

# **TEST METHODS TO PREDICT MICROBIAL ATTACK OF WATER-BASED COATINGS**

## **EXECUTIVE REPORT**

PRA Reference: MAWC  
DTI Reference: MDE Programme, Project D1

March 2000

## **Introduction**

Structural materials are frequently coated with polymeric paints and varnishes to afford protection from environmental degradative process. The organic nature of such coatings make them intrinsically susceptible to spoilage by microorganisms, e.g. algae, bacteria and fungi. Infestation is progressive until a point is reached when the coating must be replaced, either for aesthetic reasons or because an invading organism has damaged the coating to an extent that diminishes its protective properties.

Predicting the ability of a coating to resist microbial spoilage is of concern to coating manufacturers and their raw material suppliers, as well as to applicators and end-users of coating products. The requirement for accurate predictive test methods is of current importance because of the increased emphasis on environmentally friendly, but potentially susceptible formulations, of which water-based systems are a particular example.

Spoilage prediction for coating products is traditionally based on short-term laboratory procedures (e.g. BS 3900:G6) which are usually augmented by several months of field trials. The former are favoured on the grounds of response time, although test conditions may not realistically match those for natural exposure. The latter approach has the disadvantages of being longer-term, and the results obtained are known to be sensitive to the local conditions of the selected field location. Correlations between tests and real lifetime records are frequently poor. There is limited understanding of much of the basic science underpinning present trial protocols, a fact that is most certainly responsible for the poor predictive responses from current test methods in dealing with initially “resistant” coatings.

Recognising these shortcomings and the importance of reliable test methodology to industry, the DTI instigated and fully funded a programme to develop novel fungal spoilage test protocols for applied coatings. Particular attention was to be given to those studies which enhance understanding of the science that underlies any test procedure.

A research contract was awarded to the PRA, and an Industrial Advisory Group (IAG) was formed from individuals with experience in the coatings field to oversee and advise on the technical progress.

## **The Work Programme**

The programme of work undertaken by the PRA in fulfilment of the project contract was set out in The Proposal, subject to certain refinements as recorded in the First Quarterly Report.

In summary, the programme has involved:

- Field trials at three climatically distinct sites
- Intensive local microclimate monitoring at each site
- Identification of the significant factors influencing the natural site spoilage

- Development of realistic short-term laboratory test procedures to replicate field exposures
- Investigation of non-subjective methods for assessment
- Studies for early spoilage detection methods, which have the potential for long-term prediction

### **The Deliverables**

The project findings have been recorded in a set of twelve technical Quarterly Reports together with a Final Report which comprehensively covers the investigative programme.

The Final Report, of which this is a summary, sets out the advances made in the scientific understanding of the problems and states the recommendations and guidelines for revision of existing test procedures and the implementation of new protocols.

### **Statistical Design and Analysis**

In view of the complexity of the systems to be studied, the PRA sought expert advice and guidance in the use of experimental design and statistical analysis.

Although such statistical techniques are established, the detailed use of design theory and analysis was generally unfamiliar to the members of the IAG. Introducing the experimental design and analysis concept to the IAG and others in the industrial community is seen as an achievement of the dissemination programme which had not been foreseen in the proposal. Use of this approach clearly increased the efficiency and effectiveness of the field trials and has allowed novel, and unexpected, findings to be extracted from the experimental data.

### **Experimental Design for Natural Exposure Trials**

The experiment was constructed around three exposure sites, with 120 test panel positions at each site. A number of factors were considered for study, with a 2-level factorial design chosen as being the most efficient. It was thus possible to include as factors, site, binder, substrate, biocide type, biocide concentration, angle and orientation (the latter two described jointly as aspect). Inception time/seasonality was also included by replacing test panels at intervals of 1, 3, 7, and 12 months. The statistical plan generated for exposure at each site was:

<b>Block 1 *:</b>	<b>8 systems x 5 replicates x 3 substrates</b>	<b>=</b>	<b>120 panels</b>
<b>Block 2:</b>	<b>8 systems x 4 replicates x 3 substrates</b>	<b>=</b>	<b>96 panels</b>

\* One replicate set was on-going, the other four were replaced by Block 2 coatings at 1, 3, 7 and 12 months.

