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Prediction of Toxigenic Fungal Growth in Buildings by Using a Novel Modelling System

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There is growing concern about the adverse effects of fungal bioaerosols on the occupants of damp dwellings. Based on an extensive analysis of previously published data and on experiments carried out within this study, critical limits for the growth of the indoor fungi *Eurotium herbariorum*, *Aspergillus versicolor*, and *Stachybotrys chartarum* were mathematically described in terms of growth limit curves (isopleths) which define the minimum combination of temperature (T) and relative humidity (RH) at which growth will occur. Each growth limit curve was generated from a series of data points on a T-RH plot and mathematically fitted by using a third-order polynomial equation of the form $RH = a_3T^3 + a_2T^2 + a_1T + a_0$. This fungal growth prediction model was incorporated within the ESP-*r* (Environmental Systems Performance [*r* stands for "research"]) computer-based program for transient simulation of the energy and environmental performance of buildings. For any specified location, the ESP-*r* system is able to predict the time series evolution of local surface temperature and relative humidity, taking explicit account of constructional moisture flow, moisture generation sources, and air movement. This allows the predicted local conditions to be superimposed directly onto fungal growth curves. The concentration of plotted points relative to the curves allows an assessment of the risk of fungal growth. The system's predictive capability was tested via laboratory experiments and by comparison with monitored data from a fungus-contaminated house.

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In developed countries, people spend a substantial proportion of time indoors, and it is now generally accepted that indoor air quality can have a significant impact on human health (8, 13–15, 18, 22, 27, 41, 50, 52, 53). The indoor environment can contain numerous potentially harmful substances, such as dust mite and cat allergens, formaldehyde, ozone, and volatile organic vapors (1, 28, 38, 42). In the present context, attention is drawn primarily to the presence, growth, and prediction of the xerophilic fungus *Eurotium herbariorum* and the mycotoxigenic fungi *Aspergillus versicolor* and *Stachybotrys chartarum* (15, 20, 22, 27, 34).

There is currently a substantial body of evidence to support the view that fungi in buildings can have severe and wide-ranging effects on the general health of occupants (7, 14, 15, 20, 22, 43, 53). Respiratory, allergenic, and other symptoms, including nausea and vomiting, have been diagnosed (14, 15, 18, 22, 43). Several major investigations have concluded that there is a significant correlation between the incidence of high levels of airborne fungal spores containing mycotoxins, particularly from *A. versicolor* or *S. chartarum*, and ill health (13–15, 18, 20, 22, 27, 29). For example, in some damp and moldy buildings, airborne concentrations of viable *S. chartarum* spores containing stachybotryotoxins can reach levels of up to 18,000 CFU/m^3 (23). Recent research has focused on the health status of workers in water-damaged office environments after exposure to fungal bioaerosols (13, 28, 42), especially *A. versicolor* or *S. chartarum* and their toxigenic metabolites (22, 27). It was concluded that prolonged and intense exposure to these toxigenic fungi is associated with reported disorders of the respiratory and central nervous systems and of the mucous membranes and the cellular and humoral immune system, suggesting a possible immune competency dysfunction (22, 27).

Clearly, the prevention of fungal development and mycotoxin production within buildings is a priority. While the use of biocidal compounds may be appropriate to prevent the problem from occurring in new buildings and to alleviate existing problems, it is generally agreed that the preferred strategy is the elimination of conditions which can lead to fungal growth (1, 46). A key element in such a strategy would be a model which could predict the likelihood and extent of toxigenic fungal growth for any given set of conditions (24). Such a model could be used to critically evaluate a building at the design stage for inherent problems, allowing appropriate changes to be made early in the project. It could also be applied to existing problematic buildings to determine the most effective remedial action.

Through the International Energy Agency's Annex 24 program, advanced computer models which can be used to simulate the moisture behavior of structures have been developed (25). However, the main focus of that research has been on the passage of moisture through walls and the prediction of moisture content and condensation within them (25). Until recently, little consideration was given to the prediction of fungal growth within an integrated building simulation model, probably because of the perceived difficulties involved in combining the biological and physical parameters which contribute to the conditions suitable for fungal development.

The present interdisciplinary study was undertaken to develop a prototype fungal prediction program for the built environment. First, growth limit curves for the fungi *E. herbariorum*, *A. versicolor*, and *S. chartarum* were mathematically described within a fungal growth prediction (FGP) database. Second, the FGP database was incorporated into the ESP-*r* environmental modelling system to produce a model that can

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identify local environmental conditions under which fungal development may occur. Third, the efficacy of the system's predictive capability was tested by laboratory-based experiments and by comparison of real and simulated data from a building exhibiting visible fungal growth.

