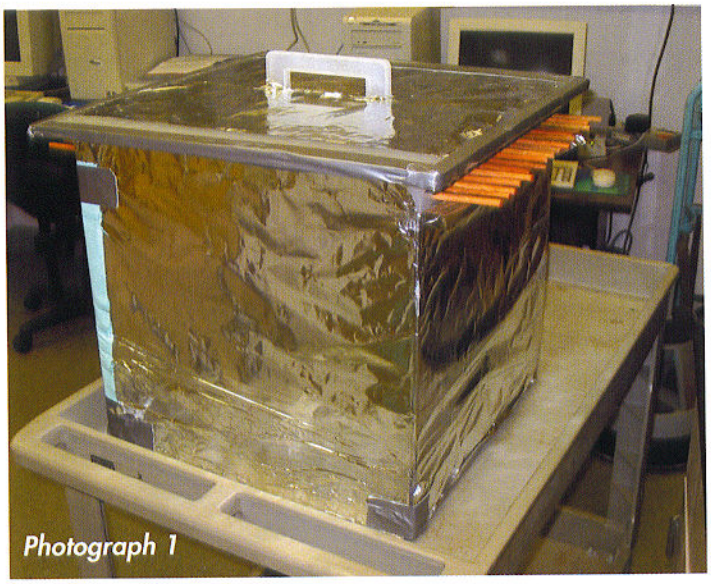
PART

MOLD-CONTAMI

Cleaning Effectiv

By Ralph E. Moon, Ph.D., CHMM, CIAQP; De-wei Li, Ph.D., and Chin Yang, Ph.D.

Editor's Note: This is the first in a two-part series based on a presentation at ASCR's All Kinds of Contents, Damage & Solutions conference. Part 2 will appear in the November 2004 issue of Cleaning & Restoration.



Photograph 1

Photograph 2

METHODS

- **Preparation and Fabric Incubation**

Four fabrics (i.e., cotton, wool, polyester and silk) were tested for fungal removal using four cleaning methods (i.e., perchloroethylene *aka* perc, petroleum distillates, laundry and laundry with bleach). Individual pieces of fabric (18" by 30") were labeled and inoculated with either *Stachybotrys chartarum* or *Aspergillus versicolor* spores provided by P&K Microbiological Services, Inc. (P&K) Cherry Hill, New Jersey. Exposure and growth by native colonizing fungi on the test fabrics was unavoidable.

Three incubation chambers (two inoculated, one control, *Photograph 1*) were prepared to contain fabric samples by covering each to prevent light penetration and placing them in the laboratory hood (24.5 C, con-

Above: One of three incubation polyethylene chambers covered with aluminum foil. Bottom: Silk fabric removed from incubation after one month.

Next page top: Silk fabric removed from incubation after nine weeks. Bottom: Confirmation of Aspergillus-like spores in the fabric after incubation (200x).

RT 1

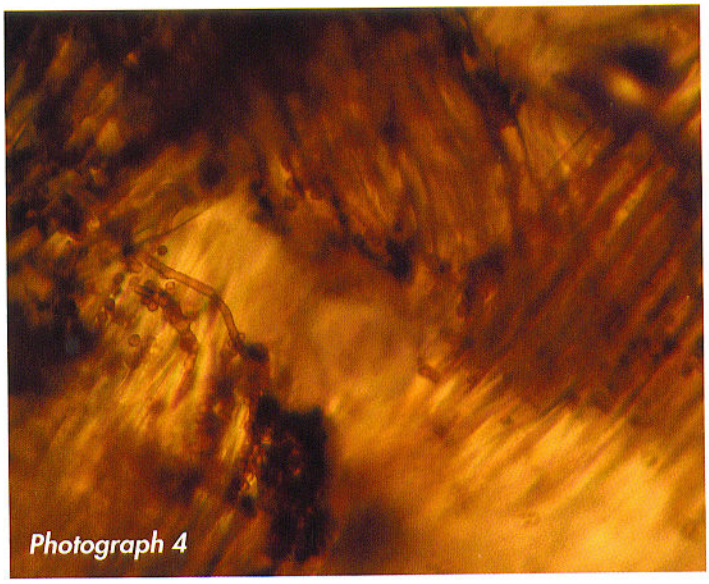
NATED FABRICS

veness Comparison

stant flowing air). The chamber lids were removed weekly to observe the fungal growth and maintain the moisture content. A detailed description of the experimental methods can be obtained from the International FabriCare Institute (IFI) in Silver Spring, Md.