Monthly visual assessments of test coatings was undertaken. An examination of historical data showed that the 0 to 5 visual rating scale defined in BS 3900:G6 was satisfactory for statistical analysis.

## **Local Climate Weather Monitoring of Selected Natural Sites**

Intensive local climate monitoring (and more importantly the microclimate above the coating surface) is a procedure not typically found on exposure testing sites. An achievement of the project has been the installation of automatic climate monitoring units at each exposure site, and the collecting and processing of the data. The results show there can be significant variation in microclimates on test panels exposed at three sites, located over a relatively small geographical area. It is believed that the project's weather records form a useful data set in their own right which will be of interest elsewhere in the scientific community.

The statistical treatment of test data allowed significant "signals" to be identified above the background noise and, probably uniquely for microbial spoilage studies, it allowed significant interactions to be identified.

One of the three selected sites was situated ca 25 miles south of the PRA, in an arboreal ~ rural setting. A second site was located on the roof of the PRA's laboratories in south west London. The remaining site was designated abnormal in that the exposure array was close to the ground (i.e. below 1 metre) beside a sheltering wall on PRA's grounds.

The hypothesis was put forward that microbial colonisation of a coated surface should be strongly influenced by the microclimate at, or just above, the surface in question. A range of climatic factors were selected for monitoring, including total pyrometry, UVA and UVB, air temperature and humidity, panel temperature and wetness, rainfall. An automated "data hog" recording facility was chosen (one at each site), which was downloaded for analysis at PRA each month.

Comparison of weekly wetness duration showed that the rural-arboreal site (Holmbury St Mary) was ca 3 hours per day wetter during the spring/summer period (most certainly dew rather than rain) than the Teddington roof and ground sites. Interestingly, panel temperatures (on wood) reached higher temperatures at Holmbury in July and August 1998 than at Teddington, although average temperatures were not significantly different. Radiation levels at Holmbury appeared to fluctuate with tree foliage presence, giving an overall lower radiation level at this site. A method investigated for analysis of the weather data involved quantifying it in terms of conditions favourable or unfavourable to fungal growth. On this basis the Holmbury site appeared the more favourable, although the higher temperatures recorded may have created a survival problem.

Despite examining a number of different statistical techniques for partitioning the weather data in a variety of ways, the attempts to correlate the observed growth with microclimate conditions was unsuccessful except in the case of fungal counts at the rural site.

## **Analysis of Visual Ratings**

The field exposure trials have allowed investigation of coatings on three typical constructional substrates, wood, metal and an exterior board. The experimental design has involved phased exposure, which allowed seasonal effects as well as inter-site factors to be probed. Additionally, the exposure rack (the design of which was developed for the project) has allowed exposure direction and angle to be investigated.

Each month of exposure, 360 coatings (120 per site) were assessed to obtain visual score ratings. Analysis of variance (ANOVA) was used to statistically partition the observed values between the various effects. Graphical presentation of the analysis was widely used and is considered a better summary of interaction effects. This involved Tukey plots of the data, giving 90% confidence bars which overlap when there is no significant difference between conditions. The statistical procedures provide a hierarchy of effects which allows comparison to be made for similarities/differences between different experiments.

The site-month interaction plot differentiates Holmbury from the sites at Teddington roof and ground, in that a second sharp increase in growth level occurred. The ANOVA table and time trajectories for each site (i.e. board, metal, wood) show the same order of significance (site>month>site/month interaction) and all three substrates showed this second growth curve (termed the “Holmbury effect”).