MATERIALS AND METHODS

The ESP-*r* system. ESP-*r* is an advanced transient energy and environmental simulation computer package (11, 26, 56) which is used extensively in building performance appraisal. Since its inception, the system has been the subject of a comprehensive development and validation process. This has included long-term involvement in the research portfolio of the European Community, which resulted in the selection of ESP-*r* as the European reference model for two major building and environmental research programs (10, 11, 26, 51, 56). The ESP-*r* system is comprised of the following elements: the "project manager," an interface that allows the user to define the problem being investigated, within the context of the computer language required for processing, and the displaying of results once the problem has been solved; the "simulator," a numerical solver which mathematically solves the problem under investigation; and a number of supporting programs and databases containing information required by the numerical solver (e.g., thermophysical properties of building materials, glazing systems, climate).

ESP-*r* is capable of analyzing the heat, power, and air flow in a building and the operation of the associated environmental control systems (e.g., air-conditioning, heating). Typically, the building being investigated is configured within the system in terms of geometry, construction, layout, and usage. Flow paths, which represent air and moisture transfers in the building and the distribution of environmental systems and electrical power, are defined. This configuration is then analyzed under specified climate and control conditions (e.g., thermostat and time-clock settings), and the results are fed to the project manager for user interpretation. This analysis technique is equally applicable to both existing buildings and new designs, and it allows the efficacy of measures aimed at improving the energy and environmental performance of a building to be specifically quantified.

In the present context, a building can be modelled within ESP-*r* at any specified level of resolution. An enhanced resolution can be used at locations of particular concern (e.g., where there are local moisture sources or where insulation levels are low). Taking explicit account of moisture movement through walls, internal moisture generation, and air movement, ESP-*r* can predict changes in local surface temperature and relative humidity at a specified location(s) for any climatic condition. ESP-*r* is widely available at no cost to researchers, and further information and access can be gained through ESRU@strath.ac.uk.

ESP-*r* model of the test house. Comparative environmental monitoring and mycological studies were conducted for a selected fungus-contaminated surface in a late-1940's prefabricated three-bedroom, semidetached dwelling located on a housing estate in Edinburgh, Scotland. Information relating the house's design, form, and fabric (e.g., hygrothermal properties); occupancy behavior (e.g., moisture production); building environmental systems (e.g., heating, ventilation); and outdoor climate were incorporated and simulated in ESP-*r*. The house was of steel frame construction, which is prone to condensation. The windows were steel framed, with high resultant air infiltration rates, and insulation levels were generally poor. The lower floor consisted of a hall, kitchen, bathroom, storeroom, and living room, while the upper floor consisted of three bedrooms and a hall. The house was heated by a 3-kW electric heater in the living room and a 1-kW electric heater in the upstairs hallway. During the study, two people resided in the test house.

Environmental monitoring of the test house. The selected test location was a fungus-contaminated surface at the junction of a north-facing wall and ceiling in one of the bedrooms. The local environmental conditions were monitored for surface temperature and relative humidity at 1.5-h intervals over a 7-day period in March by using a dedicated thermocouple (±0.5°C) and relative humidity sensor (±0.5%) attached to a recording device (data logger, model XT 102; ACR Systems Inc., Shepshed, United Kingdom). Simultaneous monitoring of external climatic conditions also took place, by using an on-site weather station consisting of global horizontal and diffuse solar irradiance measurement (Kipp and Zonen [St. Albans, United Kingdom] type CM11 pyranometers and shadow band), wet and dry bulb temperature measurement (Vector Instruments [Rhyl, United Kingdom] type H301 aspirated psychrometer), and wind velocity and direction measurement (Vector Instruments type A100R switching anemometer and type W200P potentiometer windvane). These instruments were connected to a data logger (model DL2; Delta-T Devices Ltd., Cambridge, United Kingdom).

