Water Condensation promotes Fungal Growth in Ventilation Ducts

Pertti Pasanen¹, Anna-Liisa Pasanen¹ and Matti Jantunen² University of Kuopio, Kuopio, Finland

Abstract

In a subarctic climate the diurnal variation in temperature may cause water condensation in ducts placed in the unheated spaces of a building. In this study, germination time and sporulation of a fungus, Penicillium verrucosum, were studied on dusty. galvanized steel sheet under different moisture conditions at room temperature. The effect of condensed water in a supply air duct on spore amplification was studied in an experimental ventilation set-up. In the field, air temperatures and the dew point temperature of air in the duct were monitored continuously for a week. P. verrucosum germinated on steel surfaces during five-hour incubation of the surface under humid conditions; when the surface had been moist for half an hour, germ tubes appeared within 17 hours. During 24-hour incubation under moist conditions, P. verrucosum produced hyphae and spores. In the experimental set-up the airborne spore counts increased when the air passed through a water-condensing section of the duct. Penicillium was the most abundant fungus sporulated on the moist duct surface. In the field, during humid weather, the surface temperature on the air stream surface decreased to the dew point temperature of the air in the duct. Thus water condensation in air ducts may promote fungal growth.

KEY WORDS:

Airborne fungi, Fungal growth, Subarctic climate, Moisture, Ventilation, Water condensation

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- ¹ University of Kuopio, Department of Environmental Sciences, P.O. Box 1627, SF-70211 Kuopio, Finland
- ² National Public Health Institute, P.O. Box 95, SF-70701 Kuopio, Finland

Introduction

Fungal contamination in heating, ventilating and air-conditioning (HVAC) systems is commonly described as a cause of building-associated illness (Binnie, 1991; Bernstein et al., 1983; Morey et al., 1984; Morey and Feeley, 1990; Schata et al., 1989). Unusually high spore counts have often been caused by water condensation after cooling coils (Morey et al., 1984; Morey and Shattuck, 1989) or humidifiers (Morey et al., 1984) in the ventilation system. Despite air filtration, some dust containing living microorganisms passes through the air handling unit (AHU), and some of this dust settles on surfaces inside the ventilation system. Dust accumulated on porous insulation materials reduces indoor air quality; if the dusty insulation material becomes wet, fungal spores in this material will grow and release spores into the ventilation air (Morey and Williams, 1991).

To conserve energy, office buildings are often ventilated only when the rooms are occupied. During the air-conditioning season, the intermittent use of ventilation causes extreme variation in the relative humidity of indoor air and may result in absorption of water into porous materials (Morey and Williams, 1991). In a subarctic climate, air-conditioners and humidifiers are rarely used, and the problems caused by water sprays, condensation and leaks in the ventilation system are uncommon. Water condensation is possible, however, in ducts placed in unheated spaces. The condensation problem may arise when diurnal variation in the temperature is wide and the weather is humid, e.g. in autumn, and when the ventilation is shut off for nights and weekends. During the HVAC shut-down, natural draught forces humid indoor air into cold ventilation ducts, and may cause water condensation on their inner surface. Water condensation is not a problem as such, but if the temperature is sufficient and organic material is present, viable fungal spores will have an opportunity to germinate and proliferate. The optimum temperature for the most common species of fungi in outdoor air, *Cladosporium*, *Penicillium* and *Aspergillus*, is 20-35 °C, but the minimum reported growth temperature for *Aspergillus fumigatus* is 12 °C (Ayerst, 1969) or 9 °C (Pasanen et al., 1991a), for *Penicillium* -3 °C and for *Cladosporium* as low as -6 °C (Domsch and Gams, 1972). The minimum water activity of the growth medium required by *Penicillium* and *Aspergillus* is 0.63-0.9 (Magan and Lacey, 1984; Andrews and Pitt, 1987).

Pasanen et al. (1992) observed that *Penicillium verrucosum* germinates on nutritionally poor media, e.g. on wallpaper within five hours at room temperature when the surface was continuously or repeatedly moistened. Germination took about three times as long when the surface had been moist for only half an hour (Pasanen et al., 1992). If water condensation occurs in the ventilation system, both the temperature and the time will be sufficient for germination and production of fungal spores.

The first purpose of this study was to investigate development of fungal growth and sporulation on a material commonly used for ventilation ducts, galvanized steel sheet, in conditions which may occur in poorly designed ventilation systems. The germination and sporulation of *Penicillium verrucosum* were studied in small-scale laboratory experiments. The second purpose was to investigate the amplification of spores in a simple experimental ventilation system where water condensed during night-time HVAC shut-down periods. The third purpose was to determine whether, in a subarctic climate, water condensation occurs in real ventilation ducts placed in unheated spaces.