After one month, little or no visible growth was observed (*Photograph 2*). A dilute solution (1:2 parts) of Gatorade® (lemon lime) was sprayed once onto the fabric (20-25 ml. per piece) to promote fungal growth. Visible fungal growth was observed two weeks later. The incubation period extended for nine weeks. The fabrics supported a variety of fungal species (*Photograph 3*). Microscopic examination of the fabric confirmed fungal hyphae and spores present within the fibers before cleaning (*Photograph 4*). The fabric samples were cleaned using standard methods developed by IFI.

Photograph 3



Photograph 4

- **Fungal Culturing and Identification**

The cleaned fabric samples were shipped to P&K for extraction and culturing. Pieces of each fabric were removed, extracted and a portion of the cell/spore extract suspension was plated onto three agar plates (*i.e.*, Malt Extract Agar, Corn Meal Agar and DG18 agar) for incubation for seven days at 25 C (77 F).

RESULTS

- **Species Identification**

A variety of fungal species were identified from cleaned fabric samples. The most dominant fungal species were identified based on the amount of fungal growth [colony forming units per gram of fabric (CFU/g)] reported from each piece of fabric. Among

the control fabric samples, a ranking system identified *Trichoderma harizianum* as most frequent (85%) and most abundant followed by *Penicillium spp.* (*Table 1*).

- **Fungal Removal from Fabric**

Fungal growth on pieces of fabric varied despite efforts to sustain uniform incubation conditions. Mycological tests for fungi conducted on all control fabric samples after cleaning, reported the following results:

Silk: Silk samples (controls) that supported mixed invasive species contained low fungal concentrations (<3,000-4000 CFU/g) when laundered with bleach, laundering alone and with perchloroethylene. Cleaning with petroleum distillates resulted in the highest residual fungal concentrations (up to 297,000 CFU/g) (*Table 2*). Culturing on three different agar media reported consistent results. Silk fabric samples inoculated with *S. chartarum* spores and airborne fungal species and then cleaned, produced remarkably similar results. Petroleum distillates were the least effective cleaning method.

Polyester: Polyester fabrics performed similarly to silk; perchloroethylene and laundering with and without