Mycological examination of the test house. During the same 7-day monitoring period in March, the types of fungi on the test surface and their minimum relative humidity (RH) growth requirements were determined by using dichloran rose bengal chloramphenicol agar (DRBCA) and 2% malt extract agar (MEA) contact plates (Oxoid Products). The equilibrated relative humidity (ERH) (which is equivalent to the more commonly used biological term water activity [a_w]) was adjusted for the agar to 98.7, 94.5, 93, 90.5, 88.5, 84.5, 81, 78.5, 76.1, 74.5, 71.2, and 67.8% by the addition of glycerol. The final ERH was confirmed with an a_w-Wert Messer Chamber (Lufft). During the monitoring study, the DRBCA and MEA plates were pressed against areas of confluent fungal growth at the test location. The contact plates were positioned on metal rack supports over 50 ml of appropriate saturated salt solutions, as described by Grant et al. (19), in crystallizing chambers (100 mm in diameter by 60 mm in depth) which controlled the ERH in the culture media at the aforementioned levels. In preparing and maintaining the humidity chambers, the stipulations made by Wexler and Hasegawa (54) and Winston and Bates (55) regarding the control and accuracy of ERH were carefully observed. A check was made on the ERH level attained in each chamber by using a model DP680 hygrometer (Protimeter Ltd., Marlow, United Kingdom) and Solomat (Bishops Stortford, United Kingdom) model MPM2000 and was found to agree within a 1% margin. The contact plates were subsequently incubated in the above-mentioned atmospheric controlled chambers at 20°C for 25 days. The plates were examined periodically for the presence of fungal growth, and the emerging yeasts and molds were identified by conventional mycological techniques (44).

Lowest relative humidity value supporting growth of building-isolated fungi on woodchip wallpaper. This study was designed to compare the minimum RH requirements for the growth of fungi isolated from the test surface on nutritionally rich laboratory-based culture media with the minimum water requirements when grown on the nutritionally inferior building material woodchip wallpaper. The test molds were grown and sporulated on MEA slants after 10 days at 25°C, while the yeast cultures were grown on MEA for 3 days at 25°C. Strips of woodchip wallpaper (40 by 40 mm) were placed in minimal salt solutions, as described in Grant et al. (19), after autoclaving at 109°C for 10 min and drying overnight. The squares of woodchip wallpaper were positioned in atmospheric chambers that were controlled at the above-mentioned series of ERH values. After equilibration for 10 days, duplicate squares were separately centrally inoculated with the test fungi by using a sterile needle. The chambers were incubated and examined for growth over 110 days at 20°C. The identities of the emerging fungi were confirmed as described earlier.

Statistical analysis. Analysis of variance, balance model (Minitab software release 11; Minitab Inc., State College, Pa.), was used to compare the minimum relative humidity requirements for the test fungi growing on MEA, DRBCA, and woodchip wallpaper. The studies were performed in duplicate with duplicate samples examined at each trial. Analysis of variance, two-way model, was used to compare minimum moisture requirements reported by previous researchers with limiting RH values obtained for the same fungi during this study. A paired *t* test was used to compare simulated and real RH and temperature data from the test house. All significant differences were reported at the 95% ($P < 0.05$) confidence interval.

RESULTS

Development of the fungal growth prediction program. An analysis of previously published data (1–6, 9, 12, 16, 17, 19, 21, 23, 24, 30–32, 34, 36–40, 48, 49) and experiments conducted in this study were used to derive growth limit curves (isopleths), which define the minimum combination of local-surface relative humidity and temperature for which growth of the toxigenic fungi *A. versicolor* and *S. chartarum* and the xerophilic atoxigenic fungus *E. herbariorum* will occur (Fig. 1). Each growth limit curve was generated from a series of data points on a temperature-versus-relative-humidity (T-RH) plot and was mathematically fitted by using a third-order polynomial equation of the form $RH = a_3T^3 + a_2T^2 + a_1T + a_0$. Curve fitting for the isopleths was undertaken by using the curve-fitting package within Microsoft Excel 97. The above-mentioned third-order polynomial gave both the closest match (for all the data analyzed) and required profile for the control data points used ($R^2 = 0.96$).

Mycological verification of fungal growth limits incorporated within ESP-*r*. The types of fungi isolated from the test house and the lowest RH levels at which each fungus grew at 20°C on MEA, DRBCA, and woodchip wallpaper after 25 and 110 days of incubation are given in Table 1. There was no significant difference ($P < 0.05$) between the lowest RH values supporting growth of the test fungi on MEA and DRBCA (Table 1). Due to the absence of *S. chartarum* in the test house, the minimum RH limit for the growth of *S. chartarum* IMI 032542 (obtained from the International Mycological Institute, CABI International, Egham, Surrey, United Kingdom) was examined with MEA, DRBCA, and woodchip wallpaper under the ERH-controlled atmospheres described earlier. The re-