Material and Methods

Growth of *Penicillium verrucosum* on Dusty Steel Plate

Fungal species which belong to a *Penicillium verruco*sum complex (e.g. P. aurantiogriseum) have been one of the most common and abundant species found from air, dust and surface samples, both indoors and outdoors (Verhoeff et al., 1992; van Reenen-Hoekstra et al., 1991). *Penicillium verrucosum* Samson (DSM 62878) was chosen to represent the molds in a HVAC system. To follow the colony formation and spore production of *P. verrucosum* under favourable conditions, about 20 spores were first inoculated on malt extract agar (Blakeslee, 1915). Germination and growth of the fungus were observed at 20-23 °C under a light microscope (Olympus BH-2) at 200x magnification.

After the spore production had been observed on agar, spores were inoculated on 2l samples of unsterilized galvanized steel sheet (1 cm^2). The sheet metal pieces were cut from a supply air duct of a nonproblem building where dust had accumulated over a three-year operation period. Three samples were used for controls and examined immediately. Six samples were moistened once by adding about 50 µl sterilized water with a thin pointed pipette; three of





them were examined after 3 hours' incubation at 20-23 °C in a dry petri dish, and the remaining three were incubated for 17 hours under the same conditions. Three other samples were moistened at the beginning and twice during a five-hour incubation. The remaining nine samples were moistened once, and then incubated in petri dishes at near saturated relative humidity at 20-23 °C for 6, 24 and 28 hours. Fifty to one hundred fields of each sample were examined by scanning electron microscope (SEM) (JEOL JSM-35) at magnifications of 2 400x and 12 000x. A detailed description of the examination procedure is presented by Pasanen et al. (1992).

Simulations of Water Condensation in an Experimental Ventilation Set-up

In order to study amplification of fungal spores on a surface of the ventilation duct, a small-scale ventilation system with a cooled section of duct was built (Figure 1). A 1.2 m long, nonporous piece of a used duct (diameter 100 mm) with 50 mm polystyrene external insulation (K-value 0.78 W/m² °C) was inserted into a freezer. The temperature of the freezer was regulated by on/off operation to ± 3 °C. Room air was blown through the air duct at 5-6 m/s daily between 6:00 h and 17:00 h as is usual in non-industrial buildings. To reduce ingoing spore levels, the air was filtered (HEPA) during sampling. At night and on weekends the surface temperature of the cooled duct decreased to and below the dew point temperature of the air in the duct, so the nonporous inner surface of the duct was wetted diurnally. Temperatures of the freezer air, the air in the duct, and on the inner surface of the duct were monitored (Datataker 100 and resistance sensors). Relative humidity (Vaisala HMI 21) and flow rate (Pitot tube) of the air in the duct were also monitored.

Fungal spores from the air entering and leaving the cooled duct section were collected simultaneously on malt extract agar by modified six-stage Andersen samplers (N6) (Jones et al., 1985) on every work day. Collection of the first 10-minute samples was started when the ventilation fan was turned on, and the second sampling was started 13 minutes after that. The samples were incubated at room temperature, 20-23 °C, for five to seven days. Spore counts were calculated according to the method of Andersen (Andersen, 1958). The most common genera of fungi were identified with the aid of a light microscope (Olympus BH-2).

Field Measurements

Water condensation and fungal growth in a real ventilation system were studied in a modern office building built in 1980. The building was equipped with a mechanical ventilation system. The constant air volume (1.908 m³/s) air handling unit (AHU) ventilated one third of the building volume. The AHU was equipped with G90 (EU4) classified glass fibre bag filters, and with a cross flow heat recovery unit. The room for the AHU was situated on the roof of the building. Supply and exhaust air ducts (both 33 m) were located on the roof in an unheated space and insulated externally with 150 mm of glass fibre (K-value 0.313 W/m² °C). All the exhaust air was let out of the building through the heat recovery unit. The ventilation system was operated from 5:00 h to 20:00 h every day.