The analysis provides a means for ranking the importance of the factors studied. Thus, when the wood substrate is considered, analysis of variance shows an order of significance of aspect>resin>biocide type/concentration interaction>site. Initial exposure month was significant, but at a comparatively low level. By comparison, the order of significance for masonry coatings on board was aspect>site>biocide type interaction>biocide type/concentration interaction, and for metal coatings it was site>aspect>resin>site/resin interaction.

Studies of the time trajectories showed that, overall, the month or season for starting an outdoor trial was important for the rate, but not the final level of spoilage.

The project identified problems with correlating results from different observers, which will need further investigation.

## **Image Analysis**

The subjectivity of the traditional method of spoilage assessment, by a visual rating on a 0-5 scale was questionable, particularly when different observers were used. The project developed and tested electronic image capture and analysis methods as a means for more objective measurement. This approach had the additional benefit of producing archivable and retrievable pictorial records. The task proved more difficult than expected. Nevertheless, the image capture microscopy technique potentially provides opportunities for the manipulation and quantitative measurement of digital images taken (sequentially) from test coatings. Percentage area of blackness and percentage of images containing hyphae were investigated as approaches to quantifying surface colonisation, however poor correlation

with visual score data was observed. The two methods of assessment differed, with the presence of fungal hyphae in a field of view perhaps being a more definitive indicator of colonisation.

The microscopy system experienced early problems, however once established it proved possible to reproducibly image contiguous areas of ca 1.5 x 1.0 mm each month. Thus, changes with time were readily visualised, including growth, degradation and disappearance of fungal structures. It was apparent that while visual rating takes account of all “physically present” fungal material, the imaging system was able to record life, death and disappearance processes.

This image assessment method has produced ratings which may be more closely related to the microbial ecology assay studies than the normal visual assessment. It is probable the technique is monitoring the life and death cycles of the organisms in a way not discernable by visual observation.

### **Microbial Ecology Analysis**

Fungal taxa associated with the test surfaces (both as active colonisers and as possibly adventitious presences) were collected separately by PRA and by CABI-Bioscience. PRA sampling was selective, and involved only random swab sampling of panels onto potato dextrose agar, and was intended to give “snapshots” of the primary colonising fungi present on plates during the exposure.

For the CABI-Bioscience work complete panel sets were removed for isolation, enumeration and identification (using different isolation assay techniques) at 1, 3, 7 and 12 months.

Statistical analysis of all wood coating data showed that the assay technique was the most significant factor influencing detection of fungi. In fact, the techniques fell into homogenous groups according to whether they were looking at just the film surface, penetrated fungi, or surface and sub-surface. Each site was significantly different. Months 7 and 12 data fell into the same homogenous group (i.e. not significantly different), and south facing coatings gave more growth than north facing.

In total, 100 different fungal taxa were isolated. Fungal trends (tested statistically by the Chi-squared test) suggested that some fungi (e.g. *Aureobasidium pullulans*) may be relatively unaffected by a factor such as aspect, while others (e.g. *Alternaria alternata*, *Cladosporium cladosporioides*) show a marked difference in their response. This same type of variation in response was also produced by site, by substrate and by biocide type. It was apparent that certain fungi tend to be ubiquitous, common presences on exposed coatings, including *A alternata*, *A pullulans*, *Cladosporium spp*, *Epicoccum nigrum*, as well as pink/yellow yeasts.

An interesting phenomenon occurred during the exposure programme, and involved the initial and later growth periods observed at the Holmbury St Mary site. One primary presence identified on the September exposed, 2-3 months old coatings, was the fungus *A pullulans* growing in short, twisted torulose chains on certain paint films. The later sudden growth increase which occurred in July-August also appeared to be due primarily to (a relatively massive inoculation) of *A pullulans*, however on this occasion it appeared as a propagular non-spreading presence (possibly in response to the hotter/drier conditions at the time).

## **Laboratory Test Procedure**

Using the knowledge gained in the field studies, new approaches to short-term test procedures were formulated. The construction of special test cabinets incorporating exposure angle, and use of test panel preconditioning (artificial weathering) were introduced into the experimental design.