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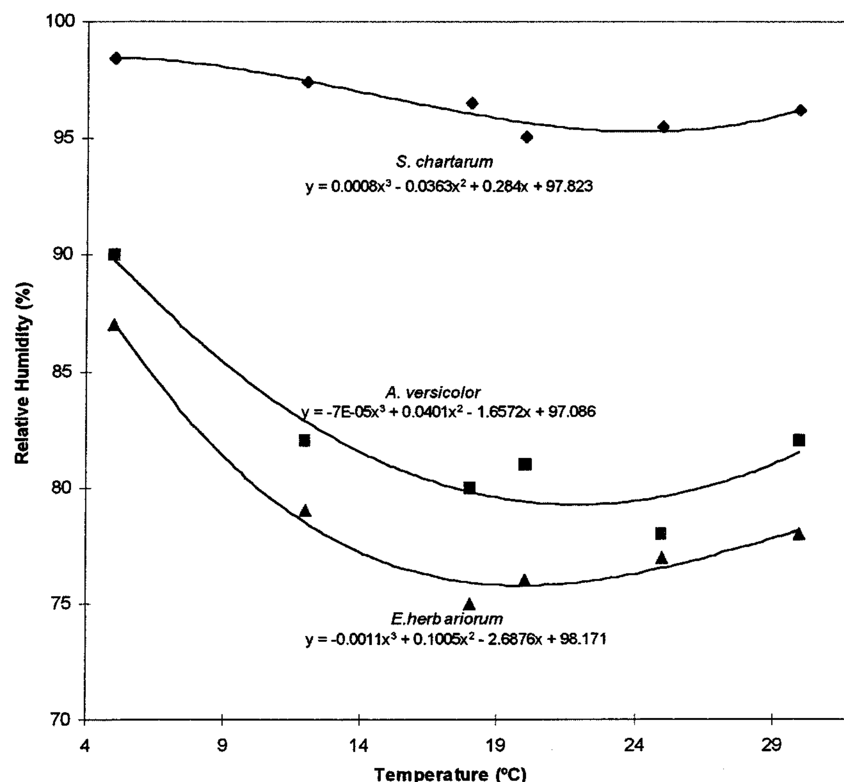

FIG. 1. Third-order fit of relative humidity and temperature which limit growth of *E. herbariorum*, *A. versicolor*, and *S. chartarum* on building materials (data points obtained from this study and from previously published research).

sults of this study are consistent with the isopleths shown in Fig. 1, where the minimum RH levels supporting growth of *E. herbariorum*, *A. versicolor*, and *S. chartarum* are fully consistent with the predictions of the model, as is the lack of any fungal growth at 74.5% and below (Table 1).

Ten different mold species and two yeasts (*Hansenula anomala* and *Rhodotorula glutinis*) were isolated from the test surface (Table 1). Significant variation ($P < 0.05$) in the abilities of the test fungi to grow at different humidity levels was apparent (Table 1). This ranged from growth at ≤81% RH for the molds *A. versicolor*, *E. herbariorum*, *Penicillium brevicompactum*, and *Penicillium spinulosum* to failure for some molds (*Mucor plumbeus*, *Phoma herbarum*, and *S. chartarum*) and the aforementioned yeasts to sustain growth at less than 93% RH. Despite prolonged incubation of inoculated woodchip wallpaper (110 days) at 20°C, the fungi *Cladosporium cladosporiodies*,

TABLE 1. Minimum relative humidity requirements for growth of different building-isolated fungi on MEA and DRBCA after 25 days and on woodchip wallpaper after 110 days of incubation at 20°C

Fungus isolated from test house	Minimum RH (%) supporting growth on[a]			Significant difference ($P < 0.05$)[b]
	MEA	DRBCA	Woodchip wallpaper	
Cladosporium cladosporioides	84.5	85.5 ± 2	89 ± 1	Yes
Cladosporium herbarum	85.5 ± 2	85.5 ± 2	88.5	Yes
Alternaria alternata	88.5	88.5	89.5 ± 1.2	No
Aureobasidium pullulans	88.5	89 ± 1	88.5	No
Penicillium brevicompactum	81.8 ± 1.8	82.7 ± 2	85.5 ± 2	Yes
Penicillium spinulosum	81	81	84.5	Yes
Aspergillus versicolor	81	81	82.2 ± 1.2	No
Eurotium herbariorum	76.1	76.7 ± 1.2	77.3 ± 1.2	No
Phoma herbarum	93	93.8 ± 0.8	93.8 ± 0.8	No
Mucor plumbeus	93.8 ± 0.8	93.8 ± 0.8	94.1 ± 0.8	No
Hansenula anomala	92.4 ± 1.3	93	94.5	Yes
Rhodotorula glutinis	91.7 ± 1.7	92.5 ± 1	93	No
Stachybotrys chartarum IMI 032542[c]	94.5	94.5	96.5 ± 2.3	Yes

[a] Values are averages of four replicate samples representing two trials.

[b] Difference between lowest humidity value supporting growth of fungus on woodchip wallpaper and those supporting growth on MEA and DRBCA. No significant difference between minimum RH values obtained from MEA and DRBCA at the 95% confidence interval was found ($P < 0.05$).