To investigate the water condensation on the inner metal surface of the duct, the temperature of the inner surface of the supply duct, the air temperature inside and outside of the duct, and the relative humidity were monitored continuously. The measuring points were situated 15 m and 30 m from the air handling unit (Figure 2). Fungal spores were sam-

supply air duct unnunn Insulation 150 mm 0.3 m hannanna hRainn Galvanized steel plate 1 mm ← 1.0 m Total length 33 m SUPPLY AIR DUCT 30 m 15 m Fungal spore sampling Air and Surface temperature measurements Flow rate and RH measurements ungal spore sampling EXHAUST AIR DUCT ROOM FOR AIR HANDLING UNITS

Cross section of insulated

Fig. 2 Schematic design of the main ventilation ducts on the roof of the field building; measuring points are presented in a 33-metre long supply air duct. A cross section of the externally insulated sheet metal air duct is presented in the upper scheme.



Scale: 1 µm

Fig. 3 SEM micrograph of swelled *P. verrucosum* spores on twice-moistened galvanized steel sheet after five-hour incubation. The germ tube has appeared and hyphae have started to elongate.

pled by Andersen six-stage samplers (Andersen, 1958) at the end of the supply air duct both before and after the ventilation was switched on, and near the AHU after the ventilation was turned on. The measurements were made in October and January. The fungal spore counts and relative humidity of the outdoor air were measured each morning during the experimental period.

Results

Growth of *P.verrucosum* on Dusty Steel Sheet Samples

Spores of *P verrucosum* were swollen, and germ tubes had emerged and were elongated, indicating that the fungus had germinated during the two hours of incubation on the agar. Hyphal growth, branching and the beginning of sporulation were observed three hours after inoculation. Visible fungal colonies with spores appeared during 17 hours of incubation on agar plate.

In the fungal growth experiments on the dusty steel samples, the control samples confirmed that all inoculated spores were initially in the resting stage. The spores did not germinate either immediately or after three hours of incubation on the dusty steel samples that had been moistened only once, but the germ tubes appeared after 17 hours of incubation. In these cases the surface remained visibly moist for about half an hour. The spores were swollen and the germ tubes had emerged during the five-hour incubation on the dusty steel samples that had been moistened three times (Figure 3), and the spores of



Scale: 10 µm

Fig. 4 SEM micrograph of *P. verrucosum* growth on moist galvanized steel sheet after 28-hour incubation at 100% relative humidity. Spores have germinated and grown, and sporulation has increased the spore counts.

the fungi were in the same stage of development on steel sheet samples that had been incubated for five hours in the humid environment. When the surface of the steel sheet remained moist for 24 hours, elongation and branching of hyphae were observed; after 28 hours the final stage of development of fungi, spore production, was also detected (Figure 4). This suggests that fungal amplification can occur within 28 hours after the surface of cooling coils, drain pans, and humidifier components are moistened.

Fungal Growth on Duct Surface of Experimental Set-up

The average spore counts within the first ten minutes in the entry and exhaust air were 7.1 and 14.1 CFU/m³ (Colony Forming Units), respectively (Table 1). The corresponding average counts within

 Table 1. Spore counts in the entry and exhaust air after the first

 10 minutes and 13 to 23 minutes after start of the fan of the experimental apparatus.

Time (days)	Spore counts (CFU/m ³)				
	0-10 minutes entry exhaust		13-23 minutes entry exhaust		
8	4	4	7	1	
10	4	28	10	44	
14	7	1	22	42	
17	1	28	11	11	
18	14	30	17	31	
19	14	4	17	32	
20	6	4	7	7	
average	7.1	14.1	13.0	24.0	

 Table 2. Spore counts for different genera of fungi in entry and exhaust air after the first 10 minutes and 13 to 23 minutes after start of the fan of the experimental apparatus.

Fungal genus	Spore counts (CFU/m ³)				
	0-10 entry	minutes exhaust	13-23 entry	minutes exhaust	
4				-	
Aspergillus spp.	-	1	·	3	
Botrytis spp.	-	1		-	
Cladosporium spp.	-	3		2	
Penicillium spp.	4	15	17	27	
Sporobolomyces	4	7	22	23	
Others	5	3	4	2	

- = colonies of the genus were not present

13 to 23 minutes after the ventilation was turned on were 13 and 24 CFU/m^3 .

The common outdoor airborne fungi Aspergillus spp., Cladosporium spp. and Botrytis were not detected in the entry air, but they were present in some samples of exhaust air. Rapidly growing and proliferating Penicillium spp. sporulated the most (Table 2).

Field Measurements

Diurnal variations in the air temperature of the supply air duct are presented in Figure 5. When the ventilation was turned on, the surface temperature of the duct increased rapidly due to warming by the supply air, and when the ventilation was turned off, it decreased slowly. During the breaks, the surface temperature of the duct dropped to the dew point temperature of the air in the duct. Measurements were made in autumn when the weather was stable but humid; it was especially foggy and misty during the morning hours. In the room end of the supply air duct the surface temperature did not reach the dew point temperature of the air in the duct.