The wood coatings were used to investigate laboratory-based protocols (for simulating field behaviour). In a designed study, the full range of test systems were preconditioned by various QUV weathering regimes and then inoculated with four fungal taxa identified as primary colonisers. Coatings were also tested “as applied” (i.e. without weathering). Incubation in BS 3900:G6 type humidity chambers readily produced growth on the unweathered susceptible coatings, however no differentiation of “resistant” coatings occurred (although in the field these had different active lives). Only one artificial weathering regime (i.e. 90% UVB/10% condensation) showed any significant factors but these, and the pattern of growth behaviour, were not consistent with natural behaviour.

On natural exposure, coatings are continuously aged and inoculated. Therefore a second laboratory study was undertaken to investigate behaviour of inoculated/reinoculated films after exposure to either condensation or UV radiation. The first condition showed a better match to outdoor performance than the latter.

In terms of fungal colonisation of coatings, the foregoing laboratory studies tended to give quantitative rather than qualitative similarities to field behaviour, in that microbial selection and surface ecology were different. Subsequent microbiological work showed that different strains of the same organism varied in growth/vigour on paint films, and that growth also varied with the test substrate.

## **Early Detection by Biochemical Methods**

Work undertaken by the subcontractor (CABI-Bioscience) probed potential biochemical methods for detecting and quantifying early activity of fungi on the surface of inoculated coated panels.

Investigations were made relating to three areas of potentially relevant biochemistry, namely, enzyme profiling, ergosterol analysis and hydrophobicity.

Three groups of fungi from different sources were compared for selected enzyme capabilities, in order to determine whether paint biodeteriogens could be characterised by this process. However, the three fungal groups proved not to be significantly different.

Ergosterol is uniquely present in the cell membranes of filamentous fungi. Under laboratory conditions it was shown that ergosterol could be detected on a painted surface before the film showed visual fungal growth, and that the level present increased with time. Thus, early detection of fungal colonisation may be a practical possibility. However, aspects of the technique are still uncertain.

Hydrophobicity of fungal cells reflects their ability to adhere to a surface, and was considered as another possible means of characterising paint biodeteriogens. Once again, the three groups of fungi

from different sources were studied, but it was found that no separation based on hydrophobicity occurred.

An additional piece of work (not within the original project scope) was undertaken. A molecular biology approach was applied to amplify the DNA profiles and hence detect and fingerprint each of the species present in a contact sample, taken from the surface coatings.

## **The Recommendations and Guidelines**

### **The Design of the Experiment.**

A strong recommendation is made for the use of a formal experimental design approach to both field and laboratory microbial spoilage trials. The accessibility of the personal computer and the availability of statistical software packages leave no excuse for not embracing this means for improving the efficiency and effectiveness of test methodology.

It is recommended that field trials be undertaken at least at two climatically distinct sites in order to provide conditions for performance differentiation, (*note that the current project, while confirming performance variation between sites, has not established a definitive model for the relationship between microclimate and spoilage patterns*) and it is advised that while the season of initiating a trial exposure may have an effect on the rate of spoilage it may not significantly change the eventual pattern of spoilage.

### **Assessment Methods**

Visual assessment, using the existing standard protocol BS 3900:G6 is deemed a satisfactory “semi quantitative” means of monitoring spoilage progress in either the laboratory or the field.

It is nevertheless recommended that experienced observers should be used. If more than one observer is used to record data, then the degree of correlation between the observers should be established.

The limited study of one biochemical method (*viz. ergosterol analysis for the early detection of spoilage*) suggests this method is worthy of further consideration. During the current project however, none of the three biochemical techniques investigated were developed to the point where they would be considered for recommendation as part of a new test protocol.

It is concluded that sequential electronic image capture via a microscope can be a valuable objective tool in visualising changing microbiological spoilage patterns on coatings. Also, electronic image analysis should be considered as a means for objectively quantifying microbial colonisation.