continued on page 35

TABLE 1:
FUNGI IDENTIFIED ON CLEANED CONTROL FABRICS

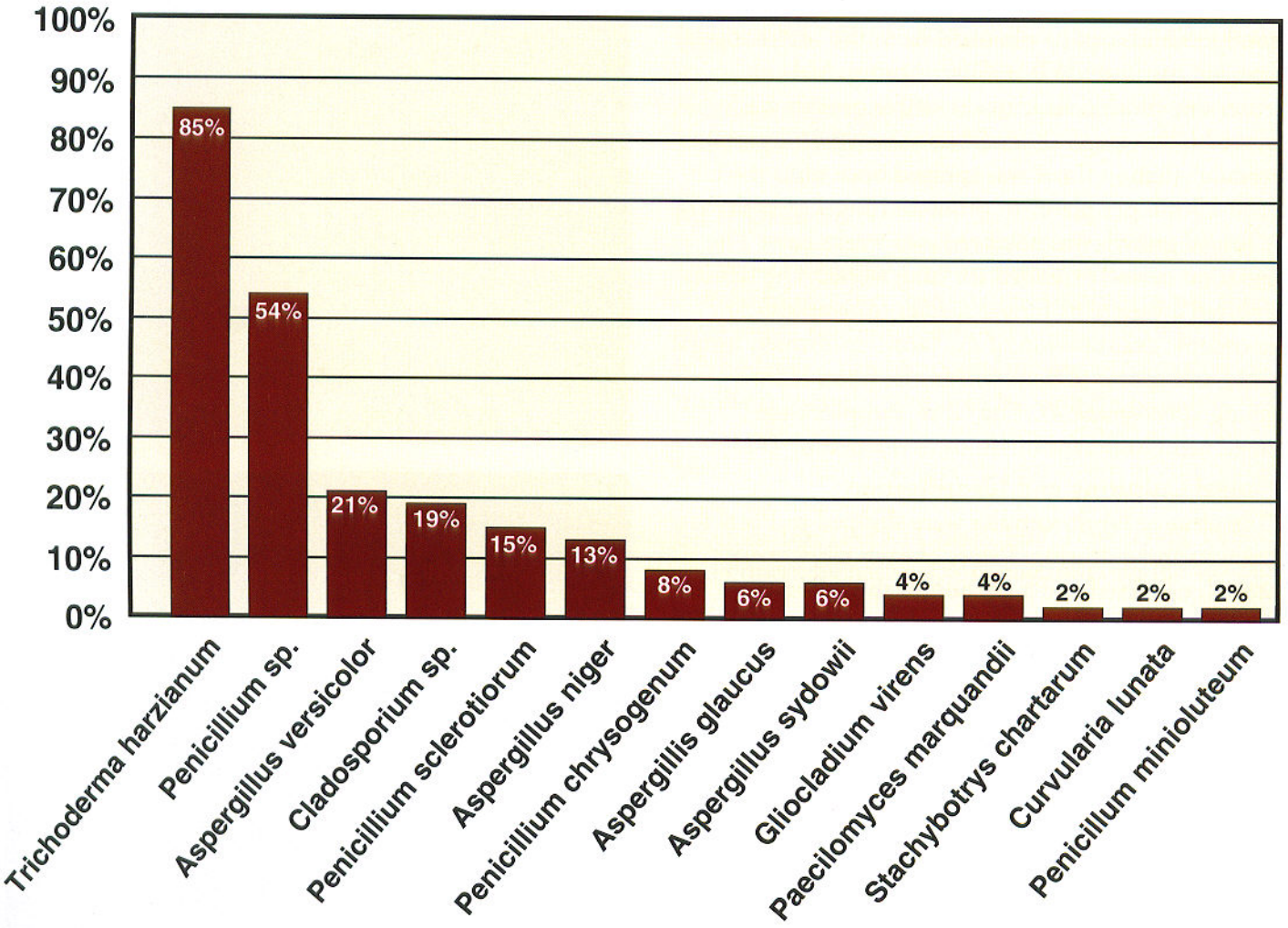
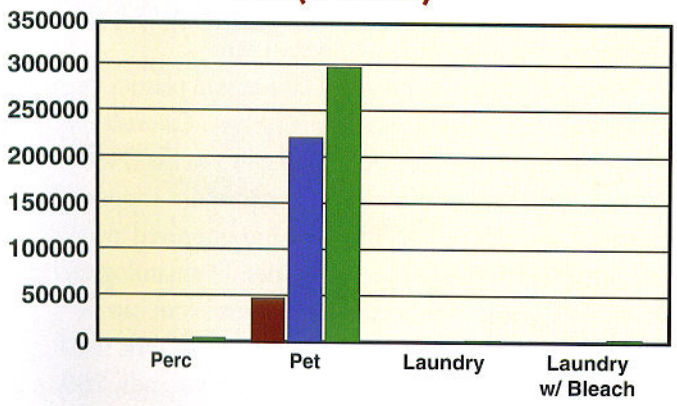
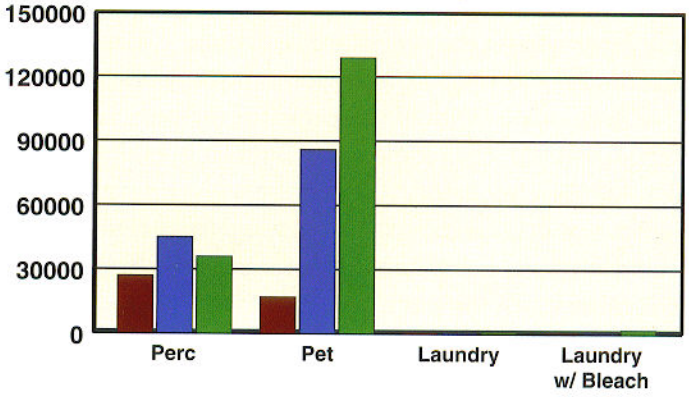