During the measuring period in January, the lowest outdoor air temperature decreased to -10.3 °C and at the same time the surface temperature of the duct was reduced to +6.5 °C. During the winter measurements, the water content of the air in the duct was too low for water to condense on the duct.

Because the air was filtered, the concentration of fungal spores decreased along the ventilation system. At the end of the duct the levels of fungal spores decreased when the ventilation was turned on, and no increase in the number of spores was observed.

Discussion

Several species of fungal spores (Cladosporium, Alternaria, Penicillium, Aspergillus) are frequently isolated from components of HVAC systems. Microorganisms and debris may be built into HVAC systems or, without sufficient filtration, they may be carried on air currents and may contaminate the surfaces of a ventilation system (Binnie, 1991). The results of our fungal growth experiments indicated that growth and sporulation of a common indoor air fungus (Penicillium verrucosum) began on dusty steel sheet at room temperature within 1-2 days, when the surface occasionally became moist. Similar results have been found on other intermittently wet, nutritionally poor surfaces in an indoor environment (Pasanen et al., 1992). The germination time (five hours) for this rapidly growing fungus on the nutritionally





Table 3. Counts of airborne fungal spores at the beginning of the supply air duct and in the end of the duct indoors and outdoors.

Season	Fungal spore counts (CFU/m ³)				
	Outdoor air	At the beginning of the duct	End of the suply duct		
			ventilation off	ventilation on	
October	478	50	79	40	
January	< 20	10	11	3	

poor surface was shorter than the operation shutdown times in the ventilation system during nighttime, and the sporulation time was shorter than operation breaks in the ventilation system on weekends, when condensed water may appear on cold surfaces. The fungal spore production and release to supply air will be more abundant in ventilation systems with wetted porous insulation where the time for growth on moist media is longer (Morey and Williams, 1991).

Fungal spore counts were increased in the air that passed through the diurnally moist ventilation duct of the laboratory set-up. The total spore counts were several orders of magnitude lower than those measured in contaminated ventilation systems with moisture problems (Morey et al., 1984; Morey and Williams, 1991), but at the same level as in uncontaminated supply air of office buildings during a subarctic winter (Pellikka et al., 1986). During the breaks, the surface temperature of the cooled part of the duct was far below the optimum growth temperature for most fungi. The spore counts of samples collected 13-23 minutes after the ventilation was turned on were higher than the counts in samples collected during the first ten minutes. Drying of the moistened air duct apparently promoted spore release into the air stream. The velocity, 5-6 m/s, in the duct was sufficient to release Penicillium and Aspergillus spores (Pasanen et al., 1991b). Spore counts for the fast-growing fungus, Penicillium, increased mostly during a three-week experiment. This result agrees with findings from contaminated HVAC- systems (Bernstein et al., 1983; Morey et al., 1984).

In the field experiments, insulation of the supply air duct could not prevent its surface temperature from decreasing to the dew point temperature of the air in the duct under nocturnal natural draught conditions, where warm and humid indoor air flowed slowly backwards from the rooms towards the AHU. When the ventilation was turned on, the fungal spore counts decreased in the ventilation system towards the room because of filtration of the outdoor air. This has also been observed in the ventilation systems of uncontaminated office buildings in Finland (Pellikka et al., 1986). Our results indicate that conditions for microbial growth in this particular duct were not favourable and were not long enough; the weather had been foggy for just a few days before the measurements.

Conclusions

The results of fungal growth experiments indicate that fungal spores are able to germinate and sporulate on the dusty steel surface of a ventilation system when the surface becomes moist for a few hours. Our brief field measurements showed that watercondensing conditions occur in real life in ducts situated in cold spaces, when air humidity is high and when ventilation is turned off for nights and weekends. In the weather conditions of central Finland this phenomenon did not result in significant emissions of spores, but we conclude, on the basis of our fungal growth experiments in the laboratory, that in warmer and more humid climates there is a real risk of fungal growth and significant release of spores in ventilation ducts located in unheated spaces.

This risk of fungal growth can be eliminated by placing all ventilation ducts in warm spaces or by maintaining continuous forced airflow through them or by increasing the thickness of insulation on ducts in cold spaces.