However, these procedures have been shown to record different spoilage characteristics than those underlying visual rating. Thus, while electronic imaging during microbial spoilage trials may provide useful information, this approach is not currently recommended as a replacement for the existing visual assessment procedure.

It is recommended that an element of ecology assay should form a part of natural exposure trial evaluations. Analysis of sequential changes in surface microflora during field exposure can yield unique information. The procedure will, for example, follow any changes in succession of the dominating taxa.

### **Inoculation in Laboratory Tests**

The primary colonising fungi were identified for the UK urban and rural sites, used in the project (viz. *Alternaria alternata*, *Aureobasidium pullulans*, *Cladosporium spp* and *Epicoccum nigrum*). It is recommended that strains of these fungi, alongside standard strains, be included in the challenge employed in laboratory testing procedures. The makeup of inoculation for use in laboratory tests will thus correspond more closely to service conditions. This approach will contrast with the use of inappropriate species, which may be specified in the current test protocols.

Caution should, however, be exercised when drawing conclusions from limited field trial results. A “singular” extreme growth event occurred at the rural site during the project. It was concluded that this might have been due to a transient, local high level fungal inoculation. To replicate such an event in a laboratory test, a challenge of ca 16,000 cfu/cm<sup>2</sup> would be needed, which is in fact similar to the level used in the BS 3900:G6 test protocol.

There is evidence that the sets of test strains used in current laboratory test procedures can lose vigour on extended storage, therefore it is recommended that the vigour of laboratory test strains should be maintained, and that ability to grow on each relevant substrate is confirmed.

### **Laboratory Test Conditions**

The work undertaken leads to a general guidance principle that, it is unrealistic to seek absolute correlation between results from short term laboratory tests and those from field exposure trials. However, it is nonetheless possible to observe a realistic correlation for the hierarchy of influence for the factors and interactions.

Where appropriate, it is suggested that a laboratory test procedure involving cycles of inoculation and humid incubation periods be considered. In the current project, it was found that this procedure differentiated resistant coatings with a hierarchy of rankings which was reasonably consistent with those found at the three UK natural exposure sites.

During natural exposure there is concurrent weathering and inoculation which is not readily replicated in the laboratory. It is not recommended that procedures to pre-condition samples by artificial weathering, prior to laboratory inoculation, are generally undertaken. It is suggested that such procedures will “jump” a coating to an unpredictable point in a natural weathering sequence, without it experiencing the consequences of early microbial activity and gradual.

Existing laboratory test procedures using high humidity incubation effectively differentiate susceptible from resistant coatings. It is recommended that for such tests, coatings of established susceptibility and known resistance should be included as performance reference points.

## **Future Work**

A revision of the current British Standard Fungal Test (BS 3900:G6) is recommended. This would require further experimentation to quantify the nature of any proposed modifications. From the results generated in this project, it is apparent that a number of potentially important factors may require detailed study. These include:

- test substrate
- test species/strains
- inoculum compositions and method of application
- incubation conditions
- combination effects

The requirement would be to establish their significance in a laboratory-based protocol and therefore the degree of control necessary for confidence in the predictive application.

Both image analysis and ergosterol analysis have shown potential for objective assay of fungal development on coatings and, in the latter case, for detecting fungal growth in advance of visual appearance. With some further development, the techniques could find a place in a standard spoilage test procedure.

Innovative molecular biological methods for “fingerprinting” fungi are available and these should be investigated in relation to early detection and quantification of coating colonisation.

It was accepted that the range of natural sites studied within the project would be small. Nevertheless the sites used were climatically differentiated and the local biological behaviour was distinct. A failure to find a definitive correlation between the local microclimate and growth pattern was disappointing. With DTI and industry funding, an extension project is underway in which more sites, offering a greater climate challenge, are being used. It is anticipated that this work will enhance understanding of the relationship between locations and spoilage patterns, which may lead to a model allowing extrapolation between results obtained at a test site and growth behaviour in another location.

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