[c] Fungal culture obtained from the International Mycological Institute, CABI International, Egham, Surrey, United Kingdom.

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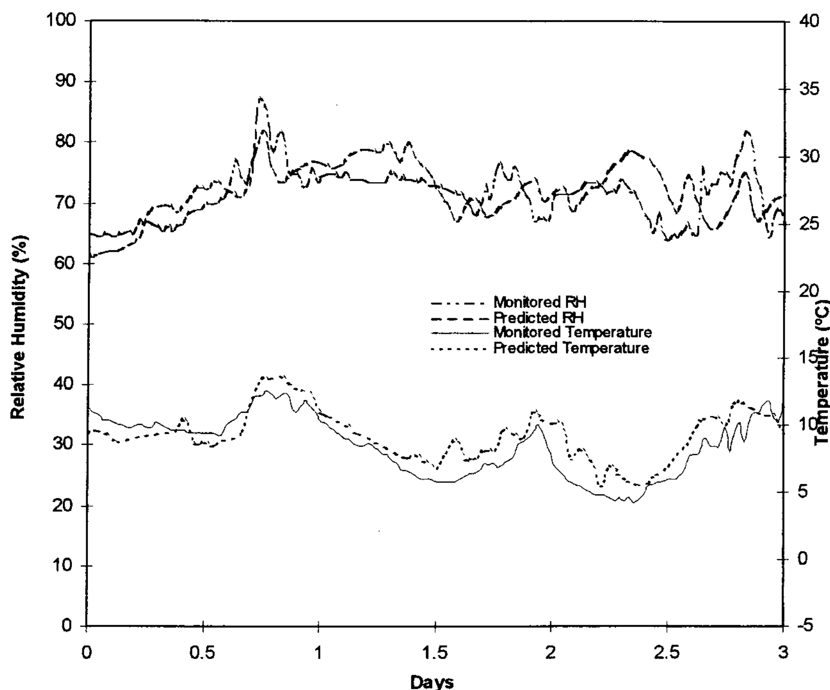

FIG. 2. Monitored and predicted relative humidity and temperature data at surface of concern in test house (period in March).

Cladosporium herbarum, *P. brevicompactum*, *P. spinulosum*, *H. anomala*, and *S. chartarum* grew at lower RH values on the nutritionally rich MEA and DRBCA over the shorter 25-day period (Table 1).

Comparison of simulated and real relative humidity and temperature data from the test house. A simulation of the mold-contaminated test house was run against the externally monitored climatic data for the 7-day period in March. The predicted-versus-real temperature and relative humidities at the test location for part of this period are shown in Fig. 2. It is evident from Fig. 2 that the simulated and real data are in relatively close agreement ($P < 0.05$). On this basis, simulated data from ESP-*r* could be taken as providing a good representation of the temperature and relative humidity occurring at the test location over any stipulated period of time.

Testing the predictions of the fungal program. In order to compare the predictions of the ESP-*r* fungal program against the types of fungal species isolated from the test location, the monitored (i.e., real) surface relative humidity and temperature data for the 7-day period in March are shown superimposed on the growth limit curves in Fig. 3. While the model successfully predicted fungal growth at the test location, on the basis of the range of plotted RH data in Fig. 3, only fungi with a growth limit of below 83% RH would have been predicted to occur. This upper RH measurement of 83% does not account for the isolation of hydrophilic molds such as *Cladosporium*, *Alternaria*, *Aureobasidium*, *Phoma*, and *Mucor* or the yeasts *Hansenula* and *Rhodotorula*, which were shown to have minimum moisture requirements of 89, 89.5, 88.5, 93.8, 94.1, 94.5, and 93% RH, respectively, on woodchip wallpaper (Table 1). In order to explain the occurrence of these molds, a simulation employing 1-h intervals and climatic data for a 3-day period in January was performed. The simulated conditions of surface relative humidity and temperature at the test location are shown superimposed on the growth limit curves in Fig. 4. On the basis of this plot (where surface relative humidity values reach as high as ~96%), a user would have correctly predicted the likely presence of the aforementioned hydrophilic fungi (Table 1), in addition to predicting the growth of *E. herbariorum* and *A. versicolor* and the absence of *S. chartarum*. The user would therefore have predicted an extensive development of different types of fungi spanning a wide range of T-RH growth categories, which is in agreement with the outcome of the mycological tests.

