, the first forms of life on earth were the anaerobic procaryotes, obtaining energy at first by the glycolytic pathway; although it is admitted that such a complex enzymic system may have been proceeded by simpler means of obtaining energy. The possible importance of the archaebacteria (e.g., Methanobacteriaceae) in this connection, first discussed in the literature in 1977, probably appeared too late to be mentioned here.

Not all would agree with some of the evolutionary ideas propounded but they do give the volume a unifying theme which has been worked out with considerable skill and well worth being studied by those interested in this aspect of microbiology.

James M. Shewan.
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Biodeterioration
Proceedings of the Fourth International Symposium, Berlin
T A Oxley, D Allsopp and G Becker

Biodeterioration is the study of any undesirable change brought about in a material of economic importance by the activities of living organisms, and biodegradation harnesses this process to transform waste products. The contributors to this book are experts from throughout the world in the biodeterioration of pulp, paper, wood, metals, polymers, rubber, paint, agricultural products, the biodegradation of biocides, and the disposal of fuel, oil, and spillages.

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