TABLE 2:
RESIDUAL FUNGAL ACTIVITY AFTER CLEANING

Silk (Control)

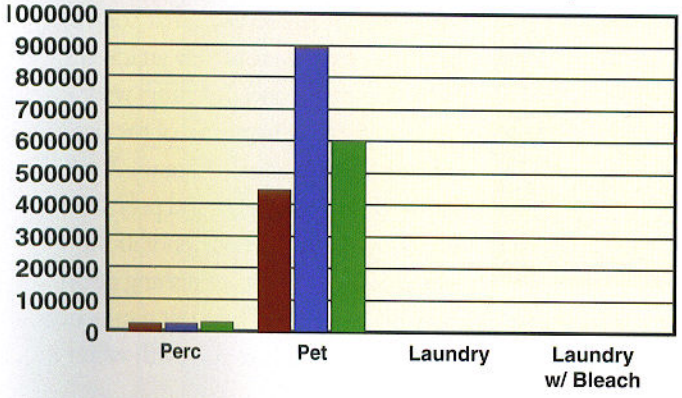


Cotton (Control)

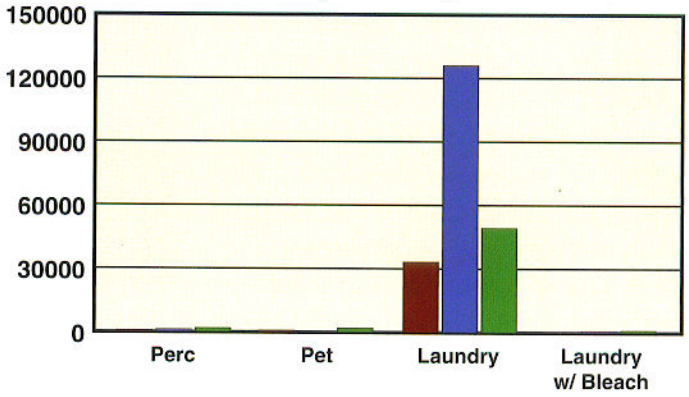


MFA CMA DG18

Silk (Stachybotrys Inoculated)



Wool (Control)



Polyester (Control)

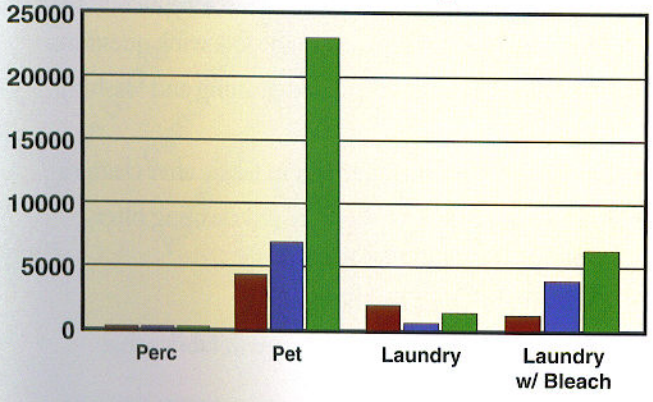
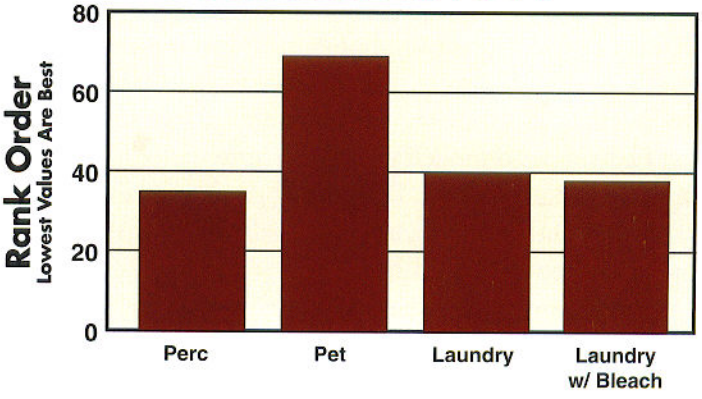


TABLE 3:
CLEANING EFFECTIVENESS FOR ALL METHODS



Continued from page 32

bleach provided the best removal methods (*Table 2*). The highest concentrations of fungi (up to 23,000 CFU/g) were reported from polyester fabric cleaned with petroleum distillates. Perchloroethylene reported the lowest fungal concentrations when used to clean polyester.