DISCUSSION

The most commonly occurring fungi which contaminate damp buildings in North America and Europe are those that form true cell walls of the group Eumycota (43, 46). While fungi from all subdivisions of Eumycota are often present in damp dwellings, the majority of these fungi belong to the class Hyphomycetes of the subdivision Deuteromycotina, such as *Penicillium*, *Aspergillus*, *Cladosporium*, and *Stachybotrys* (1, 43, 46). Fungal growth in buildings has been shown previously to be essentially a surface phenomenon (1). Fungal spores germinate and form active mycelia on hygroscopic building materials or interior finishes when certain critical growth parameters are satisfied (1, 46). On internal wall surfaces, the principal controlling factors governing fungal growth are relative humidity (which governs the free water availability) and temperature (1, 19, 24).

This study has addressed the prediction of local environmental conditions that encourage fungal growth on internal surfaces. Critical limits for the growth of the indoor fungi *E. herbariorum*, *A. versicolor*, and *S. chartarum* were mathematically described in terms of growth limit curves, or isopleths, that define the minimum combination of temperature and relative humidity for which growth will occur. Each growth limit curve was generated from a series of data points on a T-RH plot and was mathematically fitted by using a third-order polynomial equation of the form $RH = a_3T^3 + a_2T^2 + a_1T + a_0$

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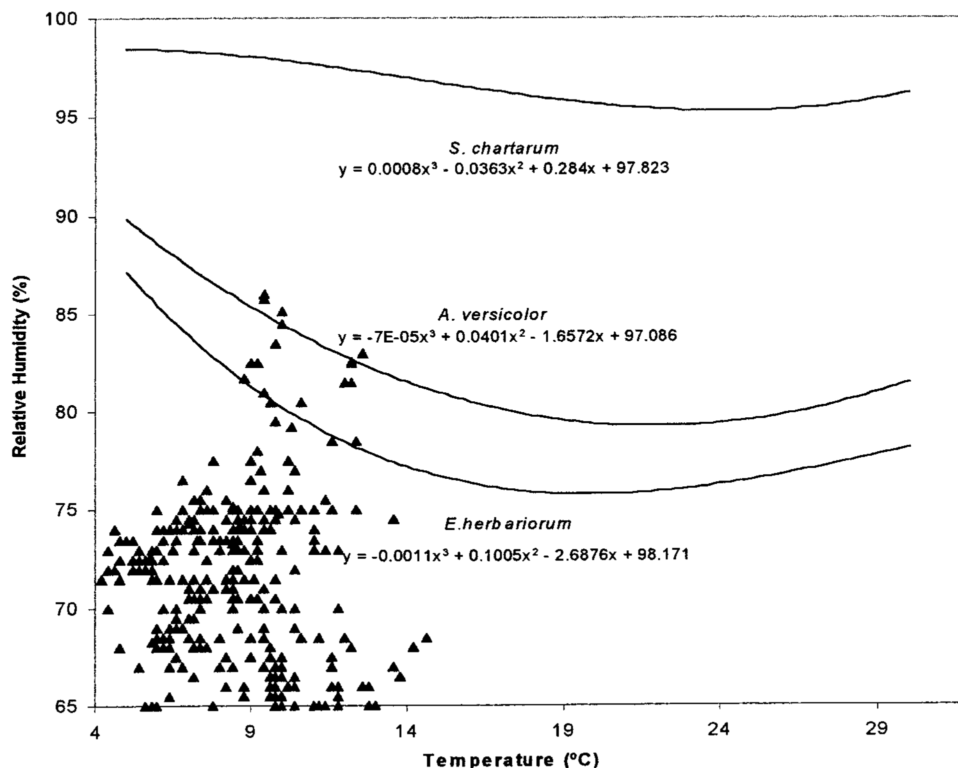

FIG. 3. Monitored environmental data for test house superimposed on growth limit curves in ESP-*r*. Each point represents a recorded temperature/relative humidity value collected at 1.5-h intervals at the test location over a 7-day period in March.

(Fig. 1). The data points were derived from previously published research data (1–6, 9, 12, 16, 17, 19, 21, 23, 24, 30–32, 34, 36–40, 48, 49) and from experiments carried out in this study (Table 1). It is evident from Fig. 1 that the three fungi differ in their minimum RH and T requirements to sustain growth on building materials. For example, *E. herbariorum* requires a minimum of 76.1% RH to sustain growth at 20°C, whereas *A. versicolor* and *S. chartarum* require a minimum of 81 and 96.5% RH, respectively, to grow at the same temperature. Local surface T-RH values occurring below the growth limit curve for each fungus prevent the organism from either initiating or sustaining growth. Both Adan (1) and Grant et al. (19) have postulated that fungal growth will be prevented if RH and surface temperature conditions within buildings are maintained such that internal wall surfaces remain below 80% RH. The limit of 80% RH for the prevention of fungal growth in buildings (1, 19) is 5% greater than the prediction limit for fungal growth set by the ESP-*r* model. Our recommendation of 75% RH for limiting fungal growth in buildings is based on our findings shown in Table 1.

