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FUNGI ASSOCIATED WITH MATERIALS' BIODETERIORATION IN THE HUMID TROPICS

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Swab sampling methods, commonly used in field microbiological inspections of equipment tested in the humid tropics, were found to provide an inaccurate indication of fungi that grow actively on a test item.

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I. BACKGROUND

Field microbiological inspections of equipment on test at US Army Tropic Test Center (USATTC) have historically involved the use of swab sampling techniques to evaluate areas of suspected fungal growth. The swab samples were returned to the laboratory and used to prepare cultures from which different fungal species could be isolated and identified. A potential problem with this method of inspection results from the fact that surfaces exposed in the humid tropics are contaminated with a wide variety of organic materials including fungal spores. The swab sampling procedures can pick up contaminant spores along with spores from species actually growing on the test item. If these contaminant spores belong to species which grow rapidly in culture and are able to outcompete the species causing degradation, then false identifications of degrading species might be made.

A second problem is also caused by contaminant spores. These spores can be picked up from surfaces that are not supporting active fungal growth. An organism will then develop from these spores, grow in culture, and give a false impression that fungal attack is occurring.

Results obtained from microbiological inspections conducted at USATTC support the possibility of incorrectly interpreting data that result from swab sampling. A small number of species have been isolated and identified from the vast majority of swab samples from past test projects. The species found could not generally be related to the nature of the test substrate. The commonly found species were prevalent soil fungi which grow rapidly on culture media. Thus, the organisms identified may represent contaminants which grow well on laboratory culture media, rather than organisms involved in material degradation.

II. OBJECTIVE

Evaluate the validity of results obtained from the use of swab sampling methods in field microbiological inspections.

III. MATERIAL AND METHODS

a. Test Materials: All test materials were obtained from other USATTC test and research projects. The original material examined was a part of the 120mm tank ammunition undergoing tropic exposure. This ammunition had an elastomer obturator band which appeared to be supporting fungal growth. This obturator band material was examined using the procedures described in paragraph IIId. The remaining materials had been exposed as part of a USATTC Methodology Investigation, Tropic Versus CONUS Deterioration Tests.[1] These

[1] Draft, Methodology Investigation: Tropic vs CONUS. Correlation Tests, US Army Tropic Test Center, Fort Clayton, APO Miami 34004, F. Chen and W. A. Dement, TECOM Project No. 7-CO-RD8-TT1-001, USATTC Report No. 820703, unpublished.

materials were exposed for 48 weeks at the McKenzie Forest Exposure Site. At the time of the current investigation, the materials had been stored in plastic bags in the laboratory for approximately one year. These materials were POL Collapsible Fuel Tank material, tropicalized cotton jungle fatigue material, cotton, mylar, latex, nylon, and cellulose acetate. Detailed information on these materials can be found in the above referenced Methodology Report.

b. Additional Tropic Exposure: Two of the Tropic Versus CONUS materials (tropicalized cotton and cotton) were subjected to two additional weeks of tropic exposure during the wet season (June 1981) at the Fort Clayton General Purpose Test Area under a jungle canopy.

c. Culture Media: Two types of culture media were used in this investigation. One was carrot decoction agar, which is the standard medium used for growing and isolating fungi at USATTC. It contains a carbon nutrient source and is able to independently support the growth of most soil fungi. The second was mineral salts agar. This medium contains no carbon nutrient source, but supplies all mineral nutrients required for fungus growth. Methods of preparing these media are presented below.

(1) Carrot Agar: Autoclave 35 grams of sliced carrots in 500 milliliters of distilled water for 15 minutes. Filter the carrot extract and add enough distilled water to make 1000 milliliters. Add 15 grams of agar and autoclave for 20 minutes.

(2) Mineral Salts Agar: Combine 0.7 gram of KH_2PO_4, 0.7 gram of K_2HPO_4, 0.7 gram of $MgSO_4.7H_2O$, 1.0 gram of NH_4NO_3, 0.005 gram of NaCl, 0.002 gram of $ZnSO_4.7H_2O$, 0.002 gram of $ZnSO_4.7H_2O$ and 0.001 gram of $MnSO_4.H_2O$. Add enough distilled water to make 1000 milliliters of solution. Add 15 grams of agar and autoclave for 20 minutes.

d. Examination of 120mm Ammunition Material: This material was examined by swab sampling, direct scanning electron microscopy (SEM), and culture on mineral salts agar.

(1) Swab sampling. Areas of suspected fungal growth on the rubber obturator ring were rubbed with a wet, sterile cotton swab. The swab was taken to the laboratory and used to innoculate sterile carrot decoction agar in a Petri dish. The Petri dish was maintained at room temperature and examined with a light microscope after two to three days and again after one week. The fungi found growing on the Petri dish were identified when possible. If a fungus produced spores but could not be identified, it was described to allow comparisons with data from other methods of analysis. Fungi which did not produce spores were not included in the data.

(2) Direct SEM observation. Small samples (2-4mm by 4-8mm) of rubber obturator band material supporting fungal growth were cut from the ammunition. A sample was mounted on an aluminum SEM stub with colloidal graphite adhesive. The sample was sputter-coated with gold. The sample was

examined with an International Scientific Instruments (SEM). Fungi found on the sample were identified or described if identification was not possible.

(3) Material sampling. Small samples (2-4mm by 4-8mm) of rubber obturator band material supporting fungal growth were cut from the ammunition. These samples were washed gently with a test tube brush in tap water and rinsed three times by shaking in tubes of sterile distilled water. The samples were then transferred to Petri dishes of sterile mineral salts agar. The cultures were maintained at room temperature in the laboratory and examined at weekly intervals for four to six weeks. All fungi found were either identified or described when identification was not possible. Only those fungi growing on or in close proximity to the material sample were included in the list of fungi from a culture.

e. Examination of Tropic Versus CONUS materials. These materials were examined by swab sampling and material sampling. No direct SEM examination was conducted because the samples had been stored for a long period of time in the laboratory and no active fungal growth was present.