Cotton: Laundry with and without bleach worked best for cotton fabrics (*Table 2*). Perchloroethylene was less effective, while petroleum distillates were the least effective.

Wool: Perchloroethylene, petroleum distillates and laundering with bleach proved effective in removing fungi from wool (*Table 2*). Laundering alone was reported to be less effective; however, variations in the extent of fungal growth may have contributed to these results.

Among the four cleaning methods, perchloroethylene and laundering, with and without bleach, were the most effective in removing fungi. Use of a petroleum distillate was the least effective cleaning method (*Table 3*). Polyester was the easiest fabric to clean while silk was the most difficult. This finding was consistent with a visual examination of the fabrics after incubation; polyester fabrics supporting little or no visible fungal growth while the silk fabrics supported the most prominent fungal growth.

- **Residual Fungi in the Cleaning Equipment**

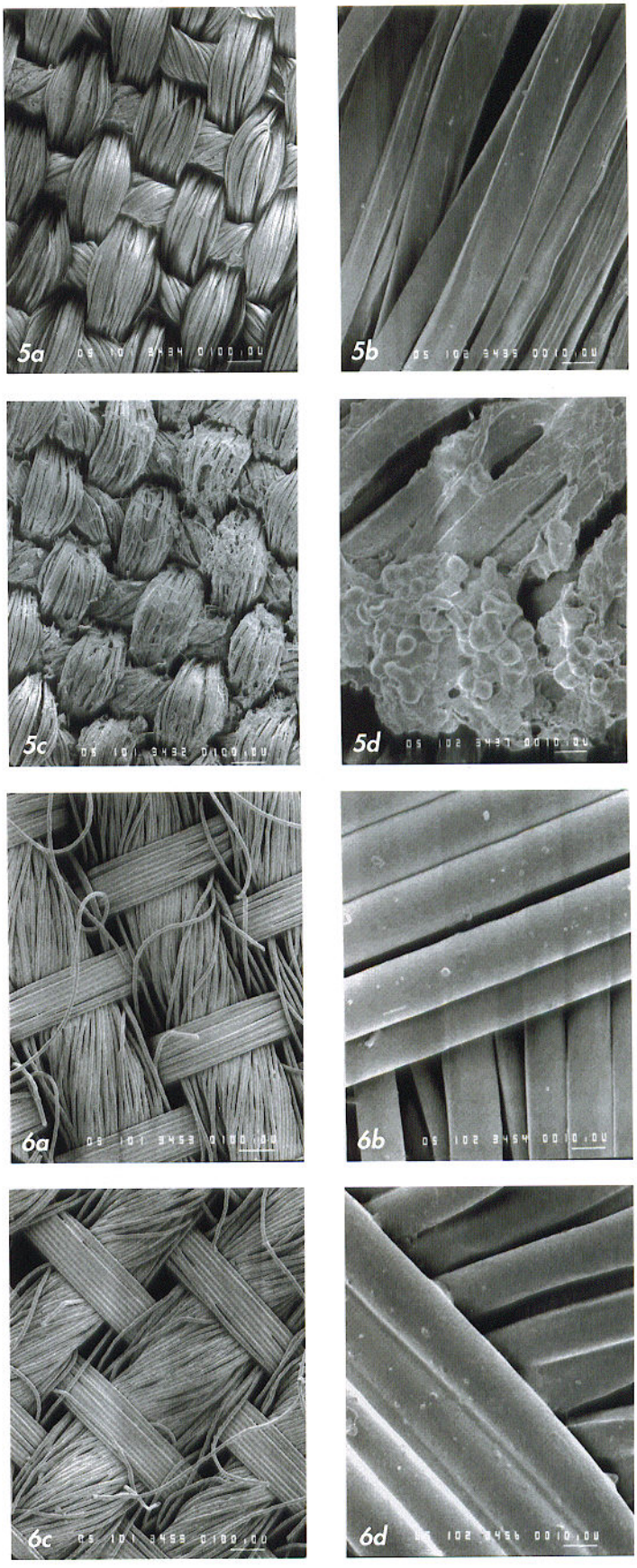
Petroleum distillates contained fungi in both the solvent and filter after cleaning. The other cleaning methods contained "no growth" (or below the quantitation limit) in the solvents, filters and wash waters.