In relation to the minimum moisture requirements for food spoilage and building-related fungi, many researchers have previously reported that the mold *E. herbariorum* can grow at very low RH values (1, 43, 44, 46). Therefore, relative humidity values occurring consistently below the isopleth for this highly xerophilic fungus will result in the indoor surface remaining free of all fungal growth. The concept of using RH-versus-T isopleth curves for predicting germination, growth, and asexual sporulation of toxigenic and nontoxigenic fungi on nutrient media and in foodstuffs has been exploited by a number of previous workers (2, 5, 6, 36). Ayerst (6) showed that growth of a wide variety of food spoilage fungi was governed by controlling the limiting combinations of a_w (ERH) and temperature, and each fungus had an optimal value for both of these parameters at which the growth rate was maximized. Adan (1) found that the maximum tolerance to low moisture conditions was exhibited on materials of a high nutritional content under optimum temperature. In the context of this study and to our knowledge, this is the first time this concept has been employed to predict fungal growth in buildings.

Minimum a_w values (converted to ERH) obtained by previous researchers for the growth of fungi on nutrient media at temperatures of 20 to 25°C are compared with the findings of this study in Table 2. These minimum relative humidity values limiting growth of the test fungi were shown to be in good agreement ($P < 0.05$) with minimum moisture requirements reported for the same fungi by other researchers (Table 2). Many of these workers showed that these fungi also differed in moisture requirements for each stage in their growth cycle, where differences of ~2% RH between spore germination, hyphal growth, and sporulation were recorded (1, 2, 24, 45). However, the findings of this study do not agree with the work of Nikulin et al. (34), who examined growth of *S. chartarum* and its toxin production on some building materials and in animal fodder under different RH conditions. Nikulin et al. (34) reported that *S. chartarum* was capable of growth (and in some instances, toxin production) on wallpaper, pine panel, and paper at 78% RH, while all previous studies reported that this fungus required a minimum RH of 91% or above to sustain growth (6, 9, 19, 23, 36). It is also recognized that *S. chartarum* is not a xerophilic organism (19, 24, 44), which, according to the definition of Pitt and Christian, is "a fungus capable of growth under at least one set of environmental conditions at 85% RH or less" (40).

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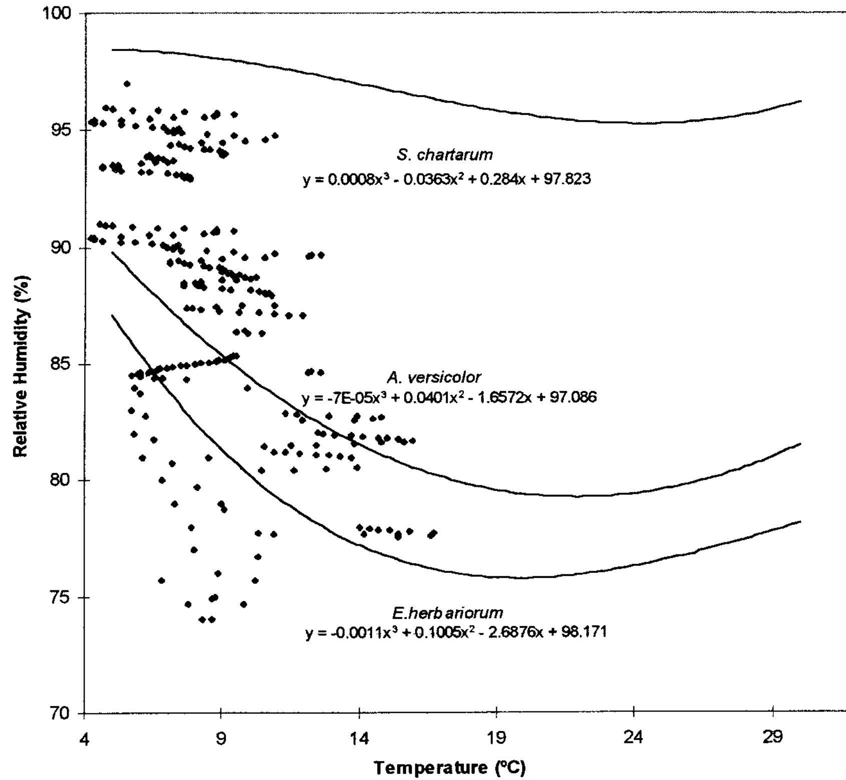

FIG. 4. Predicted environmental data for test house superimposed on growth limit curves by ESP-*r*. Each point represents a temperature/relative humidity value predicted by ESP-*r* at 1-h intervals at the test location with actual climatic data for a 3-day period in January.