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References

- Andersen, A.A. (1958) "New sampler for the collection, sizing, and enumeration of viable airborne particles", *Journal of Bac*teriology, 76, 471-484.
- Andrews, S. and Pitt, J.I. (1987) "Further studies on the water relations of xerophilic fungi including some halophiles", *Journal* of General Microbiology, 133, 233-238.
- Ayerst, G. (1969) "The effects of moisture and temperature on growth and spore germination in some fungi", *Journal of Stored Product Research*, 5, 127-141.
- Bernstein, R.S., Sorenson, W.G., Garabrant, D., Reaux, C. and Treitman, R.D. (1983) "Exposure to respirable, airborne Penicillium from a contaminated ventilation system: Clinical, environmental and epidemiological aspects", American Industrial Hygiene Association Journal, 44, 161-169.

- Binnie, P.W.H. (1991) "Biological pollutants in the indoor environment". In: Kay, J.G., Keller, G.E. and Miller, J.F. (eds) Indoor Air Pollution, Radon, Bioaerosols and VOC's, Chelsea, Lewis Publishers, Inc.
- Blakeslee, A.F. (1915) "Lindner's roll tube method of separation cultures", *Phytopathology*, 5, 68-69.

Domsch, K.H. and Gams, W. (1972). In: Hudson, P.S. (ed.) Fungi in Agricultural Soils, London, Longman Group Ltd.

- Jones, W., Morring, K., Morey, P. and Sorenson, W. (1985) "Evaluation of the Andersen viable impactor for single stage sampling", American Industrial Hygiene Association Journal, 46, 294-298.
- Magan, N. and Lacey, J. (1984) "Effect of temperature and pH on water relations at field and storage of fungi, *Transactions of British Mycological Society*, 82, 71-81.
- Morey, P.R., Hodgson, M.J., Sorenson, W.G., Kullman, G.J., Rhodes, W.W. and Visvesvera, G.S. (1984) "Environmental studies in moldy office buildings: biological agents, sources and preventative measures", Annual American Conference on Government Industrial Hygiene, 10, 21-35.
- Morey, P.R. and Shattuck, D.E. (1989) "Role of ventilation in the causation of building associated illness and the sick-building syndrome". In: Cone, J.E. and Hodgson, M.J. (eds) Problem Buildings: Building Associated Illness and the Sick Building Syndrome, Philadelphia, Hanley & Belfus, Inc.
- Morey, P.R. and Feeley, J.C. (1990) "The Landlord, Tenant, and Investigator: Their needs, concerns, and viewpoints". In: Morey, P.R., Feeley, J.C. and Otten, J.A. (eds) *Biological Contaminants in Indoor Environments*, Philadelphia, ASTM.

Morey, P.R. and Williams, C.M. (1991) "Is porous insulation in-

side a HVAC system compatible with a healthy building". In: Proceedings of Healthy Buildings, IAQ 91, 128-135.

- Pasanen, A.L., Kalliokoski, P. Pasanen, P. Jantunen, M. and Nevalainen, A. (1991a) "Laboratory studies on the relationship between fungal growth and atmospheric temperature and humidity", *Environment International*, 17, 225-228.
- Pasanen, A-L, Pasanen, P, Jantunen, M. and Kalliokoski, P. (1991b) "Significance of air humidity and air velocity for fungal spore release into the air", *Atmospheric Environment*, 25A, 459-462.
- Pasanen, A-L., Heinonen-Tanski, H., Kalliokoski, P. and Jantunen, M. (1992) "Fungal micro-colonies on indoor surfaces – an explanation for the base level fungal spore counts in indoor air", Atmospheric Environment, 26B, 117-120.
- Pellikka, M., Jantunen, M.J., Kalliokoski, P. and Pitkänen, E. (1986) "Ventilation and bioaerosols". In: Goodfellow, H.D. (ed.) *Ventilation* '85, Amsterdam, Elsevier Science Publishers, pp. 441-450.
- Reenen-Hoekstra, E.S. van, Samson, R.A., Verhoeff, A.P., van Wijnen, J.H. and Brunekreef, B. (1991) "Detection and identification of moulds in Dutch houses and non-industrial working environments", Grana, 30, 418-423.

Schata, M., Jorde, W., Elixmann, J.H. and Linskens, H.F. (1989) "Allergies to molds caused by fungal spores in air conditioning equipment", *Environment International*, 15, 177-179.

Verhoeff, A.P., van Wijnen, J.H., Brunekreef, B., Fischer, P., van Reenen-Hoekstra, E.S. and Samson, R.A. (1992) "Presence of viable mould propagules in indoor air in relation to house damp and outdoor air", *Allergy*, 47, 83-91.