- **Effectiveness of Steam Pressing**

Steam pressing appears effective in reducing the amount of fungi after cleaning. Mycological examination of the fabric pieces that were not steam pressed reported higher concentrations of fungi on all agar plates than fabric pieces that were steam pressed.

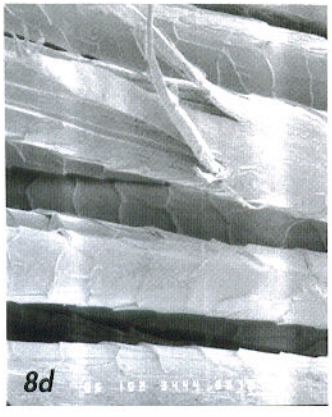
- **Tensile Strength Following Mold Contamination**

The tensile strength of wool declined when laundered with and without bleach. The tensile strength further



Top Group of 4 Photos: Scanning Electron Microscopy of silk fabric (upper-left, control @ 100x; upper-right, control @ 1000x; lower-left, test @ 100x and lower-right, test @ 1000x).

Bottom Group of 4 Photos: Scanning Electron Microscopy of polyester fabric (upper-left, control @ 100x; upper-right, control @ 1000x; lower-left, test @ 100x and lower-right, test @ 1000x).



declined when the fabric supported fungal growth (*Table 4*). Tensile strength testing was conducted for wool fabric alone.

- **Scanning Electron Microscopy (SEM)**

SEM photographic comparisons between control and test fabrics (cleaned) confirm that after incubation and cleaning, residual fungi are present in silk and cotton; wool fibers underwent structural changes. Polyester fibers showed no apparent change. Sections of the test fabrics were selected that were visibly stained or appeared to support residual growth. Among the four images presented in each photograph, the top-left and right photographs (*a & b*) are the control fabric samples, while the lower-left and right photographs (*c & d*) are the test fabric samples.

SEM photographs of silk fabric show the retention of residual fungi on and between individual fibers (*Photograph 5c*). A comparison between control and test fabrics (100 x) shows the proliferation of fungal growth in the test fabric (*Photographs 5a and 5c*). At 1000x magnification, fungi are observed to be closely bound to the silk fibers and substantiate the difficulty of removing them via cleaning (*Photograph 5d*).

Polyester fabric revealed no visible changes in the surface features of the fiber (*Photograph 6*). The appearance of the fibers is uniform in both the control and following washing and exposure to microbial growth. Small deposits of possible biological origin are present between the test fibers (*Photograph 6d*).

Cotton fabric shows a relaxed appearance when the control is compared to the test fabric (*Photographs 7a and 7c*). At 1000x magnification, residual fungal hyphae are visible (*Photograph 7d*); however, prominent changes in the fiber surfaces are not evident.

Wool fabric fiber reveals the appearance of being weakened (*Photograph 8*). At 1000x magnification, pieces of the fiber have pulled away revealing the fiber's interior structure (*Photograph 8d*). Fungal growth is also present among the wool fibers *(Photograph 8c*).

Top Group of 4 Photos: Scanning Electron Microscopy of cotton fabric (upper-left, control @ 100x; upper-right, control 1000x; lower-left test @ 100x; lower-right test @ 1000x).

Bottom Group of 4 Photos: Scanning electron Microscopy of wool fabric (upper-left, control @ 100x; upper-right, control @ 1000x; lower-left, test @ 100x; lower-right, test @ 1000x).