On the basis of the methodology described by Nikulin et al. (34), it is possible that the moisture limit for growth of *S. chartarum* was greater than the reported 78% RH. The authors inoculated the building materials with a 1-ml volume of spores suspended in a highly nutritious aqueous wash which had been equilibrated for only 3 days to reach 78% RH (the final and subsequent RH values not being monitored in the chambers). This procedure goes against the usual convention of using a dried-spore suspension to avoid altering the moisture content of the samples. It would further appear that the samples were incubated at 20 to 23°C, which may have permitted this fungus to grow before the saturated salt solution reduced the atmosphere to less than 90% RH. Therefore, it is possible that the actual RH may have been significantly higher than the reported 78%. Nikulin et al. (34) showed that *S. chartarum* was capable of growth and of producing stachybotryotoxins on wallpaper, gypsum board, hay, and straw.

The fungal prediction model used in the present study and containing the growth limit curves, which is incorporated within ESP-*r*, operates by using information from two sources. First, as previously described, ESP-*r* can predict, from an appropriate representation of the building, changes in local sur-

TABLE 2. Minimum a_w values (converted to ERH) obtained in this and previous studies for growth of test fungi over the temperature range 20 to 25°C

Fungus	Minimum RH (%) supporting growth: Previous studies: Value(s) (reference[s])	Previous studies: Avg ± SD	Values found in this study[a]	Significant difference ($P < 0.05$)
Aspergillus versicolor	78 (9, 19, 30), 79 (23), 80 (4), 83 (36)	79.3 ± 2	81	No
Alternaria alternata	85 (24, 37), 88 (30)	86 ± 1.7	88.5	No
Cladosporium cladosporioides	88 (30), 84 (19, 23)	85.3 ± 2.3	84.5	No
Cladosporium herbarum	84 (19), 88 (36), 90 (30)	87.3 ± 3	85.5	No
Mucor plumbeus	93 (9, 24)	93	93.8 ± 0.8	No
Penicillium brevicompactum	79 (23), 81 (9), 82 (24), 83 (19)	81.3 ± 1.7	81.8 ± 0.8	No
Penicillium spinulosum	79 (19), 80 (9)	79.5 ± 0.7	81	No
Phoma herbarum	92 (16), 93 (19, 23)	92.6 ± 0.6	93	No
Stachybotrys chartarum	94 (6, 9, 36), 93 (19, 23)	93.6 ± 0.5	94.5	Yes
Aureobasidium pullulans	89 (23), 88 (44)	88.5 ± 0.7	88.5	No

[a] Values shown are for growth on MEA over a 25-day period at 20°C.

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face temperature and relative humidity at any specified location for any set of climatic data. Second, the mathematical functions defining the isopleths (Fig. 1) are contained within the FGP database. This allows the predicted local conditions to be superimposed directly on the growth limit curves, as illustrated in Fig. 4. The concentration of plotted points relative to the isopleths allows an assessment of the risk and extent of possible fungal growth.

Environmental monitoring study of the fungus-contaminated test house (Fig. 2) showed that the comparison between simulated and real surface temperature and relative humidity data over a 7-day period in March was relatively good ($P < 0.05$). It should be noted, however, that due to the lack of some information, this simulation could not be regarded as constituting a strict test of the model, which has been subjected to strictly controlled validation exercises (56). For example, due to the age of the building, some of the structural properties were not readily ascertainable and values for similar representative materials had to be substituted (24). Other uncertainties arose because it was not possible to obtain definite information on the influence of the occupants on the internal environment, e.g., additional heat and moisture from washing and cooking, etc. With this information, a more accurate prediction would have been possible.

Conclusions. Overall, the present study has verified the feasibility of a computer-based approach to the prediction of toxigenic fungal growth in problematic buildings and has demonstrated the usefulness of the prototype ESP-*r* program. However, the continued development of the prototype into a comprehensive prediction model will require an upgrading of the current FGP database. First, there is very limited information currently available on the effects of fluctuations in temperature and relative humidity on fungal growth, sporulation, and mycotoxin production in buildings. Second, a large number of fungi, hitherto regarded as harmless and which commonly occur indoors, have recently been implicated as the cause of human ill health; e.g., some *Fusarium*, *Acremonium*, and *Penicillium* spp. have been shown to be agents of hyalohyphomycosis (53). It is envisaged that additional isopleths for the prediction of these emerging filamentous fungal pathogens will be incorporated within the FGP database. Such information will enhance ESP-*r*'s fungal prediction capability, thus allowing the program to make a more accurate assessment of the risk or probability of toxigenic fungal growth in existing and new buildings.

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