(1) Swab sampling. The swab sampling procedures described for the 120mm ammunition material were used for all of these materials except the fabrics. Fabrics were placed in a sterile tube with approximately 2 milliliters of sterile distilled water. The material was agitated in the water with the cotton swab. This swab was then used to inoculate the carrot decoction agar culture.

(2) Material sampling. A 1- by 1-centimeter sample was cut from the material in the same area that was swab sampled. The sample was gently washed in tap water using a test tube brush. It was then rinsed three times by shaking in tubes of sterile distilled water. The sample was transferred to sterile mineral salts agar in a Petri dish and maintained at room temperature in the laboratory. The culture was examined weekly for four to six weeks using an optical microscope. Fungi were either identifed or described if identification was not possible.

IV. RESULTS

Table 1 presents the fungi found from each material using the two methods of examination, swab sampling and material sampling. The most prevalent fungi found in a culture are identified by an asterisk. The other fungi were represented by only a few colonies. Results from direct SEM examination of the 120mm ammunition obturator band material surface agreed with the results obtained from the material sampling method. In general, the swab sampling and material sampling methods provided quite different results.

V. DISCUSSION

The material sampling results were used as standards to evaluate the swab sampling methods. The mineral salts medium provides everything that a fungus needs for growth except a carbon nutrient source. The material that is placed

on the mineral salts medium represents the only potential carbon source. Any fungus which grows well in the medium containing the material sample must be using some part of that materials as a carbon nutrient source and thus would represent an organism that was degrading the material in nature. Based on the results in Table 1, swab sampling provides a poor indicator of what fungus species are actually growing on and potentially degrading a test item.

There was some concern that the long laboratory storage of materials from the Tropic versus CONUS project might be a reason for the poor agreement between the two methods. Samples of cotton and tropicalized cotton from the Tropic versus CONUS project were exposed for two weeks at the Fort Clayton General Purpose Test Area in the jungle during the wet season. Swab sampling results indeed showed more species of fungus; however, the fungi were not the same as those found in the mineral salt cultures containing the material sample. The swab samples contained common, fast-growing fungi, e.g. Cephalosphorium sp and Rhizopus sp. The mineral salts cultures again showed the presence of Trichoderma sp and Cephalosporium sp. The fungus Fusarium sp was the most common species observed on cotton and was a prevalent organism on tropicalized cotton. It is hypothesized that the long laboratory storage resulted in loss of viability of contaminant spores, but did not affect those organisms that were actively growing with the material as a nutrient source, except for certain species, e.g. Fusarium sp.

VI. CONCLUSION

Swab sampling provides an inaccurate indication of fungi that grow actively on a test item. The preferred method of inspection is to collect material samples suspected of supporting fungal growth, and to analyze these samples in the laboratory by direct SEM examination and by culture study using mineral salts agar. If material samples cannot be taken from the test item and swab sampling is required, tape should be used to pick up suspected fungus colonies from the test item surface. Direct SEM examination of this tape-collected sample will provide data to aid in interpreting swab sampling results. The identification of a fungus from both swab samples and tape-collected samples is evidence that the organism was actively growing on the test item surface.

TABLE 1. FUNGI ISOLATED FROM MATERIAL USING DIFFERENT SAMPLING METHODS

MATERIAL	SAMPLING METHOD	
	Swab Sampling	Material Sampling
120mm Obturator Band	Penicillium sp a/ Cladosporium sp a/ Monilia sp Pestalotia sp Aspergillus sp Unknown #1	Cephalosporium sp (SEM) b/ Verticillium sp (SEM) b/ Fusarium sp (SEM) Penicillium sp (SEM) Cladosporium sp (SEM) Streptomyces sp (SEM) Paecilomyces sp Unknown #2 Unknown #3
POL Tank	Cladosporium sp a/ Aureobasidium pullulans	Cephalosporium sp Gliocladium sp a/
Tropicalized Cotton	Pestalotia sp a/	Trichoderma sp #1 a/ Cephalosporium sp a/ Curvularia sp Phoma sp Unknown #?
Tropicalized Cotton (2 weeks additional exposure)	Cephalosporium sp a/ Rhizopus sp a/ Aureobasidium pullulans Penicillium sp Fusarium sp	Trichoderma sp #1 a/ Cephalosporium sp a/ Fusarium sp a/ Gliocladium sp a/ Streptomyces sp Spicaria sp
Cotton	None	Trichoderma sp #2 a/ Fusarium sp a/ Phoma sp a/ Cephalosporium sp Curvularia sp
Cotton (2 weeks additional exposure)	Cephalosporium sp a/ Aureobasidium pullulans Trichoderma sp #2 Spicaria sp	Fusarium sp a/ Cephalosporium sp a/ Trichoderma sp #2 a/ Streptomyces sp
Latex	Penicillium sp a/ Unknown #3	Curvularia sp a/ Penicillium sp a/ Cladosporium sp a/ Spicaria sp a/
Nylon	Pestalotia sp a/ Unknown #1	Unknown #4

Table 1 (concluded)

MATERIAL	SAMPLING METHOD	
	Swab Sampling	Material Sampling
Cellulose Acetate	*Pestalotia* sp a/	None
Mylar	None	None

a/ Most prevalent fungi found in the culture. Other fungi listed were represented by only a few colonies.

b/ SEM-Identified by direct scanning electron microscope examination of the material.

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