DISCUSSION & CONCLUSIONS

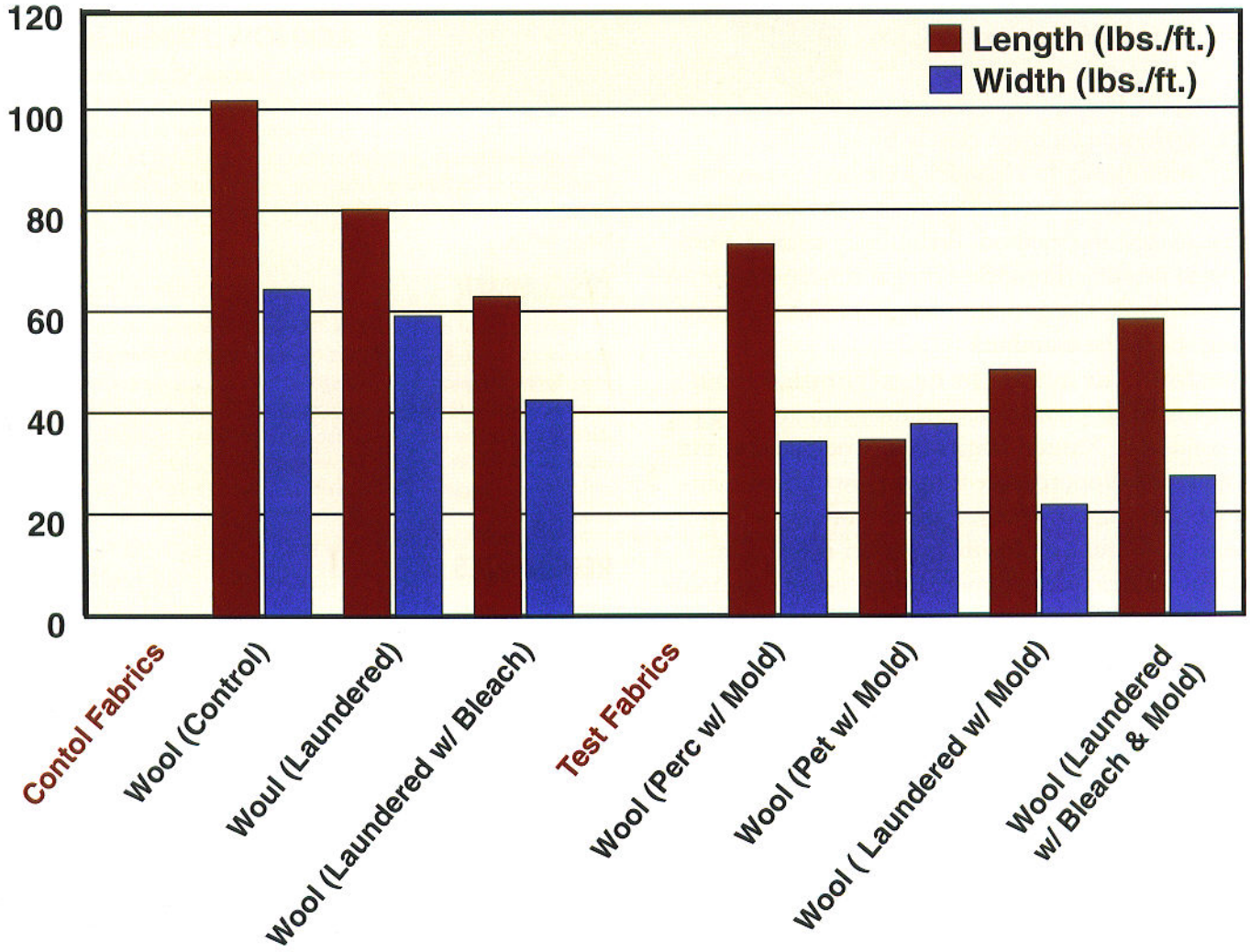
Several factors affect the removal of fungi from fabric. The extent of hyphal growth (i.e., active fungal growth), fabric type and cleaning method influence the completeness of removal. Fabrics that are visibly moldy will be difficult to clean regardless of fabric, cleaning method or species present.

The analytical results support the need for further study. Though the experimental design was uniform, the extent of fungal growth varied making absolute comparisons between the sample results difficult. Despite this variation, the effectiveness of each cleaning method was reproducible and allowed meaningful interpretation of the results.

The diminished effectiveness of cleaning mold-contaminated fabrics through use of petroleum distillates is noteworthy to professional cleaners. Segregation or the refusal to accept mold-contaminated clothing provides a simple way to avoid contaminated equipment and minimize cross contamination.

Clean fabrics do not easily support active fungal growth without the addition of nutrients. This finding was consistent with evidence of fungal growth on fabric-covered furniture and contaminated clothing left in

TABLE 4:
TENSILE STRENGTH TESTS: WOOL



water-damaged homes. Fabrics that absorb soft drinks, milk, food and sweat are most likely to support visible microbial growth.

The tensile strength test results show that changes occur in wool fabric after fungal contamination. Measured changes in tensile strength support the interpretation that fungi begin to obtain nutrition from the host fabric after two months of incubation.

Examination of the fabrics during incubation revealed that the slender fungal hyphae attach by wrapping around the fabric fibers (*Photograph 3*). This observation helps explain why silk and wool are difficult to clean when they support fungal growth.

Among the four cleaning methods, perchloroethylene followed closely by laundry with bleach and laundry were best to remove fungi. Use of petroleum distillates were the least effective fungal cleaning method. Preliminary testing shows that steam pressing diminishes fungal concentrations. A determination of the optimum length of time for steam pressing should be examined.

Once fabrics are invaded by fungal growth, various cleaning methods may not return the garment to its previous condition. Natural fabrics (i.e., wool, cotton and silk) appear to support more fungal growth than man-made fabrics (i.e., polyester). Furthermore, once contaminated with fungal growth, polyester is easiest to clean. Wool fabrics that support fungal growth for more than two months may experience a change in tensile strength. Garments that support visible mold growth should be segregated and cleaned separately or returned to the owner.

Fungal-contaminated garments that are dry-cleaned with perchloroethylene or cleaned by laundering and laundering with bleach are less likely to result in fungal contamination of the equipment and solvents than the use of petroleum distillates. ■

Dr. Ralph E. Moon received his Doctoral of Philosophy degree from the University of South Florida in Tampa, Florida, and currently serves as the department manager of the Building Sciences Department at HSA Engineers & Scientists. He lectures extensively to insurance companies on the environmental consulting liability, cause and origin of water damage and building sciences.

Dr. De-Wei Li is conducting research on indoor fungi at the Connecticut Agricultural Experiment Station. Prior to joining CAES, he worked for P&K Microbiological Services, Inc. for three years. He is author or co-author of 20 research papers and two books.

Dr. Chin S. Yang received his Doctoral of Philosophy degree from the State University of New York (SUNY), College of Environmental Science and Forestry in Syracuse, New York. He has worked extensively with allergists, physicians, industrial hygienists and health professionals on microbiology-related problems and on Legionella *and Legionnaires disease outbreak investigations. He has published and lectured extensively.*

ACKNOWLEDGEMENTS

Chris Edwards, ACW Management Corp.; Adrienne Beckta-Rybak and IFI employees; and Dean F. Martin, University of South Florida, supported the research effort. The FabriCare Foundation, the Alliance of Professional Restoration Drycleaners, the International Fabricare Institute and the Certified Restoration Drycleaning Network funded the research.

DISCLAIMER

This study did not attempt to define the best available technology for the removal of fungal growth and mycotoxins. It was intended to determine how the typical dry cleaning and laundering processes work on mold removal. During the cleaning processes, we chose cationic dry cleaning detergents that are common to the industry; however, there are vast differences in detergents available in the industry. The authors believe that significant improvements can be made with further study of products and processes.

REFERENCES

Seves, A.; Romano, M.; Maifreni, T.; Sora, S.; Ciferri, O.1998. The microbial degradation of silk: A laboratory investigation. *International Biodeterioration & Biodegradation*, 42 (40): 203-211.

Klintworth, R.; Reher, H.J.; Viktorov, A.N.; Bohle, E. 1999. Biological induced corrosion of materials II: New test methods and experiences from Mir station. *Acta Astronautica*, 44 (7-12): 569-578.

Neely, A.N. and Orloff, M.M. 2001. Survival of some medically important fungi on hospital fabrics and plastics. *Journal of Clinical Microbiology*, 39(9): 3360-3361.

Guther, A. and Effendy, I. 2002. In vitro growth of human pathogenic fungi on different stocking fabrics. *Mycoses*, 45: 22-26.

Szostak-Kotawa, J. 2004. Biodeterioration of textiles. *International Biodeterioration & Biodegradation*, 53 (3): 165-170.